

CHEMICAL FISSURE TREATMENTS

S. BUCHANAN

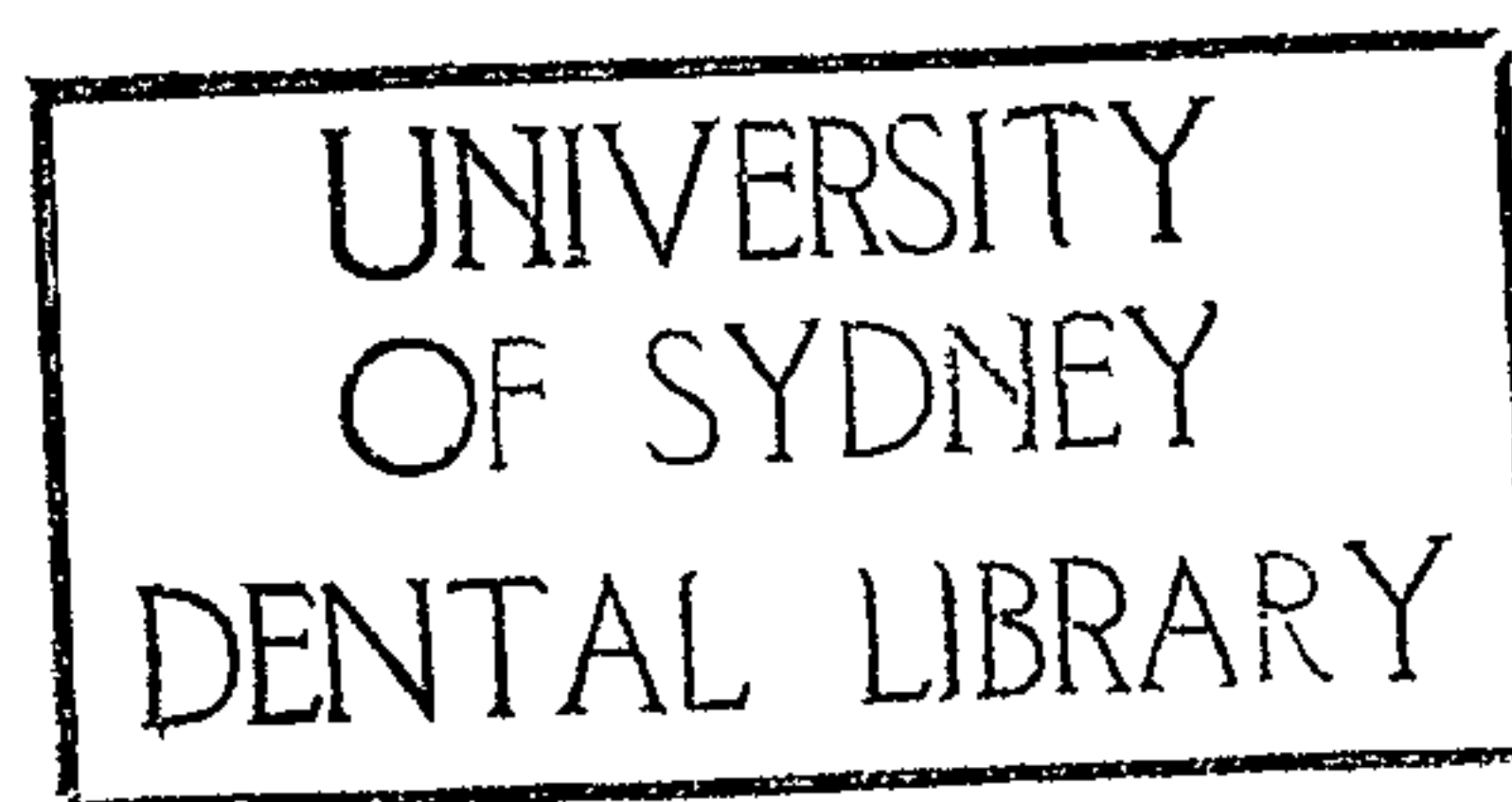
MDS

1988

EFFECT OF CHEMICAL AGENTS ON ACID PRODUCTION IN, AND THE
MICROBIAL CONTENT OF, PITS AND FISSURES

SUSAN BUCHANAN B.D.Sc.

A thesis submitted in partial requirement for the degree of
MASTER OF DENTAL SURGERY



Department of Preventive Dentistry,
Faculty of Dentistry,
University of Sydney

1988

ACKNOWLEDGEMENTS

The author would like to express her gratitude to:

Professor Noel D. Martin, Dean of the Faculty of Dentistry and Head of the Department of Preventive Dentistry, for permission to conduct this study in his Department.

Associate Professor Graham G. Craig, Department of Preventive Dentistry, University of Sydney for his valuable guidance and supervision during the course of this study.

Dr. Keith Powell, Senior Lecturer in Preventive Dentistry, University of Sydney, for his advice on a number of occasions during the course of this study.

Associate Professor Keith Godfrey, Department of Preventive Dentistry, University of Sydney, and Dr. Amrik Manku, Head of Department of Orthodontics, United Dental Hospital of Sydney for making available the facilities of their respective orthodontia departments and for providing the patients for this study.

Dr. Thomas Higgins for his advice and assistance on a number of occasions.

Dr. J. Wilkinson, Deputy Superintendent of the United Dental Hospital of Sydney for arranging the use of hospital facilities without which this study could not have been carried out.

Dr. Ilsa Pratt, Head of Admissions Department, United Dental Hospital of Sydney, and her staff for their kind cooperation during my time in her department.

Mr. Ted Foote, Coordinator of Hospital Maintenance, United Dental Hospital of Sydney, and his staff for their constant maintenance and servicing of the autoclave.

Robert Van Luym and Peter Holleman, Department of Photography, United Dental Hospital of Sydney, for the preparation of some of photographs used in this thesis.

Mrs. Joan Thwaite, and Mrs. Debra Crowe, librarians, Faculty of Dentistry, University of Sydney, for their assistance with the library research for this thesis.

Dr. Janine Kirkwood for reading and correcting the final manuscript.

My family for their support and understanding throughout the course of this study.

TABLE OF CONTENTS

	Page
Title Page	i
Acknowledgements	ii
Table of Contents	iii
List of Figures	vi
List of Tables	vii
 <u>INTRODUCTION</u>	 1
 <u>REVIEW OF LITERATURE</u>	 5
 PITS AND FISSURES	 5
Morphology of Pits and Fissures	5
Methods of Investigating Pits and Fissures	8
<u>Microscopy</u>	8
<u>Bacteriological Sampling</u>	9
<u>Biochemical Methods</u>	12
Contents of Pits and Fissures	13
Development of Plaque in Pits and Fissures	16
Pit and Fissure Caries	20
Use of Antimicrobial Agents on Pits and Fissures	22
 <u>SECTION I: EFFECT OF PROPRIETARY AGENTS ON INHIBITION OF</u>	
<u>ACID PRODUCTION IN VIVO</u>	27
 DEVELOPMENT OF EXPERIMENTAL METHOD	28
Indicator Dye	28
Revelance of Colour Changes to pH of Dental Plaque	31

MATERIALS AND METHODS	34
MATERIALS	34
Test Agents	34
Indicator Dye	34
Patients	36
METHODS	36
RESULTS	38
DISCUSSION	40
<u>SECTION II: EFFECTS OF SILVER FLUORIDE AND SILVER IODIDE ON</u> <u>INHIBITION OF ACID PRODUCTION IN VIVO</u>	41
MATERIALS AND METHODS	42
MATERIALS	42
Test Agents	42
Indicator Dye	42
Patients	43
METHODS	43
Clinical Procedures	43
Statistical Analysis	43
RESULTS	45
DISCUSSION	45
<u>SECTION III: EFFECTS OF SILVER FLUORIDE-SILVER IODIDE ON</u> <u>THE MICROBIAL FLORA OF PITS AND FISSURES IN VIVO</u>	47
MATERIALS AND METHODS	48
MATERIALS	48
Media	48
Test Agents	49
Indicator Dye	50

Plaque Collection Materials	50
<u>Needle</u>	50
<u>Construction of an Instrument for Core</u>	
<u>Sampling of Pits and Fissures:</u>	50
Patients	54
METHODS	54
Clinical Procedures	54
<u>Treatment Appointment</u>	54
<u>Sampling Appointment</u>	56
Laboratory Procedures	57
<u>Sample Dispersion and Dilution</u>	57
<u>Microplating Technique</u>	57
<u>Counting</u>	59
Statistical Analysis	59
Sensitivity of Plaque Microbes to Bromcresol Purple	59
RESULTS	60
DISCUSSION	70
<u>GENERAL DISCUSSION</u>	71
<u>SUMMARY AND CONCLUSIONS</u>	87
<u>APPENDICES</u>	89
<u>BIBLIOGRAPHY</u>	95

LIST OF FIGURES

	Page
Figure 1 Colour changes produced in a series of 10 ml aliquots of standard buffer solutions(pH range 4.3 to 7.8) following the addition of 0.1 ml of (a)0.37% bromcresol purple, (b)0.37% bromcresol purple containing 10% sucrose, and (c)standard bromcresol purple indicator solution.	29
Figure 2 Electrode system used for measuring plaque pH.	32
Figure 3 Examples of colour changes seen following the application of BCP/sucrose indicator to the fissure systems of sound lower permanent second molar teeth.	35
Figure 4 Method of isolation of lower second molars used in this study.	37
Figure 5 Schematic diagrams showing the method of construction of the fissure sampling instrument.	51
Figure 6 Instrument devised for core-sampling the contents of the depths of pits and fissures.	53
Figure 7 A 26-gauge hypodermic needle and an 06 endodontic K-file placed in the same fissure of a section of a lower third molar.	55

LIST OF TABLES

	Page
Table 1 Inhibition of acid production by antimicrobial agents.	39
Table 2 Inhibition of acid production by AgI alone and in conjunction with AgF.	44
Table 3 Total bacteria recovered from fissures on sheep blood agar.	63
Table 4 Streptococci recovered from fissures on MSA agar.	64
Table 5 <u>Streptococcus mutans</u> recovered from fissures on MSB agar.	65
Table 6 Lactobacilli recovered from fissures on LBS agar.	66
Table 7 Lactobacilli recovered from fissures on LBS-TJ agar.	67
Table 8 Specific groups of bacteria expressed as a percentage of total bacteria recovered from teeth receiving four minute treatments.	68
Table 9 Specific groups of bacteria expressed as a percentage of total bacteria recovered from teeth receiving sixty minute treatments.	69

INTRODUCTION

Pits and fissures occur predominately in molar and premolar teeth, but may also occur in anterior teeth, for example cingulum pits. They result from the folding of the inner enamel epithelium, of the enamel organ, during the morphodifferentiation stage of tooth development(57). The importance of these pit and fissure systems as predilection sites for dental caries was first recorded in 1841(146), and since that time substantial amounts of research work have supported this early observation(12,15,45,51,79,94,112,137). The reduction in the incidence of dental caries which has been witnessed in recent times, due to the widespread use of fluorides, has occurred mainly in free smooth surface and approximal surface caries(14,33,41,66,83). As a result of this drop in caries, pit and fissure lesions are now the predominant carious lesions, and have been found in several studies to account for up to seventy six percent of all carious lesions in 12-13 year olds(14,33). The occlusal surfaces of the molar teeth are particularly susceptible and seem to be at maximum risk during the years immediately after eruption. Eklund and Ismail(41) in 1986 reported that approximately 70% of all occlusal surfaces of permanent molars became decayed or filled within ten years of eruption.

Despite the obvious importance of pit and fissure systems as sites for dental caries, the bulk of the research, which has been conducted for over a century on the relationships between dental plaque, bacteria, diet, and oral hygiene and the occurrence of dental caries, has been carried out on smooth tooth surfaces(18,62,72,115,128,167,198,199). The pit and fissure systems have received inadequate attention presumably because of the poor accessibility to these areas for sampling and experimental procedures. Some studies have highlighted important differences between fissure plaque and smooth surface plaque(55,157,169,179,186,188,191). Zobell(209), and Berry and Henry(11) have also shown that cells adsorbed on surfaces, such as would occur in fissures, may express different glycolytic and growth activities. In view of these facts, care should be taken when extrapolating information obtained from experiments conducted on smooth surfaces to pit and fissure systems.

A number of theories have been postulated as to the reason for the susceptibility of pits and fissures to dental caries. These include:

1. The irregular and retentive nature of the fissure acts as an ideal biological niche for highly cariogenic bacteria such as Streptococcus mutans and lactobacilli(61,62,80,81,109,180).
2. Because of its inaccessibility, the dental plaque in a fissure would be left, in all probability, relatively undisturbed by mechanical cleansing forces, either by the toothbrush or the action of the tongue or saliva(62,100).

3. The confined nature of fissures and the densely packed mass of bacteria which they contain, may present penetration barriers to salivary buffers and other substances such as fluoride. On the other hand, substances formed within the plaque of the fissure, or those which do penetrate, may remain for a long time(118,121).
4. The irregularity and hypomineralisation of the enamel surrounding the fissure, particularly at its base, may make it more susceptible to dissolution and permit the subsequent ingress of bacteria(31,50).

Because bacteria are required for the initiation of dental caries, antimicrobial agents may have some preventive role. A large number of antimicrobial agents have been tested for their plaque inhibiting properties, however, the bulk of this research has been carried out in relation to periodontal disease(95,129). The fissure ecosystem may be considered as a closed ecosystem in that the physical nature of the space probably prevents new bacterial colonisation once it is fully inhabited(169,183). Thus in fissures it may be possible, with antimicrobial agents, to alter a cariogenic flora to a non-cariogenic one. It could also be considered desirable to sterilise a fissure prior to placement of a fissure sealant because, although a lesion does not progress under an intact sealant(69,71,85), the total elimination of the fissure microflora in an incipient lesion would be an additional safeguard(117).

To date only short term studies have been carried out to determine the effect of antimicrobial agents on pit and fissure plaque. In addition only relatively few agents including stannous fluoride(154,170,171), chlorhexidine(153,154), acidulated phosphate fluoride gel(110) and iodine solutions(27,118,153) have been used. Furthermore in view of the importance of acid production in the initiation of dental caries(113,165) it is noteworthy that none of these studies have examined the effects of these agents on acid production in pits and fissures. This latter aspect is of importance because an agent that can cause a prolonged suppression of acid production in pit and fissure sites could be of value clinically.

This investigation was carried out to determine the effect of stannous fluoride, chlorhexidine, acidulated phosphate fluoride gel, silver fluoride and an iodine/copper sulphate preparation on acid production in pits and fissures in vivo. A further study was carried out to assess the acid-inhibiting properties of a sparingly soluble salt, silver iodide, when used alone and in combination with silver fluoride. In addition, the influence of the AgI/AgF combination on the microbial composition of pit and fissure plaque after twelve weeks was examined.

REVIEW OF LITERATURE

PITS AND FISSURES

Morphology of Pits and Fissures

Because of the difficulty of obtaining sound first and second molars the majority of morphometric analyses of pits and fissures have been carried out on premolars and impacted third molars. Most of the present knowledge has been based on examinations of ground sections of pits and fissures using light microscopy(3,4, -45,63,132). Transmission electron microscopy has also been used to study replicas of ground sections of fissures from molar and premolar teeth(3). All these studies have shown a great diversity in the form of pits and fissures. Ngano(132) and Awazawa(4) attempted to categorise fissures into a number of types and demonstrated that there can be a wide variation within the fissure system of one tooth. Ngano(132) classified them as

1. V-type: wide at the top and gradually narrowing towards the bottom.
2. U-type: almost the same width from top to bottom.
3. I-type: an extremely narrow slit.
4. IK-type: extremely narrow slit associated with a large space at the bottom.
5. Other types.

Theuns and Pot(189) modified the above system and classified

fissures into six categories. However, in contrast to Ngano who used ground sections, Theuns and Pot categorised their fissures solely on the basis of tactile examination with a probe. In order to overcome the problems of sectioning, Galil and Gwinnett(53) developed a vinyl resin replicating technique which allowed them to demonstrate not only the shape and the distribution of pits and fissures in eighty human permanent unerupted molar and premolar teeth but also the relationship of pits and fissures to each other. The specimens were examined by light and scanning electron microscopy. In their study(53), an even greater diversity of form was shown than in earlier investigations. Divergent pits and fissures were particularly abundant in molar teeth, in fact, some of the third molars showed as many as ten pits arising from a single fissure.

Several workers(4,15,94,138) have attempted to ascertain whether tooth morphology predisposes to caries and have found that a relationship exists between the steepness of the cusps and the depth of the fissure. In an effort to provide a more precise morphometric definition to the categorisation of fissures, Fejerskov et al.(45) measured thirteen parameters on each of four to eight serial sections ground from 51 maxillary first premolars. Among the parameters they measured were occlusal angle(cusp steepness), depth and width of fissure, and the thickness of the enamel adjacent to the fissure. He found that the only measurements which showed any positive correlation to each other were steepness of cusp and depth of fissure. He concluded that occlusal fissures of human teeth could not be

categorised into specific groups on the basis of morphological features. Juhl(86), using premolar teeth in a similar study to that of Fejerskov et al.(45), could not only find no correlation between the different measurements, but also none of the parameters measured correlated in any way with the caries initiation sites which existed within the fissures of the teeth examined.

In the study of Fjerskov et al.(45) the depths of fissures ranged from 43 μm to 1216 μm and the widths of fissures from 6 μm to 180 μm . These measurements correspond well with those of other workers(4,5,31). Two studies(45,142) have found that their measurements corresponded to a normal distribution. Gillings and Buonocore(63) and Crabb(31) measured the thickness of enamel overlying the dentine at the base of fissures in molars and bicuspid. These workers found that this value varied greatly and could be less than 500 μm . Gillings and Buonocore(63) found that in many areas of the fissures, deep unusual invaginations occurred which went to the dentinoenamel junction. These authors(63) hypothesised that if they had had access to newly erupted caries free posterior teeth they may have seen these deep invaginations to an even greater extent than in the caries free molars which they used.

Frank(50), using transmission electron microscopy and undecalcified sections, showed the superficial enamel of fissures in thirty permanent molars and premolars to be either prismatic or aprismatic and no different from other coronal surfaces.

Ripa et al.(144) reported that an aprismatic outer layer of enamel was visible when they examined longitudinal undecalcified sections of twenty-eight erupted deciduous teeth, eighty-eight erupted and forty unerupted permanent teeth. The study involved the use of polarised light, phase contrast microscopy and microradiography. The aprismatic layer was present in seventy percent of permanent teeth, and was about 30 μ m thick.

Crabb(30,31), using reflected and polarised light and scanning electron microscopy, noted a superficial porous enamel layer and concluded that it was 180-300 μ m thick in unerupted teeth but was less in partially erupted teeth(100-180 μ m). A significant finding in the study of Crabb(30,31) was that the porosity of outer enamel was quite evident in fissure areas at the time the tooth erupted into the mouth, even though mineralisation had occurred at other sites. Awazawa(3) studied the enamel at the base of fissures using transmission electron microscopy and found marked areas of hypomineralisation in which the distinction between enamel prisms and interprismatic substance could not always be identified. There was also an increase in organic material in these areas.

Methods of Investigating Pits and Fissures

Microscopy: The structural development and composition of the contents of pits and fissures have been studied with decalcified and undecalcified sections of extracted teeth and mylar strip fissure models(3,4,45,50,52,55,63,88,130,133,157,187,188). These sections have been examined by light, scanning electron, and

transmission electron microscopy. In addition the morphology of pits and fissures has been studied using a replicating technique, developed by Galil and Gwinnett(53). It was designed to overcome the problem of sample loss during sectioning. In the technique a homogenous mixture of acetone and vinyl resin was applied to the crown of a tooth under vacuum pressure for thirty minutes. After the resin had set to a rubbery consistency, the tooth was dissolved away in a solution of concentrated hydrochloric acid. The replicas provided a three dimensional outline of the pits and fissures and were examined with optical and scanning electron microscopy.

Bacteriological Sampling: A variety of methods have been developed in order to study the bacterial ecology of pits and fissures. A widely used method for obtaining fissure samples is the placement of fissure models into the mouth in either a partial denture or by inserting the model into a large molar restoration(103,124,181,182,183,191). 'Models' which have been used have been constructed from mylar strips(103,169,182) or have consisted of fissure like spaces constructed in either bovine enamel or human enamel(166). In addition, enamel fissures from impacted third molars have been placed in the mouth using the techniques just described(181,183,191). Natural fissure systems as plaque collection devices are anatomically superior to the more artificial 'models'(103,124,166,169,182) but artificial fissures can be made which ensure volumetric uniformity in studies where multiple samples are required for comparison(124). Artificial fissure models made with mylar strips eliminate the

effects of any interaction which may occur between dental plaque and tooth substance(103).

It is possible that 'models' made with bovine enamel probably display different interactions with dental plaque than human enamel. However Strassler et al.(166) in a comparison of human and bovine enamel 'models' showed that the plaque was similar in both types of 'model'. None the less the plaque differed from natural fissure plaque in several microbial categories. The levels of Streptococcus mutans, veillonella species and Actinomyces viscosus were higher in the 'models', and the levels of Streptococcus salivarius were lower.

Commonly used methods of bacterial sampling are those of scraping across the fissure openings with needles(21,81,105,109,154), pointed wires(108,110), dental explorers(27,61) or cotton wool swabs(87,124). Svanberg(168) collected samples with a hypodermic needle from a groove that had been cut between an occlusal amalgam restoration and the tooth with a No.0 round bur. The samples collected by any of the above methods are subsequently analysed in the laboratory, either as individual site samples, or sometimes samples from several sites are pooled to provide a larger quantity of material to work with. Meiers et al.(119,120) developed a suction apparatus for the collection of the entire contents of a pit and fissure system as the system was removed with a high-speed handpiece with water-air coolant spray.

Needles, wires, explorers and cotton wool swabs may not collect all the fissure contents because of their inability to penetrate the depths of the fissure. In a study with rats, cotton wool swab samples showed a much greater diversity of microorganisms, probably because of contamination by bacteria from other areas, than samples obtained by splitting the molar teeth transversely in the plane of the fissure(78). Meiers and Schachtele(119) found that the commonly used method of needle scraping recovered only 18.2% of the total flora obtained with their total fissure removal technique. They also concluded that the needle scraping method of sampling was not adequate for studies on the effects of antimicrobial agents on fissure plaque because of its inability to provide information about the effects of such agents on the plaque in the depths of the fissure(119). This technique of Meiers et al.(120) is highly invasive of tooth structure and could only ethically be used on carious teeth, thereby providing a sample representative of the carious rather than the non-carious state.

Rats have also been used to study the bacteriology of fissures(77,78,87,202). It has been suggested that the early microbial colonisation of occlusal fissures in man follows a pattern similar to that in rats(77,87,188). However, because of dissimilarities between rat and human tooth morphology, eating habits and salivary characteristics, it has been questioned to what extent various findings in rats may be related to the formation and composition of plaque in occlusal fissures of human teeth(78,188).

Biochemical Methods: Only one study(123) could be found which examined the metabolism of fissure plaque in situ. This was conducted on fissure models constructed from bovine enamel and mounted in groups of three on each side of the midline of a U-shaped removable appliance(six fissure models on each appliance). Three of the fissure models on one wing of the appliance were exposed to a 10% sucrose solution in normal saline for 20 minutes and three on the other side to normal saline(control) for the same period. This was repeated eight times per day for twenty-one days. Then one each of the test and control 'models' was exposed directly, without disturbing the 'fissure' plaque, to 50 µl of uniformly-labelled [^{14}C]-sucrose for 60 minutes at 37°C. Another pair of the test and control 'models' was taken and their plaques dispersed by sonification prior to being incubated with uniformly labelled [^{14}C]-sucrose for 60 minutes at 37°C. The 'fissure' plaques were then analysed for metabolic products. The results of this experiment indicated that unique metabolic activities might exist in plaques produced in such an environment. It also indicated that, similar to smooth surface plaque, the pattern of metabolic products is related to the amount of glucose substrate available(24,36,114).

Several attempts have been made to produce a satisfactory 'model' to mimic the formation of fissure caries in vivo(166) and in vitro(9,34,89,160). These 'models' were constructed of either human or bovine enamel. The in vivo 'models' showed significant decreases of enamel microhardness after three weeks but the plaque which had developed in these fissure models differed

significantly from natural fissure plaques in that higher levels of the bacteria S. mutans, veillonella species and A. viscosus and lower levels of S. salivarius were present(166). In the three in vitro 'models'(34,89,160) caries developed at the base of the fissure and not in the walls as probably occurs in natural fissures in vivo(31,50,86,127). Strassler et al.(166) indicated that a 'fissure caries model' system would be useful in caries research of pit and fissure systems in that caries activity could be simulated within a relatively short period of time.

Contents of Pits and Fissures

Very few studies could be found in which the contents of the pits and fissures of unerupted teeth had been investigated. Galil and Gwinnett(54), in 1975, studied one hundred and ninety-three unerupted molars and premolars using light microscopy and scanning electron microscopy. The teeth were extracted with their follicles intact. The fissures were shown to contain ameloblasts lining the walls of the fissures, remnants of the enamel organ and red blood cells. It was suggested that these exist in a viable state until shortly after tooth eruption. Their ultimate fate is unknown and evidence of remnants of these components have not been reported in many post-eruptive studies of fissure contents(50,52,55,187). However, Newman(130) in studies of decalcified sections of human molar and premolar teeth under light microscopy reported that remnants of the enamel organ may persist in the fissure and that a cuticular structure may be interposed between this and the surface enamel. In rats the

enamel organ and connective tissue occupying the fissure space degenerated within four to nine days of tooth eruption(87).

In fully erupted human teeth the contents of pits and fissures have been reported to consist of bacteria embedded in an amorphous, slightly fibrillar matrix(55,157). In this thesis these contents shall be referred to as plaque. This dental plaque varies in composition and structure with increasing age of the plaque and with the depth of the fissure in humans(50,55,157) and in rats(77). Galil and Gwinnett(55) sectioned five hundred and seventy-four erupted permanent molars and premolars and observed with light microscopy, and scanning and transmission electron microscopy that the bacteria of the plaque became progressively more indistinct from top to bottom of the fissure. Several workers(52,157,187) using transmission and scanning electron microscopy have reported that, at the orifice of the fissure, there is a wide variety of viable organisms including cocci, filamentous organisms, and short rods. These organisms form a plaque with a structure similar to that of smooth surface plaque. In the middle third of the fissure several workers(50,55,157) have observed, using scanning and transmission electron microscopy, that the bacterial cells are more densely packed and contain intracytoplasmic vesicles and intracellular polysaccharides. The intracytoplasmic vesicles stain with thiosemicarbazide-osmium indicating the presence of polysaccharide(50,157). In the depths of the fissure Schroeder and de Boever(157) observed, using light microscopy and transmission electron microscopy, a condensed, lysed and modified

plaque layer which exhibited numerous ghost-like cell wall structures. This densely packed layer was also seen by Frank(50), and Galil and Gwinnett(55). The bacteria in this densely packed layer contained granular material and were separated by a fibrillar matrix which contained polysaccharide(50,55,77,157). These findings are consistent with the observations of Meiers and Schachtele(118) who found that very few viable cells could be cultivated from the bottom third of fissures over one millimetre deep.

Several workers(50,55,157,187) have demonstrated that the densely packed layer of cells in the bottom third of a fissure may become calcified. Deposition of calcium and phosphate may be in evidence within seven days after initial colonisation of the fissure(187). However, in another study by Theilade et al.(188), with artificial fissures, mineralisation did not occur for 3-4 weeks after initial colonisation. Frank(50) concluded from his transmission electron microscope studies that this calcified layer was identical with mineralised smooth surface plaque(supragingival calculus). The deposition of the calcium and phosphate appears to begin in the fibrillar interbacterial matrix of this dense layer(50,55). Two percent of all fissures studied by Galil and Gwinnett(55) were totally mineralised. In their study, when they stained for calcium using alizarin red, they found that the calcium concentration increased towards the base of the fissure. In fact seventy percent of the fissures examined were highly positive for calcium in the depths of the fissure.

The acquired enamel pellicle, which occurs under smooth surface plaque(62), has been found by Frank(50) to occur only at the entrance to the fissure. In artificial fissures Theilade et al.(188) found pellicle also restricted to the entrance of fissures. Furthermore, in the remainder of the fissure a number of workers(50,55) have observed that the bacteria and fissure contents are closely adherent to the enamel wall with bacteria often being directly in contact with the enamel wall. Sveen et al.(174) found that the acquired enamel pellicle, while present initially, was rarely observed in plaques over nine days old. Nevertheless, Theilade et al.(187) in work with human fissures and seven day old plaques observed an electron dense substance which contained fine crystal-like structures separating the bacteria from the enamel surface throughout the fissure. The bacterial surface of this layer was similar in structure to the pellicle described by Frank(50) at the entrance of the fissure. Theilade et al.(187) postulated that this organic surface layer observed under their seven day plaques could disappear with time through bacterial action(185).

Development of Plaque in Pits and Fissures

The study of plaque in pits and fissures is hampered by the poor accessibility of the site. As a result of this the information available about bacterial activity in pits and fissures is very limited. In contrast, considerable data are available on all aspects of the ecology of smooth surface plaque because of the ease of access to many smooth surfaces(18,62,72,106,115,128,

While many of the factors affecting smooth surface plaque will affect pit and fissure plaque, care should be taken when extrapolating information. For example, there may be a variation in the permeability of pit and fissure plaque in contrast to smooth surface plaque(17,68,118), whilst the glycolytic activity of bacteria in fissures may differ from that of bacteria in plaque on smooth surfaces(11,55,157,169,186,188,209). As a result of these difficulties a number of ingenious methods, including the use of 'model' fissure systems, have been employed to obtain more information on the development and properties of pit and fissure plaque(46,103,124,160,166,168,169,174,180,182, 191).

Svanberg and Loesche(169), using artificial fissures implanted in mandibular first molars, estimated that for a single S. mutans to be isolated from a fissure the salivary concentration of this organism needed to be about 10^3 cells per millilitre of saliva. In contrast the colonisation threshold for smooth surfaces, estimated by Van Houte and Green(201), in humans, was 10^4 cells per millilitre of saliva. Similar estimations were made by Duchin and Van Houte(39) for fissures and smooth surfaces also in humans. That the value for fissures is lower is not surprising as these areas provide a stagnant space to which there is limited access to salivary flow and any 'detergent' effects of mastication. The findings of Svanberg and Loesche(169) supported an earlier suggestion by Ikeda and Sandham(80), from the results of a clinical study, that fissures would be the first tooth surfaces to be colonised by S. mutans.

In smooth surface plaque, bacterial adherence to the surface of the tooth is a paramount factor in its development and considerable research has been carried out in an attempt to discover the nature of this attachment(42,61,62,67,72,106,109, 200). However in fissures it has long been assumed that bacteria are physically entrapped thereby enabling organisms with little or no adherent capability to accumulate. This theory is supported by the work of Loe, Karring and Theilade(103) with artificial fissures. Recent work by Van Houte and Russo(202), in rats, appears to minimize the importance of physical entrapment in rat fissures and support the significance of cellular adhesion and glucan forming ability in bacterial colonisation of these fissures. Nevertheless, care should be taken when extrapolating experimental findings from rats to man, because of the dissimilar morphology of the rodent dentition, the different properties of rat saliva and the habit of coprophagy(77).

A number of workers(181,182,191) have shown, in research with artificial or human fissures, that the fissures become populated within twenty four hours following exposure to the oral environment. Theilade et al.(180) showed, in natural fissures implanted in the mouth, that within a few days bacteria proliferate to occupy the whole fissure space and after seven days areas of degenerating bacteria and foci of mineralisation can be found. The microflora of dental plaque in natural fissures, 7-21 days after implantation in the mouth, is dominated by gram-positive cocci and rods with smaller proportions of gram-negative cocci and rods; fusiforms, filaments, spirilla and

spirochaetes are virtually absent(179,181). Other studies with artificial and human fissures support these findings for the early colonisation of fissures(124,181,182,188,191). This is in sharp contrast to smooth surface plaque, in which increasing numbers of fusiforms and filaments are found after three days(16,106,111,128,163,184). In old fissure plaque(>200days old), obtained from natural fissures implanted in the mouth, counts of viable organisms are similar to those for young fissure plaque grown under comparable conditions and the flora remains largely gram-positive and facultatively anaerobic(179). Certain changes in the bacterial flora in fissures do occur with time such as an increase in S. mutans and a decrease in Streptococcus sanguis and S. salivarius(179,181). According to Theilade and Theilade(183) the relative abundance of organisms in older fissure plaque is total streptococci(including S. mutans) > gram-positive rods > A. viscosus and Actinomyces naeslundii > lactobacilli > Veillonella parvula > gram-negatives (Figures calculated from the mean percentages of 592 isolates, from ten natural fissures, implanted in molar teeth for 200-275 days.). These findings are in sharp contrast to smooth surface plaque where, with time, filamentous and anaerobic forms of bacteria become more prominent(16,106,111,163,128,145). Theilade and Theilade(183) postulated that such changes as do occur in fissure plaque with age may be due to different growth rates and the different rates of degeneration of the various species present. Furthermore it may be difficult for new bacteria to enter the already filled space. Data published by Svanberg and Loesche(169), in fact support this theory.

It has been postulated that S. mutans and lactobacilli preferentially colonise retentive areas of the teeth such as pits and fissures(61,80,81,87,109,180). Further substantiation of this theory has come from work by Scheie et al.(156) and Balenseifen and Madonia(7) who showed that orthodontic banding and a consequent increase in retentive areas lead to an increase in the number of streptococci and lactobacilli in plaque and saliva. Interestingly, it has been suggested that pits and fissures may constitute the major reservoir of S. mutans for colonisation by this bacteria in other areas(27,80).

Pit and Fissure Caries

The pit and fissure systems of teeth are prime sites of carious attack particularly the pits and fissures of permanent molars(14,33,41,51). Bohannon(14) states that 54% of all dental caries in 5-17 year old individuals in the U.S.A. occur in the occlusal surfaces of posterior teeth. In a survey of a group of 12 year old children in Australia, 75.6% of all decayed or filled surfaces were pit and fissure lesions in occlusal surfaces(33). More recent analysis(41) of surface and tooth specific data from dental surveys has shown that the pattern of dental caries differs from tooth to tooth and from surface to surface. Eklund and Ismail(41) analysed data collected in a National Health and Nutrition Survey in the U.S.A. and reported that at least seventy percent of the occlusal surfaces of permanent molars became decayed or filled within ten years of the eruption of these teeth. It was also noted in this survey that caries development

in the second permanent molars was very similar to that for the first permanent molars, except that the second molars were six years behind. The occlusal surfaces of the bicuspid presented a different picture with only 45% of their pits and fissures becoming decayed or filled. Theuns and Pot(189) suggested that occlusal fissure systems in first permanent molars were not all equally susceptible to caries because of variations in the form of the pits and fissures. In fact in the first permanent molars that they studied, 20% had a fissure pattern which they believed was not caries susceptible. Half had fissure patterns in which only the central fossa region of the fissures was carious, while 30% had a fissure system in which all sections were carious.

Numerous studies in animals(48,49,87,96,177,207) and humans(20, 21,40,61,81,105,108,109,120,167) have implicated a number of bacteria as possible cariogenic bacteria. In a number of longitudinal studies(21,97,105,109), carried out over periods of two to three years, a positive relationship between a high S. mutans level and the development of fissure caries has been found. In two of these studies(21,97), however, the relationship was such that the significant increase in S. mutans levels in the fissure samples occurred 6-12 months prior to the clinical diagnosis of fissure caries. Although the results in these studies(21,81,97,105,109) implicate S. mutans in fissure decay, all of these studies found in some instances mouths with high levels of S. mutans and no decay. Also, in two of the studies(97,109) clinically obvious fissure caries occurred in the absence of detectable levels of S. mutans. In a number of recent

studies(8,19,25,195) high numbers of S. mutans have been found not only in populations of high caries prevalence but also in populations of low caries prevalence. Lactobacilli have also been detected in fissures in association with carious lesions(81,105,109). Loesche and Straffon(109) observed that in those fissures in which caries developed in the absence of S. mutans relatively high levels of lactobacilli were present.

Several studies of the sites of caries initiation in fissures in humans have reported the walls and not the base of the fissure are the vulnerable areas(31,50,86,127). From an ultrastructural point of view, the initial stages of the carious lesion in fissures are the same as for smooth surface lesions(50). The spread of the carious lesion appears to be closely related to the prism structure and direction. The available evidence suggests that the lesion proceeds along the interprismatic substance towards the dentinoenamel junction(50,127). Additional studies have shown that carious lesions may develop independently at different sites along the fissure system and then coalesce(86,94,127).

Use of Antimicrobial Agents on Pits and Fissures

Dental plaque is essential in the initiation of dental caries, and gingival inflammation(95,100,102). Consequently, considerable experimental work has been conducted in an attempt to find a means of controlling dental plaque with antimicrobial agents(for reviews see 28,95,102,104,129). However, the bulk of

the research has focused on the chemical control of gingival inflammation as distinct from dental caries. Only one study was found which concentrated solely on the chemical control of plaque and early caries in pit and fissure systems(37).

An early suggestion that the effects of an antimicrobial agent on fissure plaque might be different from its effects on smooth surface plaque was given by Caulfield and Gibbons(27). Teeth were treated with a 2% I₂-KI solution in a 53% glycerine base for 4-5 minutes after an initial prophylaxis, and subsequently at three and five days without further prophylaxis. The results were compared with a control group who received only a prophylaxis. The S. mutans levels were significantly reduced in fissure and approximal surface plaques and saliva. Reductions persisted in the salivary and approximal samples for 20-24 weeks after cessation of treatments. However one week after treatment significant numbers of S. mutans could be isolated from the fissures and by 4-6 weeks the levels in the fissures had almost returned to pretreatment values. Caulfield and Gibbons used a dental explorer to collect their fissure samples. In a more recent study, Schaeken et al.(153) found that, after treatment with a comparable iodine solution applied once, the S. mutans levels in fissure and smooth surface plaques had returned almost to pretreatment levels in twenty-one days. Fissure samples were collected with a hypodermic needle. Meiers and Schachtele(118) found that after topical treatment of the occlusal surface of molar teeth with a solution of 0.8% I₂-KI, 5.2% CuSO₄ and 1.5% ethanol for five minutes that only the outer portion of the

fissure plaque was sterilised. A total fissure removal technique was used for collecting the plaque samples.

Loesche et al.(110) had promising results with APF gel(containing 1.23% fluoride ion) applied for five to ten minutes in applicator trays on ten consecutive days. The APF treatment caused a persistent 45-75% reduction in the percentage of S. mutans in occlusal samples and this effect was still apparent twelve weeks after the last treatment. The occlusal samples were collected with a pointed wire from the fissure systems of one premolar and one second molar in each quadrant and pooled(scrapings from eight teeth formed one occlusal sample). However, in this study the APF gel did not reduce the S. mutans levels in the approximal surface plaque, which were also pooled samples. Loesche et al.(107) had found in an earlier study that APF gel did reduce S. mutans for at least twelve weeks in approximal surface plaque, and it was suggested that these anomalous results were due to limitations in the experimental design(110). More recently de Leifde(37) conducted a longitudinal study over a period of three years on the effects of intensive topical applications of 2% NaF on early caries in pits and fissures. She found no significant differences between the test and control groups.

Schaeken et al.(153,154) studied the antibacterial effects of chlorhexidine on fissure plaque particularly in relationship to S. mutans levels. They found that after topical applications of chlorhexidine gel(5% chlorhexidine digluconate in 4% methyl cellulose gel) that S. mutans could be suppressed in occlusal and

smooth surface plaque for approximately seven days but had returned to pretreatment levels after twenty-one days. If these topical applications were supplemented with mouthrinsing with 0.2% chlorhexidine solution twice daily or flossing with dental floss impregnated with 5% chlorhexidine once daily at home, S. mutans was suppressed during the time that these procedures were used. Occlusal samples were collected in their study with a hypodermic needle.

Schaeken et al.(154) also studied the effects of topical applications of 8% SnF₂ on S. mutans in fissure and smooth surface plaques. Similar to chlorhexidine it was found that the SnF₂ treatments suppressed S. mutans for seven days but, twenty-one days after cessation of treatment S. mutans had returned to pretreatment levels. Svanberg and Rolla(170) assessed the effects of mouthrinsing morning and night with 0.2% SnF₂ solution on the levels of S. mutans in saliva, and approximal surface plaque, and fissure plaque. While the levels of S. mutans were reduced in fissure plaque the reduction was much more marked in approximal surface plaque and saliva samples. No assessment of the longevity of the reduction was made in their study. In a later study by Svanberg and Westegren(171), 8% SnF₂ applied topically for two minutes three times at weekly intervals, initially reduced S. mutans levels in fissure plaque, smooth surface plaque and saliva. However the levels of S. mutans had returned to pretreatment levels in the occlusal plaque samples within two to four weeks but the levels were still significantly reduced in the approximal surface plaque samples. In all the

above studies in which SnF_2 was used the authors used hypodermic needles to obtain their fissure plaque samples.

In summary the majority of the studies(27,110,118,153,154,170, 171) have come to the same conclusion. S. mutans may be reduced in fissure plaques initially following treatment with iodine solutions, chlorhexidine, or SnF_2 but within four to six weeks the S. mutans levels have returned to pretreatment levels. This is despite the different modes of application used. A number of the studies(27,170,171) have also demonstrated that iodine solutions and SnF_2 may be more effective in reducing S. mutans levels in approximal surface plaques than fissure plaques.

SECTION I

EFFECT OF PROPRIETARY AGENTS ON INHIBITION OF ACID PRODUCTION

IN VIVO

The measurement of acid production in the pits and fissures of molar teeth is hampered because of the confined nature of the fissure system and the position of these teeth in the posterior regions of the mouth. No pH electrode systems were found which could easily reach these inaccessible sites. Furthermore, even if a suitable instrument was available, extreme difficulties would undoubtedly be experienced in obtaining patient cooperation over the periods of time likely to be required to obtain a full Stephan Curve(164,165).

In this section of the investigation a pH indicator dye, bromcresol purple($C_{21}H_{16}Br_2O_5S$)(122), was used to monitor the initial pH drop in pits and fissures after a sucrose challenge. Bromcresol purple has a nominal pH range(5.2-6.8)(122) which is within the range of the 'critical pH' for enamel dissolution in saliva(82,128,).

Sound lower second molars were treated topically with either APF gel(containing 1.23% fluoride ion); 0.5% chlorhexidine in 70% alcohol; 40% AgF; 0.8% I_2 -KI and 5.2% $CuSO_4$ in 1.5% ethanol; or 10% SnF_2 . Acid production in the pit and fissure systems of

these teeth was monitored seven days and again six weeks after the topical treatment. Although each of these agents possesses antimicrobial properties(13,44,104,107,110,118,190,193,194), as far as can be determined from the published literature none have been tested for their effect on acid production in pits and fissures.

DEVELOPMENT OF EXPERIMENTAL METHOD

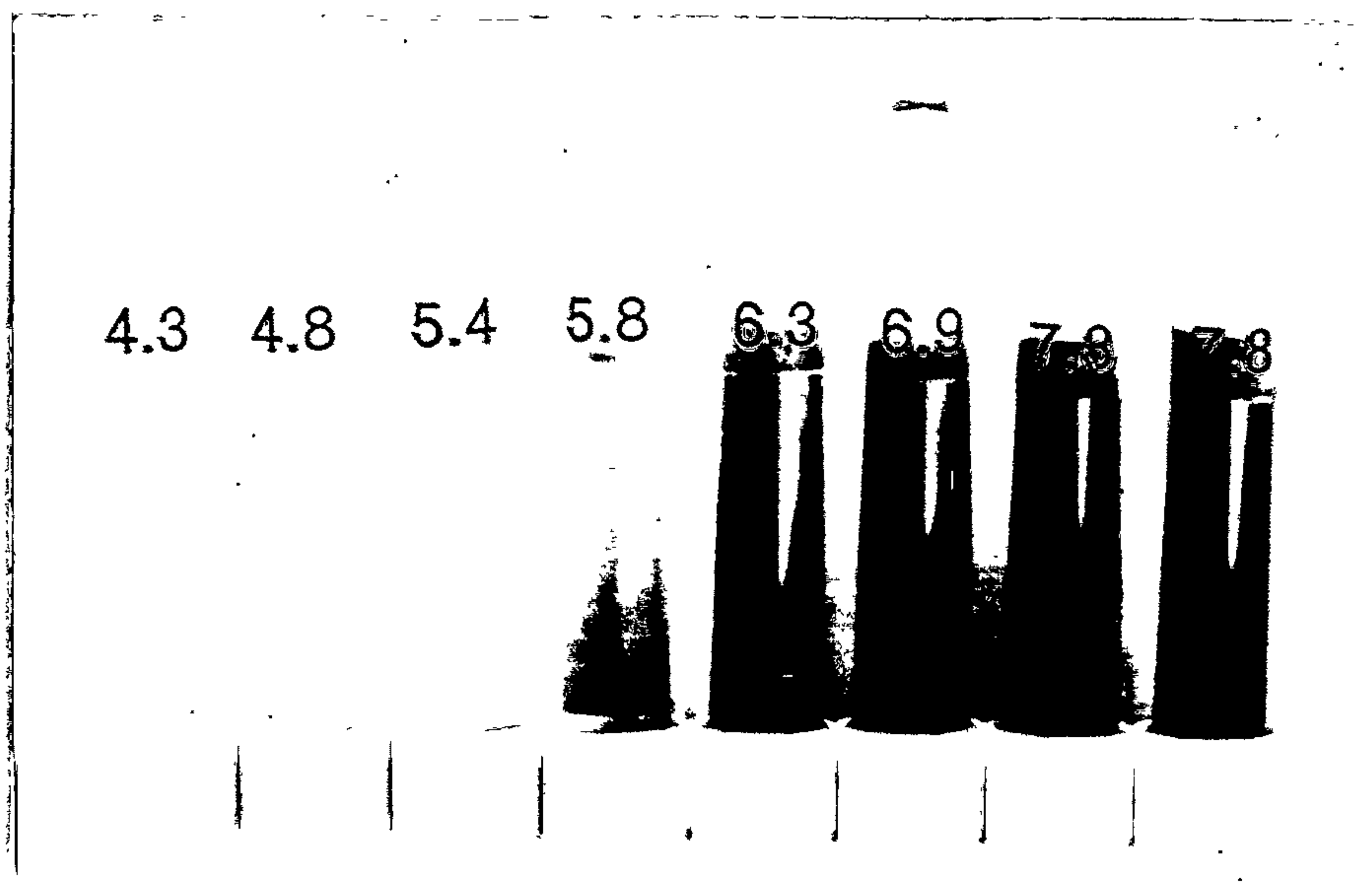
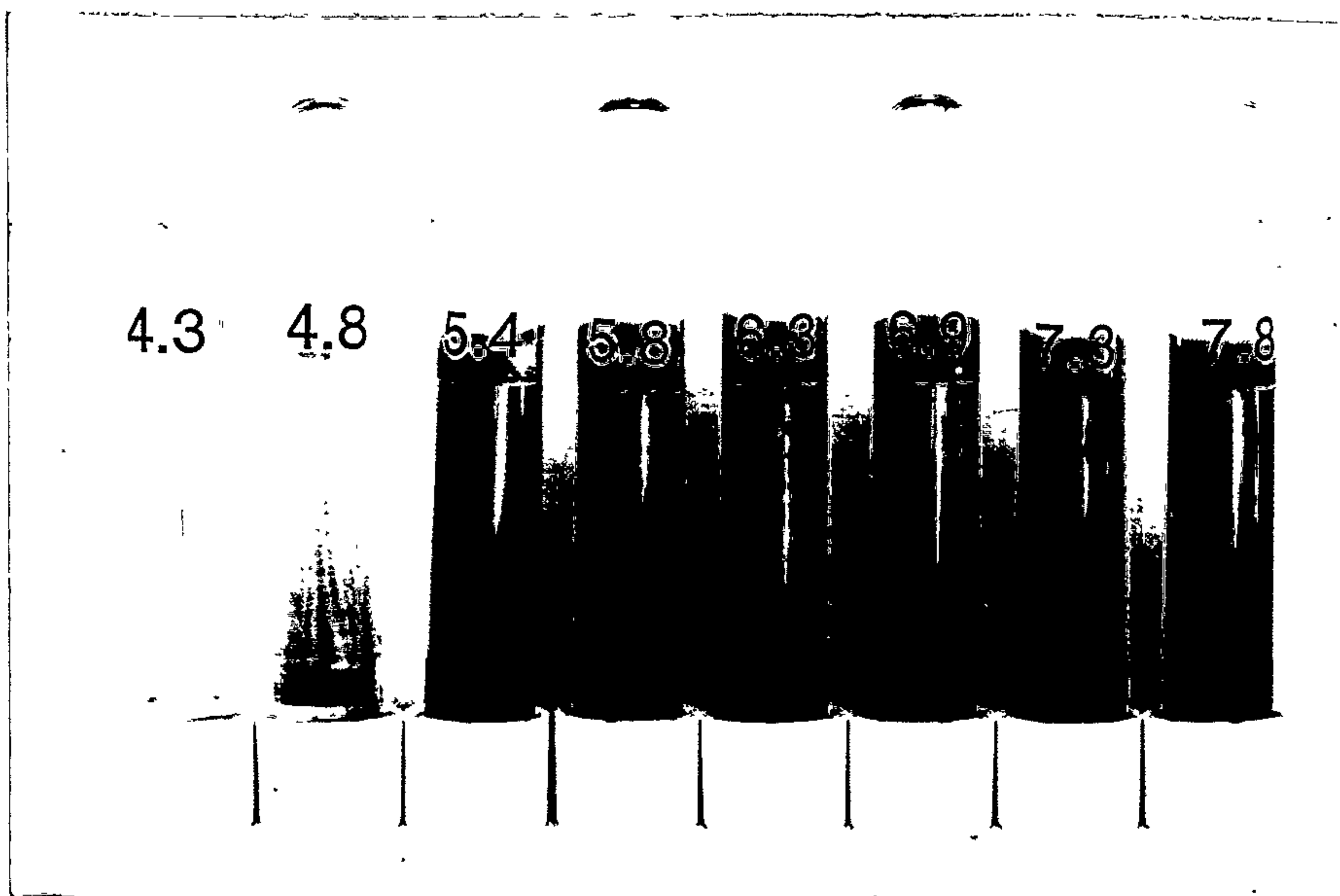
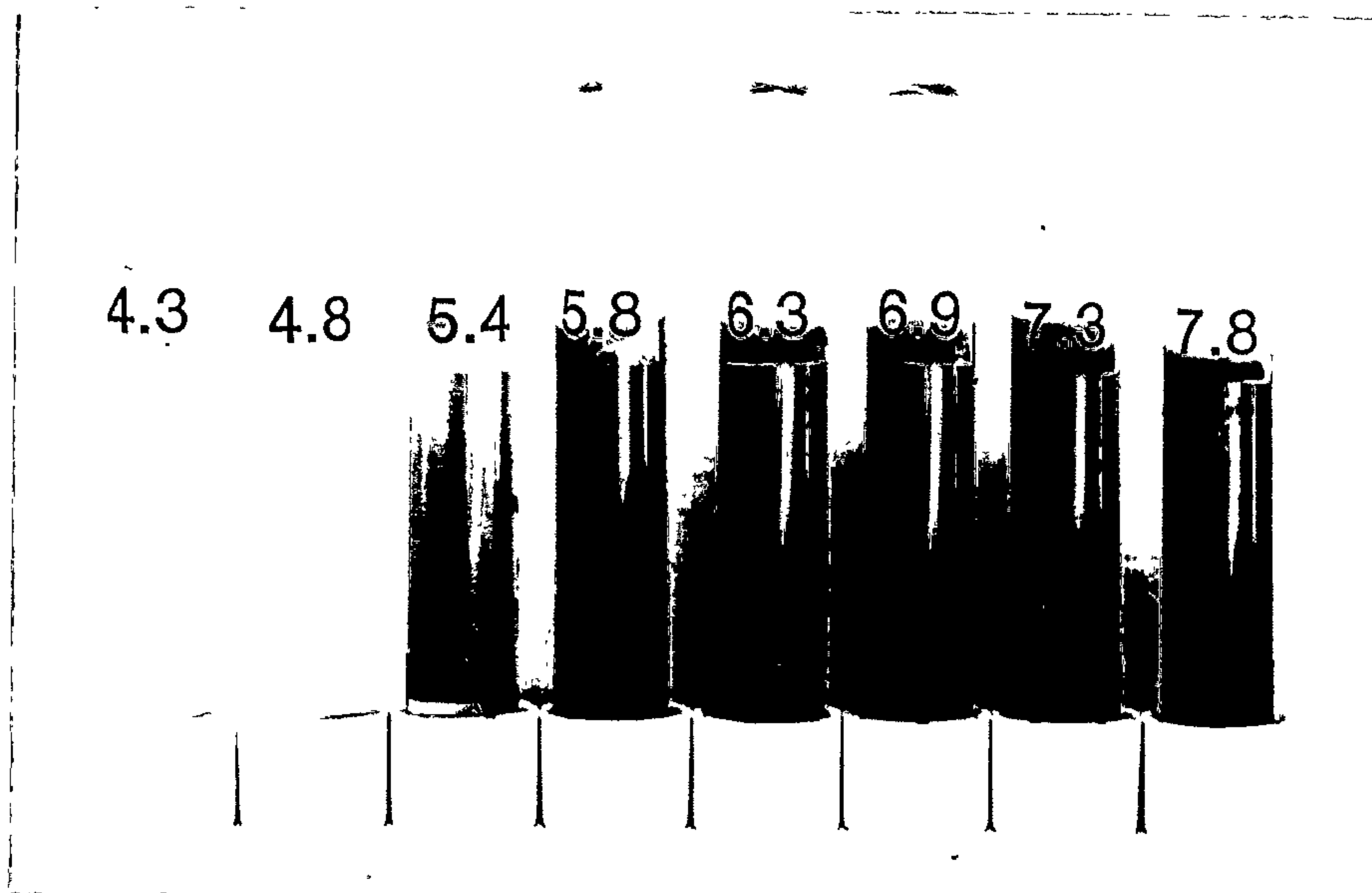
Indicator Dye

Preliminary studies showed that standard 0.1% bromcresol purple solution(159) was unsuitable because it failed to change colour when applied to fissures that had been subjected to a sucrose challenge. The difficulty was traced to the sodium hydroxide component of the standard solution*.

A solution responsive to changes in the acidity of pit and fissure plaque was obtained by eliminating the sodium hydroxide and raising the bromcresol purple concentration to 0.37% to produce a saturated solution. Instead of applying the sucrose challenge separately it was found practicable to incorporate sucrose into the bromcresol purple solution.

* 1.0 gm of the dye, dibromo-o-cresolsulfonephthalein, dissolved in 18.5 ml of 0.1 M NaOH diluted to 1 litre with water(159).

Figure 1. Colour changes produced in a series of 10 ml aliquots of standard buffer solutions(pH range 4.3 to 7.8) following the addition of 0.1 ml of (a)0.37% bromcresol purple(top), (b)0.37% bromcresol purple containing 10% sucrose(centre), and (c)standard bromcresol purple indicator solution(bottom).



The final indicator dye solution selected for the study contained 0.37% bromcresol purple and 10% sucrose in deionised filtered water*. The colour changes produced when 0.1 ml of this solution was added to 10 ml aliquots of standard buffer solutions(Figure 1) was comparable to those obtained when 0.1 ml of 0.37% bromcresol purple in distilled water was added. Both these bromcresol purple solutions gave more intense colours than seen when an equivalent volume of standard 0.1% bromcresol purple was used(Figure 1). As can be seen in Figure 1 the more concentrated bromcresol solutions give more distinctive colour changes in the pH 4.8-5.8 range.

The standard buffer solutions (pH 4.3, 4.8, 5.4, 5.8, 6.3, 6.9, 7.3, 7.8) were prepared according the method of Shugar et al.(159).

Relevance of Colour Changes to pH of Dental Plaque

Freshly extracted teeth were immediately transported to the laboratory where adherent plaque was disclosed using an aqueous solution of 0.37% bromcresol purple without sucrose. Plaque with a distinct purple colour(high pH) was then exposed to 10% sucrose, which was applied one drop at a time. At the point when the plaque changed from a purple to a yellow colour, the plaque was removed and placed in a Radiometer dental electrode(No. G2221C)**. The pH of the plaque was measured using a

* Referred to as BCP/sucrose throughout this text.

** Radiometer, Copenhagen, Denmark.

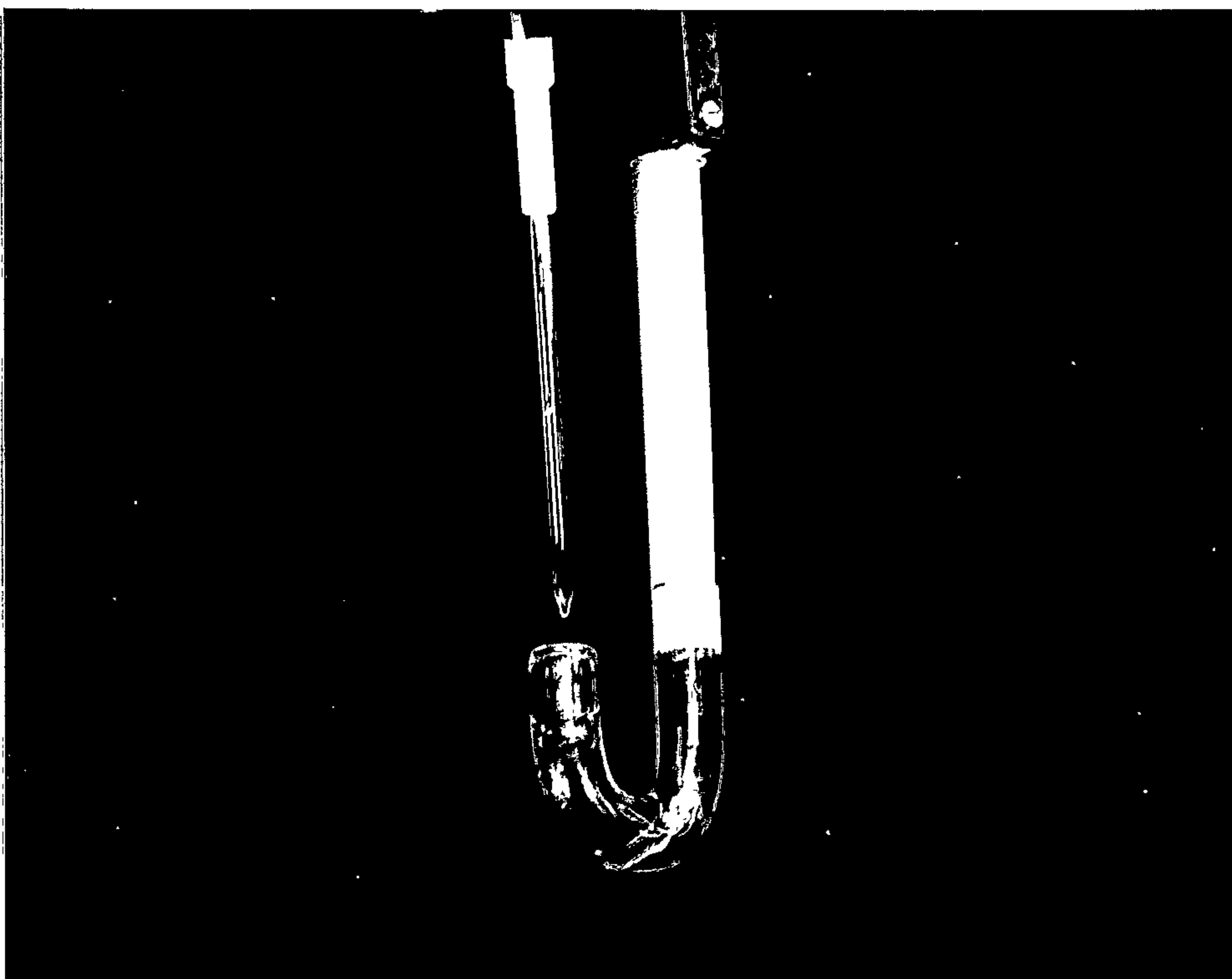


Figure 2. Electrode system used for measuring plaque pH. Reference electrode is photographed above the measuring cup located at the end of the arm of the other electrode.

Microelectrode reference electrode(No. M1-401)* in conjunction with the Radiometer electrode as indicated in Figure 2. These electrodes had been previously calibrated using Radiometer** standard buffer solutions.

The mean pH of dental plaque at the purple to yellow change point for ten samples was 5.42(S.D. = 0.19).

Another group of freshly extracted teeth with adherent plaque were treated in the same manner as described above, except that the plaque was not collected at the time of the purple to yellow colour change. Instead, at this point, the plaque was immediately exposed to freshly collected whole saliva applied one drop at a time. The diffusion of the saliva through the plaque was facilitated by gently pricking the surface of the plaque with the point of a fine dental probe. When the plaque changed from yellow to purple it was collected and the pH measured utilising the electrode system described above and illustrated in Figure 2.

The mean pH of dental plaque at the yellow to purple change point for ten samples was 6.02(S.D. = 0.24).

* Microelectrodes, Inc., Londonderry, New Hampshire, U.S.A.

** Radiometer, Copenhagen, Denmark.

MATERIALS AND METHODS

MATERIALS

Test Agents

Five preparations designed for topical treatments were evaluated:

1. **APF gel** -- Acidulated phosphate-fluoride gel(1.23% fluoride ion) (Orapham Products Ltd., Melbourne, Vic., Australia).
2. **40% AgF** -- 40% Silver Fluoride(Creighton Dental, Double Bay, N.S.W., Australia).
3. **10% SnF₂** -- 10% Stannous Fluoride Spot Application Paste(Creighton Pharmaceuticals, Kingsgrove, N.S.W., Australia).
4. **I₂-KI/CuSO₄** -- 0.8% I₂-KI and 5.2% CuSO₄ in 1.5% ethyl alcohol(based on Ora-5 formulation, Premier Dental Products, Norristown, Pennsylvania, U.S.A.).
5. **0.5% chlorhexidine** -- 0.5% chlorhexidine(ICI, Melbourne, Vic., Australia) in 70% alcohol(analytical grade ethyl alcohol).

Indicator Dye

The indicator dye solution(BCP/sucrose) contained 0.37% bromcresol purple(C₂₁H₁₆Br₂O₅S)(Bromocresol Purple, Labchem, Ajax Chemicals, Sydney, Australia) and 10% sucrose in filtered, deionised water.



Figure 3. Examples of colour changes seen following the application of BCP/sucrose indicator to the fissure systems of sound lower permanent second molar teeth. Top: An 'acidic' reaction. Bottom: A 'non-acidic' reaction.

Patients

Orthodontic patients undergoing fixed appliance therapy(mean age 14.5 years; S.D. = 1.3 years) who had caries-free lower second molars with accessible occlusal pit and fissure systems were used. The orthodontic appliances remained in situ throughout the study.

Patients were initially screened to eliminate those with pits and fissures which did not demonstrate an 'acid reaction'(yellow colour) following the application of the BCP/sucrose solution(page 38)(Figure 3).

METHODS

The treatments were applied at random to the lower second molar teeth. Each tooth to be treated was isolated using cotton rolls in Garmer's Clamps*. A Dry Guard** was trimmed as illustrated in Figure 4. and placed between the Garmer's Clamp and the lingual surface of the tooth to be treated in order to completely retract the tongue(Figure 4). A plastic saliva ejector was used when required. A mouth prop of a suitable size was used to prevent inadvertent closure of the mouth during the procedures(Figure 4). No attempt was made to clean the tooth surfaces prior to the application of a test agent.

* Garmer Dental Instrument Co., Minneapolis, U.S.A.

** Virilium Company Ltd., Greenhill Cres., Watford, Herts, U.K.

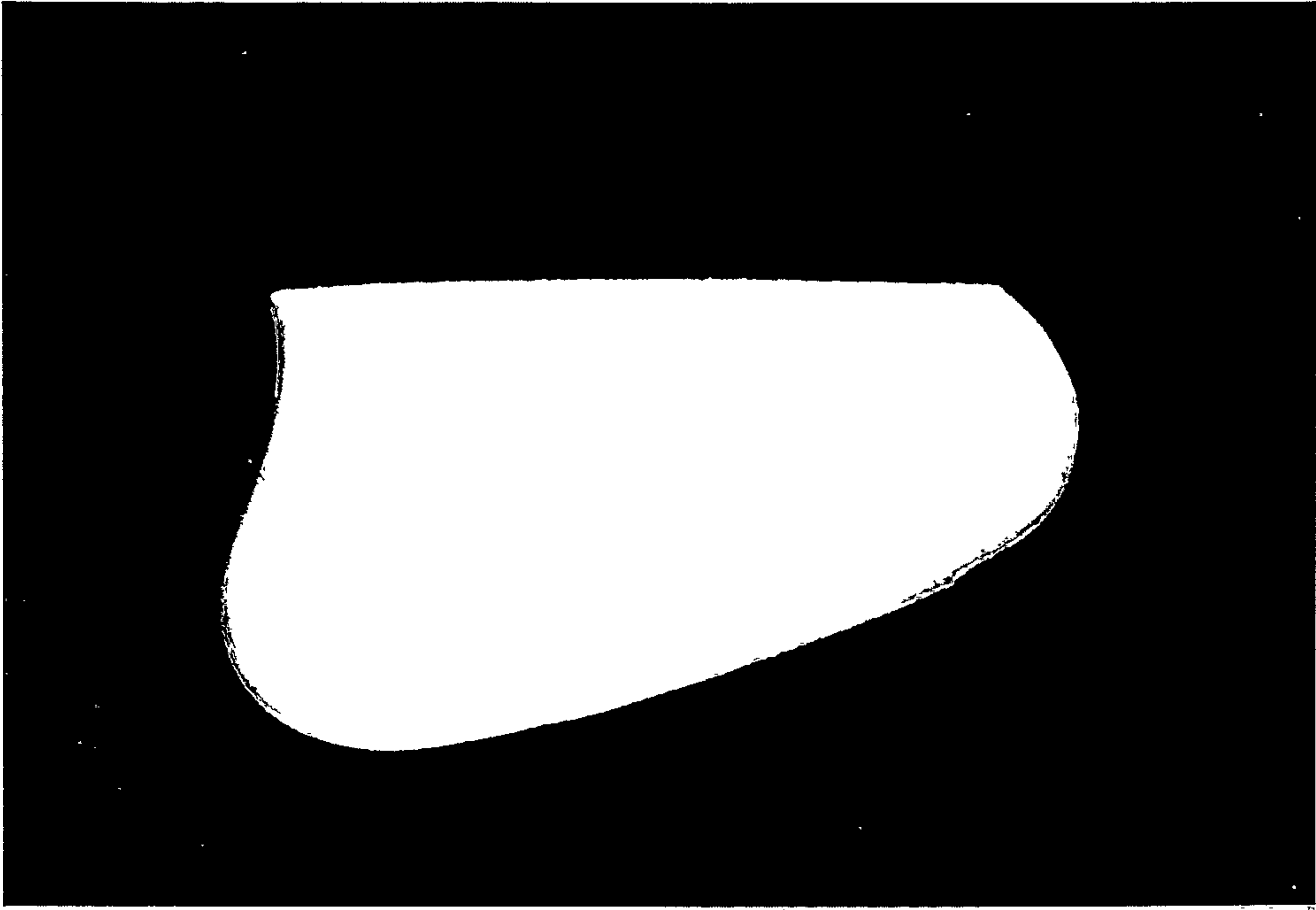


Figure 4. Method of isolation of lower second molars used in this study.

Top: A Dry Guard trimmed to the required shape.

Bottom: Garmers clamp, cotton wool rolls, and Dry Guard positioned in the mouth. A Stomahesive Wafer is in place on the second molar.

Teeth selected for treatment had a test agent applied for either four minutes or a sixty minutes. A four minute treatment consisted of applying the test preparation to the lower second molar for four minutes during which time isolation and dryness were maintained. The patients were then instructed not to eat or drink for one hour. For all sixty minute treatments the PFA method of Craig(32) was utilised and the patient was instructed not to eat or drink for one hour. The patient was also asked to remove the Stomahesive Wafer* after sixty minutes had elapsed(Figure 4).

At seven days and six weeks the patients were recalled and the teeth isolated as described above. The BCP/sucrose solution was applied after any remaining saliva had been cleaned from the tooth surface with clean dry compressed air. Excess dye was absorbed with cotton pellets. After two minutes teeth were recorded as either a yellow colour(acid reaction) or a purple colour(non-acid reaction).

RESULTS

A total of ninety teeth in forty-seven patients were treated. As Table 1 shows neither APF gel nor 0.5% chlorhexidine had any inhibitory effect on acid production at seven days.

Treatments with 40% AgF, 10% SnF₂, or I₂-KI/CuSO₄ for either four or sixty minutes had an inhibitory effect on acid

* E.R. Squibb & Sons, Noble Park, Victoria, Australia.

Table 1. Inhibition of Acid Production by Antimicrobial Agents.

Treatments	Application Time (mins)	Experimental Periods	Teeth (n)	Inhibition	
				Yes	No
APF Gel	4	7 days	9	0	9
		6 weeks*	-	-	-
	60	7 days	9	0	9
		6 weeks*	-	-	-
0.5% Chlorhexidine	4	7 days	9	0	9
		6 weeks*	-	-	-
	60	7 days	9	0	9
		6 weeks*	-	-	-
I ₂ -KI/CuSO ₄	4	7 days	9	4	5
		6 weeks	9	0	9
	60	7 days	9	7	2
		6 weeks	9	0	9
10% SnF ₂	4	7 days	9	5	4
		6 weeks	9	0	9
	60	7 days	9	8	1
		6 weeks	9	0	9
40% AgF	4	7 days	9	6	3
		6 weeks	9	0	9
	60	7 days	9	7	2
		6 weeks	9	0	9

*Discontinued after seven days.

production at seven days(Table 1). However, by six weeks each pit and fissure treated with these agents demonstrated an 'acid reaction' following the application of the BCP/sucrose solution.

DISCUSSION

None of the agents tested showed an ability to inhibit acid production over a six week period. It is possible however that there may have been some inhibition of acid production at pH levels below the non-acidic endpoint(pH 6.02) of the indicator dye used. As a result of this inherent limitation and the insensitive nature of colour indicator tests for measuring pH, the BCP/sucrose was used merely as a means of screening some of the large number of antibacterial-anticaries agents available.

Some of the teeth treated with agents containing heavy metals gave a non-acidic reaction seven days after treatment but lost their efficacy over the subsequent five weeks. The next section of the study was undertaken in an attempt to extend the length of the inhibitory action of one of the heavy metal fluorides(AgF).

SECTION II

EFFECTS OF SILVER FLUORIDE AND SILVER IODIDE ON INHIBITION OF ACID PRODUCTION IN VIVO

In the previous study no long term inhibition of acid production occurred with any of the test agents. Possibly this was because the agents were lost from the pit and fissure systems rapidly and therefore did not have sufficient time to act. It is thought that the superiority of chlorhexidine(44,64,104) and SnF_2 (192) as antiplaque agents on smooth surface plaque is due largely to the fact that these agents are adsorbed to oral structures and then slowly leached over a period of time. An early development of slow release drugs for topical use was the use of an extremely insoluble salt of silver such as silver iodide or silver chloride suspended in a high molecular weight polymer such as gelatine(13,150). The antibacterial silver ions were then released slowly over a period of time.

In this section, silver fluoride was combined with the highly insoluble silver iodide to ascertain if its effects on the inhibition of acid production could be prolonged. Silver fluoride was chosen because of the added benefits of the fluoride ion. Stannous fluoride, which showed equally promising effects in Section I and also contains fluoride, was eliminated because a compatible sparingly soluble salt, stannous pyrophosphate, is

not commercially available in Australia and an extremely complex chemical process is involved in its production(29,122).

MATERIALS AND METHODS

MATERIALS

Test Agents

Two preparations were evaluated:

1. **AgI** -- AgI slurry. 0.1 gm AgI powder in 0.8 ml filtered deionised water. The AgI (Analytical reagent grade, Labchem, Ajax Chemicals, Sydney, Australia) was ground, and sieved through a 38 μ m sieve to form a very fine powder (British Pharmaceutical Codes)(140).
2. **AgF/AgI** -- AgF/AgI slurry. 0.1 gm AgI (prepared as in item 1) in 0.8 ml 40% AgF (A.G.F. Silver Fluoride, Creighton Dental, Double Bay, N.S.W., Australia).

Indicator Dye

The indicator dye solution (BCP/sucrose) contained 0.37% bromcresol purple ($C_{21}H_{16}Br_2O_5S$) (Bromocresol Purple, Labchem, Ajax Chemicals, Sydney, Australia) and 10% sucrose in filtered, deionised water.

Patients

Orthodontic patients undergoing fixed appliance therapy(mean age 14.01 years; S.D. = 1.27 years) who had caries-free lower second molars with accessible occlusal pit and fissure systems were used. The orthodontic appliances remained in situ throughout the study.

Patients were initially screened to eliminate those with pits and fissures which did not demonstrate an 'acid reaction'(yellow colour) following the application of the BCP/sucrose solution(page 38)(Figure 3).

METHODS

Clinical Procedures

All procedures were carried out as described in Section I, pages 36-38. Each tooth receiving either a 4 minute or a 60 minute treatment with either AgI slurry or AgF/AgI slurry.

Statistical Analysis

The results were analysed statistically for differences between the two groups using Fisher's Exact Test for 2 x 2 contingency tables with small numbers(6,141).

Table 2. Inhibition of Acid Production by AgI alone and in conjunction with AgF.

	Four Minutes		Sixty Minutes	
	Yes	No	Yes	No
Seven Days				
AgF/AgI(n=9)	7	2	7	2
AgI(n=9)	5	4	5	4
Six Weeks				
AgF/AgI(n=9)	6	3	6	3
AgI(n=9)	3	6	4	5
Twelve Weeks				
AgF/AgI(n=9)	3	6	3	6
AgI(n=9)	1	8	2	7

Differences between two agents not significant at 0.05 level for 4 minute or 60 minute application times at either 7 days, 6 weeks or 12 weeks(Fisher's Exact Test for 2 x 2 contingency tables).

RESULTS

A total of thirty-six teeth in eighteen patients were treated. As Table 2 shows inhibition of acid production by both AgI and the AgF/AgI treatments was evident six weeks after treatment. Twelve weeks after treatment some teeth still demonstrated a non-acidic colour change with the BCP/sucrose indicator (Table 2). There was an obvious trend to decreased effectiveness with time. There was no significant difference at the 0.05 level between the effects of the AgI slurry and the AgF/AgI slurry, at either seven days, six weeks or twelve weeks. In addition there was no significant difference between four minute and sixty minute treatments ($P > 0.05$) at any of the observed times.

DISCUSSION

The slow release preparations tested demonstrated some improved efficacy in preventing acid production in pits and fissures. Although both preparations did extend the inhibitory period, seen with AgF in Section I, it had been markedly reduced by twelve weeks. These anti-glycolytic effects may have been because of a direct antibacterial action on acid producing organisms. False non-acidic readings may have also occurred because of the black staining of some fissures caused by the silver ions made the colour changes very difficult to discern on some teeth. Neither agent was capable of consistently producing a detectable inhibition of acid production in all pits and fissures treated. However it is possible that some inhibition of acid production

may have occurred below the level of pH 6.02 which could not be detected by the colour indicator system used.

SECTION III

EFFECTS OF SILVER FLUORIDE-SILVER IODIDE ON THE MICROBIAL FLORA OF PITS AND FISSURES IN VIVO

The combination of AgF/AgI did extend the period of inhibition of acid production, but by twelve weeks the effect had been markedly reduced. Because dental caries is a disease entity which is dependent on a number of variables, it has been suggested by several authors that a more complete assessment of caries activity can be made by comparing several indicators(76,162,196). Consequently, a microbial study was undertaken to see if the AgF/AgI treatment did in fact have an inhibitory effect on the bacteria of the fissure plaque; particularly on two important cariogenic organisms S. mutans and lactobacilli.

The antimicrobial effects of the AgF/AgI slurry developed in the last section were assessed by topically treating a lower second molar as the test tooth and by applying water to the contralateral lower second molar as the control tooth. The widely applied method of needle scraping to obtain bacterial samples of fissures was used in combination with a core sampling technique. This technique was developed expressly for the purpose of taking bacterial samples from the depths of pits and fissures.

MATERIALS AND METHODS

MATERIALS

Media

The following media were utilised in this study:

1. **RTF** -- Reduced Transport Fluid(106,149) was used for transportation and serial dilution of samples. This was prepared as described by Loesche, Hockett, and Syed(106) with EDTA as a chelating agent and dithiothreitol(Cleland's Reagent) as a reducing agent. The solution was filter sterilised(Millipore* GS filters, 0.22 μ m, Lots No. HSM 80040A & H6J79605H).
2. 5% Defibrinated sheep blood agar made up in Oxoid Blood Agar Base(Oxoid Ltd., Basingstoke, Hampshire, England. Lot No. 010 34336) for total colony counts. The base was sterilised by autoclaving at 121°C for 15 minutes and the sterile blood added aseptically when the base had cooled to 50°C(72,98).
3. **MSA** -- Mitis Salivaris Agar(Difco Laboratories, Detroit, Michigan, U.S.A.) with 0.001% Chapman Tellurite for total streptococcal counts. This was sterilised by autoclaving at 121°C for 15 minutes and adding the tellurite aseptically when the media had cooled to 50°C.
4. **MSB** -- Mitis Salivaris Agar(Difco Laboratories, Detroit, Michigan, U.S.A.) with 0.001% Chapman Tellurite, 20% sucrose

* Millipore Corp., Bedford, Massachusetts, U.S.A. 01730

and 0.2 units/ml. bacitracin for the assessment of Streptococcus mutans levels. The agar containing the sucrose was autoclaved at 121°C for 15 minutes and allowed to cool to 50°C when the tellurite and the bacitracin were added aseptically(65).

5. LBS -- LBS Agar(BBL Microbiology Systems, Becton Dickson & Co., Cockeysville, Maryland, U.S.A., 21030. Lot No. D6D0DC) with 0.132% glacial acetic acid for the assessment of Lactobacillus levels(148).
6. LBS-TJ -- LBS Agar(BBL Microbiology Systems, Becton Dickson & Co., Cockeysville, Maryland, U.S.A. 21030. Lot No. D6D0DC) with 0.132% glacial acetic acid and 20% Sterile Tomato Juice(Oxoid, Basingstoke, Hampshire, England) added aseptically for assessment of Lactobacillus acidophilus levels(148).

Test Agents

The effect of one test preparation was evaluated and sterile, filtered, deionised water was used as a control as follows:

1. AgF/AgI -- AgF/AgI slurry. 0.1 gm AgI powder in 0.8 ml 40% AgF(A.G.F. Silver fluoride, Creighton Dental, Double Bay, N.S.W., Australia). The AgI(Analytical reagent grade, Labchem, Ajax Chemicals, Sydney, Australia) was ground, and sieved through a 38 µm sieve to form a very fine powder (British Pharmaceutical Codes)(140).
2. Filtered, deionised water. The filtered, deionised water was sterilised by autoclaving at 121°C for 15 minutes.

Indicator Dye

The indicator dye solution (BCP/sucrose) contained 0.37% bromocresol purple ($C_{21}H_{16}Br_2O_5S$) (Bromocresol Purple, Labchem, Ajax Chemicals, Sydney, Australia) and 10% sucrose in filtered, deionised water. The BCP/sucrose was sterilised by filtering (Millipore GS filters, 0.22 μm , Lots No. HSM 80040A & H6J79605H).

Plaque Collection Materials

Needle: Single-use sterile 26-gauge needles* were used for obtaining scrapings of fissures.

Construction of an Instrument for Core Sampling of Pits and

Fissures: The core sampler was constructed from an 06 endodontic K-file** as illustrated in Figure 5. The spiral of a spiral root filler§ was removed from the shank using two pairs of orthodontic pliers (Figure 5a). The hole in the shank was then cleaned with an Ash $\frac{1}{2}$ round bur (No. 1/006). The hole was treated with phosphoric acid etching gel for one minute (Figure 5b). This was thoroughly washed off for at least twenty seconds with water and dried with clean, oil free compressed air. A thin layer of bonding resin was applied around the hole and around the shank's

* Single-use Neolus Needles, 26G x $\frac{1}{2}$ /0.45 x 13m/m, Terumo Corp., Melbourne, Australia

** Files K, Size 06(006): Vereginite, Dentalwerke, Zdarsky Ehrler GmbH & Co. KG., Munchen 70, W. Germany.

§ Stainless Paste Carrier Art. 93, Size No. ISO 25-40: Vereginite, Dentalwerke, Zdarsky Ehrler GmbH & Co. KG., Munchen 70, W. Germany.

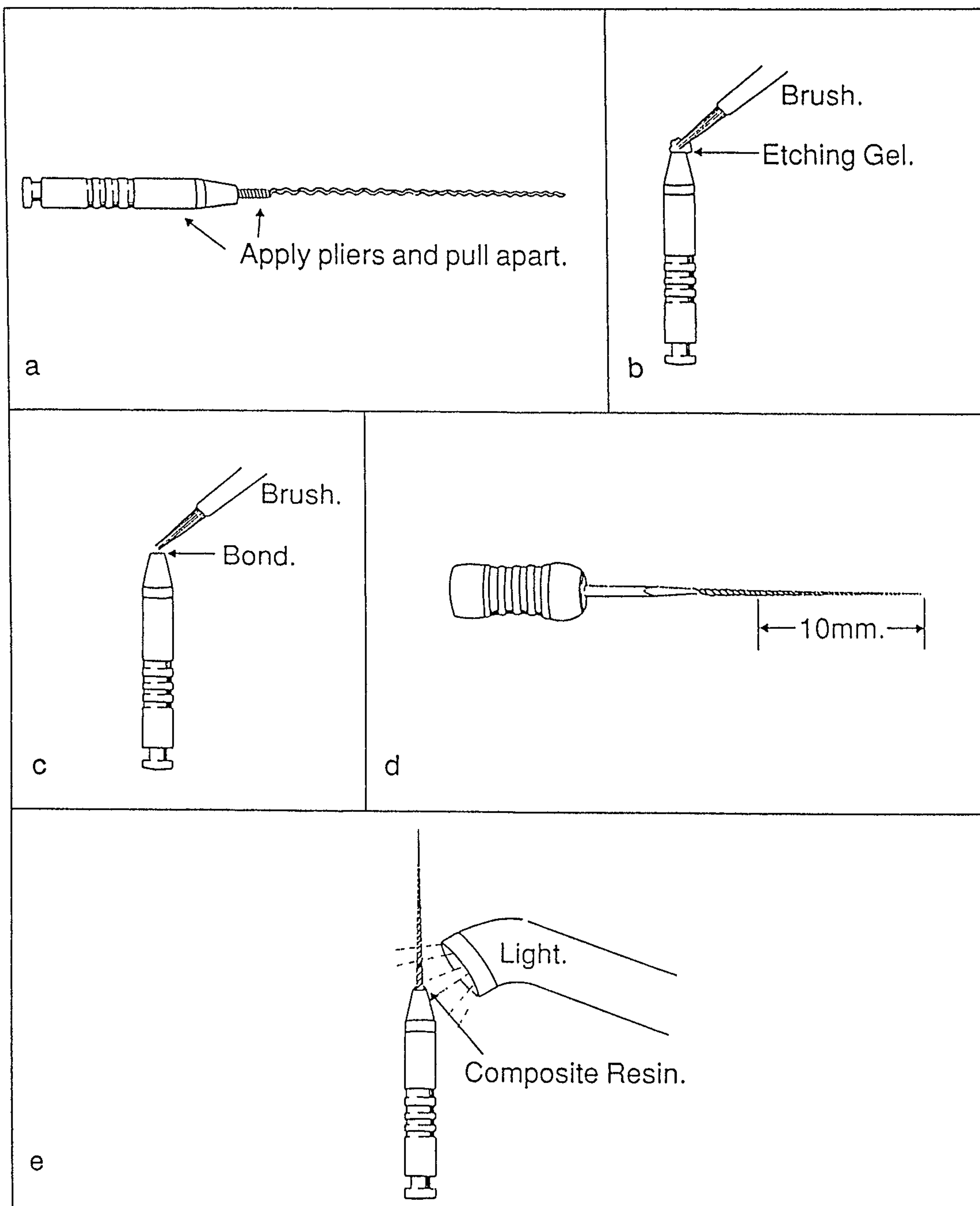


Figure 5. Schematic diagrams showing the method of construction of the fissure sampling instrument from an 06 endodontic K-file and the shank of a spiral root filler.

orifice(Figure 5c). The bonding resin was cured for twenty seconds with a white light. Both the acid and the bonding resin were worked down the hole of the shank with the bristles of a fine clean brush. The hole in the shank was then filled with light-cured composite resin*.

The point of an 06 endodontic K-file was cut from the shaft 10 mm from the tip(Figure 5d). This tip section of the endodontic K-file was placed in the composite resin filled spiral filler shank as illustrated in Figure 5e. The composite resin was cured for one minute with a white light(Figure 5e).

Each instrument was tested by holding it firmly in two pairs of orthodontic pliers and trying to pull it apart firmly but gently. The instruments were then examined under the microscope. Any instrument in which the K-file (a) had a width greater than 60 μ m at the start of the cutting blades; (b) showed evidence of stress or fatigue; or (c) was contaminated with composite resin: was discarded. The K-file was then used in an ultraslow handpiece** at minimum speed run in a forward direction. Several instruments were test autoclaved at 132°C for 5 minutes to ensure that they could be sterilised without destruction.

The instrument developed for core sampling of the fissures had an overall length of approximately 22 mm(Figure 6) and was capable

* Ful-fil Compules: The L.D. Caulk Co., Division of Dentsply International, Milford, Delaware, U.S.A. 19963.

** W & H Contra-angle Handpiece 68E (Inco Series), Dentalwerk Buermoos Ges M.B.H., A-5111 Buermoos, Austria.

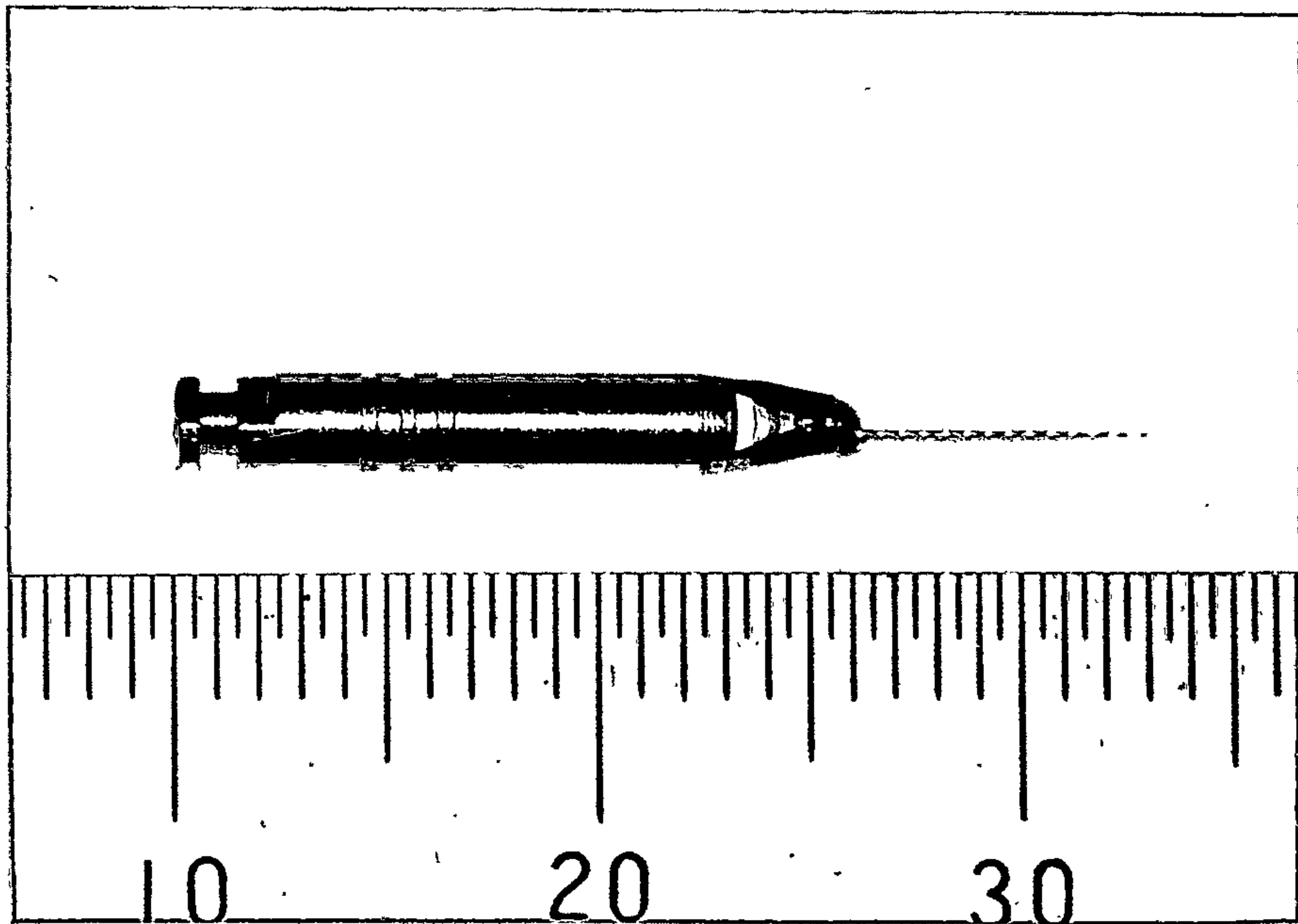


Figure 6. Instrument devised for core-sampling the contents of the depths of pits and fissures.(Scale in millimetres)

of penetrating to the base of fissures as illustrated in Figure 7.

Patients

Orthodontic patients undergoing fixed appliance therapy(mean age 14.73 years; S.D. = 0.73 years) who had two caries-free, lower second molars with accessible occlusal pit and fissure systems were used. The orthodontic appliances remained in situ throughout the study.

Patients were initially screened to eliminate those with pits and fissures which did not demonstrate an 'acid reaction'(yellow colour) following the application of the BCP/sucrose solution(page 37)(Figure 3).

METHODS

Clinical Procedures

Patients were seen at three appointments each six weeks apart. At every appointment prior to treatment or sampling the lower second molars were isolated and tested with the BCP/sucrose indicator as described previously(Section I, page 38).

Treatment Appointment: The treatment and control teeth were randomly assigned in each mouth prior to the initial examination of the patient. The tooth designated as the treatment tooth was



Figure 7. A 26-gauge hypodermic needle(left) and an 06 endodontic K-file(right) in the same fissure of a section of a lower third molar.(Magnification 32X)

treated with either a 4 minute or 60 minute AgF/AgI treatment as previously described (Section I, pages 35-36). The contralateral tooth was correspondingly treated with sterile, filtered, deionised water. The patients were instructed not to eat or drink for one hour after leaving the surgery. If they had a Stomahesive Wafer in place they were asked to remove it after sixty minutes.

Sampling Appointment: Fissure plaque sampling was undertaken twelve weeks after the topical treatments with AgF/AgI or sterile water were administered. Sterile techniques were observed during the sampling. All instruments were autoclaved at 132°C for five minutes prior to the appointment.

The patient's mouth was propped open with a suitable sized mouth prop to prevent inadvertent closure of the mouth during the procedures. The tooth was isolated with Garmers clamps, cotton wool rolls, and Dry Guard Shields as described previously (Section I, page 36). A needle point cut from its hub and held in a pair of artery forceps was moved firmly in the central fossa of the lower second molar with a rotational motion for five seconds. The needle point was then dropped into a labelled test tube containing 2 ml of RTF solution. Immediately, the prepared K-file was placed in the head of the handpiece using a pair of sterile artery forceps gripping the shank of the K-file. The K-file was locked in place in the handpiece. The handpiece was rotated at minimum speed for ten seconds in the central fossa of the tooth. The K-file was cut from the shank, with a pair of

sterile orthodontic wire cutters and dropped directly into a labelled test tube containing 2 ml of RTF solution. Samples were taken from both the test tooth and the control tooth. The samples were taken to the laboratory where they were dispersed, diluted, plated, and incubated anaerobically within two hours of collection.

Laboratory Procedures*

Sample Dispersion and Dilution: The samples were dispersed by sonification for 10-15 seconds at a setting of 4.5 with a Branson Standard Tapered Microtip** on a Branson Sonifier, Model B12L(equivalent to Model 185)**. This setting and time appear to give the highest viable count and highest colony count for plaque bacteria as determined in other studies(108,147).

The samples were then vortexed for ten seconds. Tenfold dilutions were made using sterile RTF solution with vortexing for five seconds between each dilution.

Microplating Technique: Traditionally, dispersed and diluted plaque samples have been plated in 0.1ml. aliquots on the appropriate agar plates. In plaque studies of any complexity this technique requires the preparation and incubation of enormous numbers of plates. In 1978, Westegren and Krasse(206),

* The general background to the techniques used in this section are covered in several reviews: transport of samples(23,56, 149,175), dispersion of samples(72,81,99,108,147,167), and media and plating(20,43,72,101,108,148,176).

** Branson Sonic Power co., Danbury, Connecticut, U.S.A. 06810.

developed a micromethod for the determination of microbial counts. This method offered enormous advantages in being simple, reliable and resulted in substantial savings of culture media and laboratory time and space. Studies by Matee et al.(116) and Kohler and Emilson(93) have further established that this method is accurate and reliable when compared with conventional plating techniques.

Using an Eppendorf* Micropipette, 0.25 μ l aliquots of the appropriate dilutions were plated by a micropipette technique(206). The aliquot was plated on one quarter of the plate giving a spot approximately 1-1.5 cm in diameter on the Mitis Salivaris and LBS agars. On the blood agar the surface tension was such that it was necessary to spread the spot with a spiral motion from the centre to the perimeter with a ball-ended sterile glass spreader to an approximate diameter of 1.5 cm. To avoid confluence of drops the plates were dried prior to plating and only placed in the incubation oven after the aliquots had dried on the media. Anaerobic incubation at 37°C for 72 hours was carried out using BBL Gas Pak 100 Anaerobic System with Gas Pak Disposable Hydrogen & CO₂ Generator Envelopes(4-7% CO₂ and H₂)**. Duplicate dilutions were plated on the one plate for Mitis Salivarius and LBS agars. For blood agar plates four dilutions were plated on each plate and duplicate plates were made.

* Eppendorf, Brinkmann Instruments Inc., Westbury, New York U.S.A. 11590.

** BBL Microbiology systems, Cockeysville, Maryland, U.S.A. 21030

Counting: Dilutions having 10-200 colony forming units were counted with the aid of 6.4X magnification under a Wild Heerbrugg Dissecting Microscope*. This enabled each plated area to be visualised in one field of the microscope. The mean count of the two samples of the appropriate dilution was used in the analyses.

Statistical Analysis

Data was compared statistically using a Wilcoxon's Test for signed-ranks for differences between matched pairs(139,141). Absolute numbers were not used in comparing differences between test and control groups for specific bacterial types because of the great variation in sample size and character from different sites. Consequently, only the percentage of the total bacterial count was used when analysing specific types or groups of bacteria.

Sensitivity of Plaque Microbes to Bromcresol Purple

In this section of the study all instruments used, including the weighing pans on the Cahn Electrobalance Balance**, were sterilised by autoclaving at 132°C for five minutes. The BCP/sucrose indicator was filter sterilised(Millipore GS filters, 0.22 µm, Lots No. HSM80040A & H6J79605H). Sterile gloves were worn throughout.

* Wild-Australia Pty. Ltd., Nth. Ryde, Sydney, Australia

** Cahn Ventron Electrobalance, Model No. 23: Cahn Instruments, Ceritos, California, U.S.A. 90701

A homogenous plaque sample was collected from the patient on a No. 6 plastic instrument. This was immediately taken to the laboratory where it was divided into two approximately equal portions. Each portion was placed on a teflon square--which had been tared on the balance with a similar teflon square--and weighed. One portion(to be used as a control) with its teflon square was placed in 2 ml of RTF solution forthwith. The other portion(to be used as the test) was treated with one drop of BCP/sucrose indicator for two minutes. Excess dye was absorbed with a sterile swab and the sample was placed in 2 ml of RTF solution. Samples were sonified, vortexed, serially diluted, described on pages 54-56. The mean count of duplicate platings from the appropriate dilution was used in the analyses.

The findings were subjected to a Wilcoxon's Signed Rank Test for paired observations and are presented in Appendix A. The BCP/sucrose indicator dye showed no significant antimicrobial activity($P > 0.05$).

RESULTS

Data relating to the number of colony forming units(cfu) obtained from fissure plaque samples grown on blood agar, MSA agar, MSB agar and LBS agars are presented in Tables 3, 4, 5, 6, and 7 respectively*. Findings relating to the organisms obtained by

* No attempt was made to characterise individual organisms. For the purpose of this thesis organisms grown on MSA agar are referred to as streptococci, those grown on MSB agar as S. mutans, and those grown on LBS agar as lactobacilli.

needle sampling alone and by K-file sampling alone are given in Appendices B-F. Shown in Tables 8 and 9 are data on the ratio of streptococci, S. mutans and lactobacilli to total cultivable organisms.

The samples collected from the test and control teeth displayed a wide variation in the number of colony forming units grown on the various media. The total counts on blood agar for needle samples ranged from 3.4×10^4 to 1.0×10^7 cfu from the control teeth and 6.2×10^4 to 1.6×10^7 cfu from the test teeth in the four minute treatment group. For the sixty minute treatment group needle samples the total cultivable bacteria ranged from 4.0×10^2 to 9.6×10^6 cfu for the control teeth and 3.8×10^4 to 2.8×10^6 cfu for the test teeth(Appendix B).

The total colony counts for the K-file samples from the four minute treatment series ranged from 1.2×10^4 to 7.8×10^6 cfu for control teeth and 5.8×10^4 to 8.0×10^6 cfu for test teeth(Appendix B). Total counts for the K-file samples from the sixty minute treatment series ranged from 1.5×10^3 to 2.4×10^6 cfu for the control teeth and from 1.2×10^4 to 4.0×10^6 cfu for test teeth(Appendix B).

When the K-file and needle samples were added together to give a figure for total recoverable fissure flora the range for all the control teeth was 1.2×10^4 to 1.2×10^7 cfu(Table 3). The total recoverable fissure flora from the test teeth was 1.2×10^5 to 1.8×10^7 cfu for the four minute treatments and 6.0×10^4 to

6.2×10^6 cfu for the sixty minute treatments (Table 3).

Analysing these results with Wilcoxon's Signed Rank Test for paired observations, the sixty minute treatments had no significant effect on total cultivable bacteria nor on specific groups of bacteria, however the four minute treatments caused a significant increase in the total number of cultivable bacteria in the test teeth, but not in any of the specific groups of bacteria cultivated.

The ranges for total (needle + K-file) streptococcus, S. mutans, and lactobacilli cultivated are given in Tables 4-7, and no significant differences between test and control teeth in any of the groups were seen. When these counts were analysed as a percentage of total cultivable flora, in order to overcome inherent variations in sample size and site of collection, again no significant difference between test and control teeth could be found in any of the groups (Tables 8-9).

No statistically significant antibacterial effects could be found twelve weeks after treatment of fissures with the AgF/AgI, except in the case of total colony forming units grown on 5% sheep blood agar after 4 minute treatments (Tables 3-7).

When bacterial counts of specific types of bacteria were analysed as a percentage of total cultivable flora again no significant difference between test and control teeth could be found in any of the groups (Tables 8 & 9).

Table 3. Total Bacteria Recovered from Fissures(needle + K-file samples) on 5% Sheep Blood Agar(expressed as colony forming units--cfu).

Four Minute Treatments				Sixty Minute Treatments			
Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)	Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)
SC	8.3 x 10 ⁶	7.8 x 10 ⁵	+7.5 x 10 ⁶	TA	3.2 x 10 ⁶	4.0 x 10 ⁵	+2.8 x 10 ⁶
IC	1.0 x 10 ⁷	2.1 x 10 ⁵	+9.8 x 10 ⁶	RAB	3.8 x 10 ⁵	1.2 x 10 ⁵	+2.6 x 10 ⁵
CG	1.2 x 10 ⁵	5.4 x 10 ⁴	+6.6 x 10 ⁴	RJB	1.9 x 10 ⁶	4.1 x 10 ⁴	+1.9 x 10 ⁶
DJ	4.2 x 10 ⁶	4.2 x 10 ⁶	0	TD	6.0 x 10 ⁴	6.0 x 10 ⁴	0
MK	6.2 x 10 ⁵	6.0 x 10 ⁵	+2.0 x 10 ⁴	JH	6.2 x 10 ⁶	1.1 x 10 ⁷	-4.8 x 10 ⁶
DM	1.5 x 10 ⁶	8.4 x 10 ⁵	+6.6 x 10 ⁵	JN	3.0 x 10 ⁵	5.6 x 10 ⁶	-5.3 x 10 ⁶
AN	3.8 x 10 ⁶	5.1 x 10 ⁴	+3.7 x 10 ⁶	JOP	3.1 x 10 ⁵	1.8 x 10 ⁵	+1.3 x 10 ⁵
SP	2.9 x 10 ⁶	2.3 x 10 ⁵	+2.7 x 10 ⁶	GS	1.3 x 10 ⁵	1.2 x 10 ⁵	+1.0 x 10 ⁴
ST	8.4 x 10 ⁶	1.2 x 10 ⁷	-3.6 x 10 ⁶	HS	1.6 x 10 ⁶	1.2 x 10 ⁴	+1.6 x 10 ⁶
SW	1.8 x 10 ⁷	1.0 x 10 ⁷	+8.0 x 10 ⁶	JAP	6.2 x 10 ⁵	1.1 x 10 ⁷	-1.0 x 10 ⁷
T Value*	5**			22§			

* Wilcoxon's Signed Rank Test for paired observations
 ** Difference between test and control significant at the 0.05 level.
 § Difference between test and control not significant at the 0.05 level.

Table 4. Streptococci Recovered from Fissures(needle + K-file samples) on MSA Agar(expressed as colony forming units--cfu).

Sixty Minute Treatments				Four Minute Treatments			
Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)	Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)
SC	1.9 x 10 ⁵	2.4 x 10 ⁴	+1.7 x 10 ⁵	TA	1.4 x 10 ⁵	2.6 x 10 ³	+1.4 x 10 ⁵
IC	5.1 x 10 ³	1.8 x 10 ⁴	-1.3 x 10 ⁴	RAB	2.5 x 10 ⁵	4.8 x 10 ³	+2.4 x 10 ⁵
CG	1.5 x 10 ⁵	1.5 x 10 ³	+1.5 x 10 ⁵	RJB	3.1 x 10 ⁴	1.8 x 10 ⁴	+1.3 x 10 ⁴
DJ	1.5 x 10 ⁴	1.2 x 10 ⁴	+3.0 x 10 ³	TD	1.0 x 10 ⁴	3.2 x 10 ⁵	-3.1 x 10 ⁵
MK	8.8 x 10 ⁴	3.4 x 10 ⁴	+5.4 x 10 ⁴	JH	7.2 x 10 ³	1.8 x 10 ⁵	-1.7 x 10 ⁵
DM	7.6 x 10 ⁴	1.6 x 10 ⁵	-8.4 x 10 ⁴	JN	1.0 x 10 ⁵	7.9 x 10 ⁴	+2.1 x 10 ⁴
AN	4.2 x 10 ³	5.8 x 10 ³	-1.6 x 10 ³	JOP	1.3 x 10 ⁵	8.4 x 10 ²	+1.3 x 10 ⁵
SP	2.0 x 10 ³	2.2 x 10 ⁴	-2.0 x 10 ⁴	GS	5.6 x 10 ⁴	6.0 x 10 ³	+5.0 x 10 ⁴
ST	1.5 x 10 ⁵	8.0 x 10	+1.5 x 10 ⁵	HS	2.2 x 10 ⁵	2.2 x 10 ⁵	0
SW	1.9 x 10 ⁴	3.2 x 10 ⁵	-3.0 x 10 ⁵	JAP	3.2 x 10 ⁵	3.2 x 10 ⁵	0
T Value*					14**		
					26**		

* Wilcoxon's Signed Rank Test for paired observations.

** Difference between test and control not significant at the 0.05 level.

Table 5. Streptococcus mutans Recovered from Fissures(needle + K-file samples) on MSB Agar(expressed as colony forming units--cfu).

Four Minute Treatments				Sixty Minute Treatments			
Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)	Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)
SC	3.3 x 10 ³	1.8 x 10 ³	+1.5 x 10 ³	TA	3.0 x 10 ³	9.6 x 10 ²	+2.0 x 10 ³
IC	1.4 x 10 ⁵	1.8 x 10 ³	+1.4 x 10 ⁵	RAB	ND	2.4 x 10 ²	-2.4 x 10 ²
CG	4.0 x 10 ²	2.8 x 10 ²	+1.2 x 10 ²	RJB	2.4 x 10 ⁴	2.2 x 10 ³	+2.0 x 10 ⁴
DJ	1.1 x 10 ⁵	1.7 x 10 ⁵	-6.0 x 10 ⁴	TD	4.0 x 10 ³	9.8 x 10 ³	-5.8 x 10 ³
MK	ND	1.7 x 10 ³	-1.7 x 10 ³	JH	4.4 x 10 ²	8.0 x 10	+3.6 x 10 ²
DM	9.2 x 10 ³	6.7 x 10 ³	+2.5 x 10 ³	JN	4.4 x 10 ²	4.5 x 10 ⁴	-4.5 x 10 ⁴
AN	2.3 x 10 ⁴	4.0 x 10	+2.3 x 10 ⁴	JOP	6.0 x 10	ND	+6.0 x 10
SP	4.1 x 10 ⁴	4.2 x 10 ³	+3.7 x 10 ⁴	GS	6.2 x 10 ³	2.1 x 10 ⁴	-1.5 x 10 ⁴
ST	1.1 x 10 ⁵	3.0 x 10 ⁴	+7.0 x 10 ⁴	HS	1.1 x 10 ⁵	ND	+1.1 x 10 ⁵
SW	5.2 x 10 ⁴	1.1 x 10 ⁴	+4.1 x 10 ⁴	JAP	6.4 x 10 ²	4.2 x 10 ⁴	-4.1 x 10 ⁴
T Value*	11**			25**			

* Wilcoxon's Signed Rank Test for paired observations
 ** Difference between test and control not significant at the 0.05 level.
 ND No bacteria detected at the lowest dilution.

Table 6. Lactobacilli Recovered from Fissures(cfu)(needle + K-file samples) on LBS Agar(expressed as colony forming units--cfu).

Sixty Minute Treatments				Four Minute Treatments			
Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)	Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)
TA	1.6 x 10 ²	ND	+1.6 x 10 ²	SC	1.4 x 10 ⁵	1.6 x 10 ²	+1.4 x 10 ⁵
RAB	ND	ND	-	IC	4.4 x 10 ⁴	ND	+4.4 x 10 ⁴
RJB	2.4 x 10 ²	ND	+2.4 x 10 ²	GC	ND	ND	-
TD	2.4 x 10 ³	6.6 x 10 ³	-4.2 x 10 ³	DJ	4.8 x 10 ⁴	6.2 x 10 ⁴	-1.4 x 10 ⁴
JH	1.1 x 10 ⁵	ND	+1.1 x 10 ⁵	MK	ND	ND	-
JN	ND	4.0 x 10	-4.0 x 10	DM	ND	ND	-
JOP	2.4 x 10 ²	ND	+2.4 x 10 ²	AN	6.0 x 10 ³	ND	+6.0 x 10 ³
GS	ND	ND	-	SP	1.2 x 10 ²	4.0 x 10	+8.0 x 10
HS	9.2 x 10 ²	ND	+9.2 x 10 ²	ST	1.1 x 10 ³	1.2 x 10 ²	+9.8 x 10 ²
JAP	4.0 x 10	1.1 x 10 ⁴	-1.1 x 10 ⁴	SW	ND	ND	-
				T Value*	NA		

* Wilcoxon's Signed Rank Test for paired observations
ND No bacteria detected at the lowest dilution.
NA Insufficient data for statistical analysis.

Table 7. Lactobacilli Recovered from Fissures(needle + K-file samples) on LBS-TJ Agar(expressed as colony forming units--cfu).

Four Minute Treatments				Sixty Minute Treatments			
Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)	Subject	Test(T) (cfu)	Control(C) (cfu)	Difference (T - C)
SC	1.6 x 10 ⁵	1.2 x 10 ²	+1.6 x 10 ⁵	TA	4.0 x 10 ²	ND	+4.0 x 10 ²
IC	3.9 x 10 ⁴	ND	+3.9 x 10 ⁴	RAB	ND	1.0 x 10 ³	-1.0 x 10 ³
CG	ND	ND	-	RJB	4.1 x 10 ³	ND	+4.1 x 10 ³
DJ	1.2 x 10 ⁵	1.4 x 10 ⁵	-2.0 x 10 ⁴	TD	3.0 x 10 ³	6.2 x 10 ³	-3.2 x 10 ³
MK	ND	ND	-	JH	2.9 x 10 ⁵	ND	+2.9 x 10 ⁵
DM	ND	ND	-	JN	ND	ND	-
AN	1.1 x 10 ⁴	ND	+1.1 x 10 ⁴	JOP	ND	ND	-
SP	2.8 x 10 ²	ND	+2.8 x 10 ²	GS	ND	ND	-
ST	2.0 x 10 ²	4.0 x 10	+1.6 x 10 ²	HS	1.0 x 10 ³	ND	+1.0 x 10 ³
SW	ND	ND	-	JAP	ND	7.2 x 10 ³	-7.2 x 10 ³
T Value*	NA			NA			

* Wilcoxon's Signed Rank Test for paried observations
 ND No bacteria detected at the lowest dilution.
 NA Insufficient data for statistical analysis.

Table 8. Specific Groups of Bacteria expressed as a Percentage of Total Bacteria Recovered from Teeth Receiving Four Minute Treatments(needle + K-file samples).

Subject	Streptococcus on MSA Agar		Streptococcus mutans on MSB Agar		Lactobacillus on LBS Agar		Lactobacillus on LBS-TJ Agar	
	Test (T)	Control (C)	Difference (T - C)	Test (T)	Control (C)	Difference (T - C)	Test (T)	Difference (T - C)
SC	1.69	0.33	+1.36	0.04	0.23	-0.19	1.69	+1.48
IC	2.50	2.29	+0.21	1.40	0.86	+0.54	0.44	+0.44
CG	25.83	33.33	-7.50	0.33	0.52	-0.19	-	-
DJ	0.24	7.62	-7.38	2.62	4.05	-1.43	1.14	-0.34
MK	1.16	30.00	-28.84	-	0.28	-0.28	-	-
DM	6.67	9.40	-2.73	0.61	0.80	-0.19	-	-
AN	3.42	1.65	+1.77	0.61	0.08	+0.53	0.16	+0.16
SP	1.93	2.60	-0.67	1.41	1.83	-0.42	0.004	-0.016
ST	2.62	1.83	+0.79	1.31	0.25	+1.06	0.01	+0.009
SW	1.78	3.20	-1.42	0.29	0.11	+0.18	-	-
T Value*	14**		25**	NA		NA	NA	

* Wilcoxon's Signed Rank Test for paired observations.
 ** Difference between test and control not significant at the 0.05 level.
 NA Insufficient data for statistical analysis.

Table 9. Specific Groups of Bacteria expressed as a Percentage of Total Bacteria Recovered from Teeth Receiving Sixty Minute Treatments(needle + K-file samples).

Subject	Streptococcus on MSA Agar			Streptococcus mutans on MSB Agar			Lactobacillus on LBS Agar			Lactobacillus on LBS-TJ Agar		
	Test (T)	Control (C)	Difference (T - C)	Test (T)	Control (C)	Difference (T - C)	Test (T)	Control (C)	Difference (T - C)	Test (T)	Control (C)	Difference (T - C)
TA	5.94	6.00	-0.06	0.09	0.24	-0.15	0.005	-	+0.005	0.01	-	+0.01
RAB	1.34	15.00	-13.66	-	0.20	-0.20	-	-	-	-	0.83	-0.83
RJB	7.89	3.66	+4.23	1.26	5.37	-4.11	0.01	-	+0.01	0.22	-	+0.22
TD	25.00	20.00	+5.00	6.67	16.33	-9.66	4.00	11.00	-7.00	5.00	10.33	-5.33
JH	1.42	0.31	+1.11	0.007	0.001	+0.006	1.77	-	+1.77	4.68	-	+4.68
JN	25.33	2.86	+22.47	0.15	0.80	-0.65	-	0.001	-0.001	-	-	-
JOP	1.35	3.22	-1.87	0.02	-	+0.02	0.08	-	+0.08	-	-	-
GS	1.54	18.33	-16.79	4.77	17.50	-12.73	-	-	-	-	-	-
HS	9.38	0.67	+8.71	6.86	-	+6.86	0.06	-	+0.06	0.06	-	+0.06
JAP	3.06	2.91	+0.15	0.10	0.38	-0.28	0.006	0.10	-0.094	-	0.07	-0.07
T Value*	22**			17**			NA			NA		

* Wilcoxon's Signed Rank Test for paired observations.
 ** Difference between test and control not significant at the 0.05 level.
 NA Insufficient data for statistical analysis.

DISCUSSION

The adapted K-file utilised for the collection of bacterial samples from the depths of fissures proved to be a most useful instrument. The technique employed for bacterial sampling produced similar bacterial counts to other techniques of sampling such as third molar fissure implants(179,180,181) and total fissure removal (119). A feature of the results presented in Tables 3-9 and Appendices B-F is the enormous intersample variation in the number of microflora collected. This is consistent with other studies which have also observed the same phenomena(119,120,180).

Streptococci were present in all samples but often constituted only a small proportion of the total bacterial count. S. mutans was present in all but three samples, but again was present in only small proportions. Many studies of the bacterial flora of fissures have utilised MSA and MSB media for the isolation of streptococci and S. mutans respectively(119,120,180,181,191), however, several workers have shown recently that recovery rates for S. mutans on MSB agar are low, especially for serotypes d and e(35,43,155). In view of the small numbers of bacteria which can be obtained in a fissure sample, higher and more statistically useful numbers might be obtained by utilising other media selective for streptococci such as TYCSB(203), and TSY20B(155).

GENERAL DISCUSSION

Studies on pit and fissure plaque are hampered by a number of factors that do not apply to investigations of plaque collected from smooth tooth surfaces. Some of these factors include the limited access to pits and fissures, the difficulty of obtaining adequate plaque samples, and the problems of measurement of the pH of plaque.

The design of this study took into account that there is only extremely limited access to the prime sites of dental caries, namely the pit and fissure systems of first and second molar teeth(12,15,45,79,94,112,137). Although more ready access can be gained to the pit and fissure systems of premolar teeth, the relevance of data from these sites is questionable because they do not have the same high susceptibility to caries as the corresponding areas in molar teeth(41). In addition, because first and second molars are located in the posterior regions of the mouth, not only is access restricted but the maintenance of a dry sterile field is difficult because of interference by the tongue, pooling of saliva, and, occasionally, the presence of an operculum. Another factor affecting access in this study was that all patients were undergoing orthodontic treatment and the presence of orthodontic bands precluded the use of rubber dam to ensure a dry, sterile field. In summary the techniques that can

be used for obtaining samples and taking measurements in situ are extremely limited in such a confined area.

In recent studies, pH change in smooth surface plaque following fermentation of carbohydrate has been measured by electrode systems(60,134,135,136,152,172,205). However, in earlier work and in some caries activity tests, use has been made of indicator dyes(70,73,76,90,143,161,162). The techniques used for measuring pH in recent research are the probing method(interdental touch) which takes direct readings from plaque in situ on individual tooth sites using antimony or glass electrodes(10,75,152,164,172,205), the in-dwelling electrode method(47,75,84,152) which records measurements from electrode systems housed in a denture assembly or within the dentition, and the 'one drop' method(60,152) which employs an external pH electrode system to measure small samples of plaque removed from the tooth. Objections can be raised to all these methods. It can be argued that:

1. The pH electrode may disarrange the structure and thus the diffusion rate in the dental plaque(172).
2. In the 'one drop' method the actual removal of plaque from the tooth surface may influence results(172). However, this method has an advantage over the other two, in that it can be readily used in conjunction with other analyses(59).
3. Measurements performed with the mouth open or on the laboratory bench may not reflect physiological conditions(172).

4. The experimental time is too limited(172).
5. The antimony electrodes used for the measurement may not give accurate pH values(172).

A problem of great significance with all the available electrode systems is whether the pH changes measured are occurring at the enamel plaque interface.

No electrodes appear to have been developed specifically to measure the pH changes in fissure plaques. It is impossible to obtain individual fissure plaque samples, by methods currently available for sampling, of sufficient size for the 'one drop' method of pH determination.

Because there were no viable alternatives an indicator dye was used in this study to measure pH changes at the entrance to pits and fissures. The procedure is simple and the dye can be applied directly to the fissure plaque. However, it must be emphasised that a pH indicator dye will only give an indication of the pH range at the entry to the fissure and not in the depths of the fissure. The interpretation of the colour change occurring in the indicator dye may be difficult to assess because of staining of the fissure or surrounding tooth structure, and consequently the results of indicator dye tests may be subject to some operator bias. Indicator dyes which have been used in smooth surface plaque tests are methyl red(73), bromcresol green(161) and bromcresol purple(70,90,143). Methyl red and bromcresol

green have acidic endpoints outside the so called 'critical pH'* for tooth enamel dissolution in saliva--methyl red pH 4.4 or less; bromcresol green pH 3.8 or less(204). The bromcresol purple indicator dye used in this study was found to have an endpoint between pH 5.42 and 6.02 in dental plaque collected from smooth surfaces. The acidic endpoint of pH 5.42 is well within the range of the 'critical pH' for tooth enamel in saliva of 5.2-5.7(82,128). Although the endpoints appear to be a little high, it was found in preliminary studies that an alternative indicator dye(methyl red), when used in various dilution, failed to change colour when used in conjunction with sucrose on test fissures. The higher acidic endpoint of BCP/sucrose is also more consistent with the 'critical pH' of 5.7 for tooth enamel in dental plaque, used in assessing the cariogenicity of foods(74). Because of the insensitive nature of indicator dye tests and the ambivalence in the nature of 'critical pH' the BCP/sucrose indicator was used in this study merely as a means of screening some of the large number of antibacterial agents which have been used.

* The original work of Stephan(164,165) on pH changes occurring in smooth surface plaque has lead to the development of the concept of 'critical pH'. 'Critical pH' is defined as the pH of saliva at which it ceases to be saturated with calcium and phosphate ions and at which tooth mineral dissolves(128). From the results of in vitro experiments, the range of 'critical pH' in saliva is generally considered to be 5.2-5.7(82,128). For the assessment of the cariogenicity of foods a 'critical pH' of 5.7 for tooth enamel in dental plaque has been used(74). In fissures and smooth surfaces it is the plaque, not the saliva, which constitutes the immediate environment of any tooth surface likely to be subjected to a cariogenic challenge but the 'critical pH' range for enamel in plaque fluid has not been established(22).

In this study, because of the difficulties in maintaining adequate isolation, a maximum time span of four minutes was available for accurate measurements to be taken in the mouth. This length of time is insufficient to observe the longevity of any significant plaque pH drop, either with an electrode system or a colour indicator. Thus it was not possible to obtain any indication of the buffering capacity of the fissure plaques. Several workers have concluded that a smooth surface plaque of high buffering capacity is less cariogenic than one of low buffering capacity in which the tooth surface would be exposed to a low pH for a long period of time(22,38,91,92,113). Geddes(59) has observed in smooth surface plaque that after exposure to sucrose a minimum time span of five minutes is needed before the lowest pH level is reached and that this time span may be as long as twenty minutes.

A difficulty in sampling fissure plaque is that in all probability the narrow, deep and tortuous fissure systems found in extracted premolar teeth(45,63) also occur in molar teeth. In such circumstances the collection of uniform sized and representative plaque samples could present considerable problems. Pooled occlusal plaque samples which have been used in some studies(27,61,110,154), while providing more material, may obscure important inter-site variations.

Consideration was given to adopting a widely used method for obtaining plaque samples from fissures, namely the use of a fissure model(88,103,124,160,181,182). These models are inserted

in a partial denture(191) or in a large restoration in a molar tooth(182). The fissure model may be the fissure system from an extracted third molar tooth(180,191), a folded mylar strip(88,103,169,182) or fissure-like spaces constructed in either bovine enamel or human enamel(124,160,166). However the disadvantages of this approach out weighed the possible benefits for the present investigation. Firstly it is necessary to leave the fissure models in the mouth for some time in order to develop fissure contents which are comparable to those occurring in natural fissures. Secondly there is the major problem of obtaining a sufficient number of suitable patients especially if a large number of agents are being evaluated. Finally the question remains as to how far one can extrapolate the findings obtained with fissure models to natural fissure systems in vivo.

The method of deep fissure sampling used in this study was developed to overcome some of the limitations of previous fissure sampling techniques. These have included scraping across fissure openings with hypodermic needles(21,81,105,109,154), pointed wires(108,110), or dental explorers(27,61). However, none of these instruments have the potential to collect all the fissure contents because of their inability to reach the depths of each fissure.

The importance of sampling the depths of a fissure was emphasised by the work of Meiers and Schachtele(119), who, using a total fissure removal technique, found that lactobacilli existed in

greater numbers in the depths of the fissure. Furthermore they found that bacteria were not distributed evenly throughout a fissure system. This technique of Meiers and Schachtele while providing an excellent representative sample of the fissure is highly invasive of tooth structure and could only ethically be utilised on teeth which are already carious.

The adapted K-file developed for this study undoubtedly enabled caries free fissures to be sampled to a greater depth than is possible with hypodermic needles(Figure 7). From the information provided in the Australian Standards for hypodermic needles(2) and root canal files and reamers(1), it can be calculated that one of the most commonly used implements for fissure sampling, a twenty-six gauge hypodermic needle, would penetrate a fissure of 90 μm width to 206.55 μm (2), whereas an 06 K-file, which is 90 μm wide, has the potential to penetrate to a depth of 1500 μm (1).

The value of the K-file sampling method is clearly shown in the findings from this investigation. Despite the fact that the area of the fissure to be sampled had numbers of bacteria removed by the needle scraping method first, it was still possible in every subject to obtain additional bacteria directly below the area of needle scraping(Appendix B-F). There was up to a 154-fold increase in the total number of colony forming units when the samples obtained by the K-file sampling technique were added to those obtained by needle sampling. Similar results were obtained by Meiers and Schachtele(119) when they compared needle scrapings with their total fissure removal technique. Another feature of

the K-file sampling technique was that as the K-file rotated clockwise the contents could actually be seen clinging to the file as they were withdrawn from the depths of the sampling site.

It is not possible to compare total bacterial counts between this and other studies because of the varying techniques of plaque collection and because of difficulties of expressing results in a standard manner such as colony forming units per weight of sample collected. In this study counts of total bacteria (needle + reamer samples) collected from control teeth sites ranged from 10^4 to 10^7 cfu. Needle samples from control teeth yielded 10^2 to 10^7 cfu which is similar to counts in other studies in which needle samples were used (21,105,109,119). A feature of the results presented in Tables 3-9 and Appendices B-F is the extremely wide intersample variation in the number of organisms collected. Bacterial counts varied from no colonies detected to 10^5 cfu on some of the selective media, and from 10^2 to 10^7 cfu on the blood agar. This is consistent with other studies (119,120,180) which have also observed the same phenomenon. This suggests that each fissure system is a unique ecological unit in itself, and many factors play a role in the status of an individual fissure. The cultivable bacteria obtained showed no species variation between the needle and reamer samples from one site and indicated an even distribution of the different species of bacteria throughout the fissure. This is in contrast to the work of Meiers and Schachtele (118) who found higher levels of lactobacilli in the depths of the fissures. However it should be noted that these workers used fissures with incipient lesions and

their findings regarding the numbers of lactobacilli present may have been an indication of the carious nature of the pits and fissures.

Streptococci were present in all samples taken from pit and fissure systems but generally constituted a smaller proportion of the total flora than other workers have found(119,120,180).

Streptococcus mutans was present in all but three samples but again in lower proportions than found by other workers(119, 120,180). However this may be a reflection of the fact that all teeth utilised in this study were non-carious and were in mouths of patients with very low caries activity. Other studies have generally utilised either incipient carious fissures(119,120) or placed third molar fissures in large restorations(181) which probably exist because of a previously high caries activity. Theilade et al.(181) found that S. mutans was rarely found in the fissures of Danish dental students with low caries activity while Thott et al.(191) found these organisms in all mouths and most fissures of their subjects. In fact, in a recent study by Lang et al.(97) S. mutans could not be isolated in any samples taken from the fissures of caries free individuals and its levels were extremely low in individuals with low caries activity.

Lactobacilli were cultivated from only six of the twenty control teeth and, except in three individuals who had very poor oral hygiene, the numbers were very small. This very small sample made it impossible to draw any significant conclusions about the ecology of lactobacilli and was possibly a reflection of the low

caries activity status of the majority of the individuals in this trial. In other studies lactobacilli have only occurred in substantial numbers in association with caries activity(81,105,120).

The majority of untreated non-carious fissures tested with the BCP/sucrose indicator gave an acidic response very rapidly. A very small number of individuals had fissures which gave only a non-acidic response in the initial screening of the patients. These facts perhaps indicate that the plaque in the fissure often contains a large proportion of highly acidogenic organisms. This view is supported by the work of Thott et al.(191) who found that 74% of the organisms from implanted third molar fissures grown on haemolysed blood plates were acidogenic(pH<5.2).

No significant differences between test and control fissures could be found on the selective media for lactobacilli or streptococci. However, when total colony forming units grown on 5% sheeps' blood agar were considered, the test teeth receiving four minute treatments grew significantly more bacteria. The reason for these apparently anomalous findings is not known. Even though contralateral teeth were utilised as test and control, the problem of inter-site sample variation may have obscured the effects of the treatment. However, recent work(153,154,171) on the action of antibacterial agents in fissures has shown that the effects appear to be short lived without continued application of the antimicrobial agent.

Schaeken et al.(154) found that topical applications of chlorhexidine gel(5% chlorhexidine digluconate in 4% methyl cellulose gel) or 8% SnF₂ applied once, reduced S. mutans in pooled fissure plaque samples for seven days but that it had returned to base levels by twenty-one days. In the study of Schaeken et al.(154), rinsing with chlorhexidine or flossing with chlorhexidine impregnated floss at home after the topical applications extended the period of suppression of S. mutans for as long as these procedures were followed. The rapid return to base level was despite the fact that quantities of plaque had been removed from the fissure sample sites prior to treatment to establish base levels of bacterial flora. In an earlier study by Schaeken et al.(153) in which the effects of topical applications of 5% chlorhexidine gel or 2% iodine on S. mutans levels in plaque were studied, the fissure levels of this bacteria in both groups were significantly reduced two days after treatment. However the reduction in the S. mutans levels was not significant seven days after the cessation of treatment in the iodine group and twenty-one days after cessation of treatment in the chlorhexidine group. In this study chlorhexidine was eliminated when all eighteen teeth treated with it gave an acidic reaction to the BCP/sucrose indicator seven days after treatment. In contrast teeth treated with the iodine preparation gave a non-acidic reaction to the indicator seven days after treatment in eleven of the eighteen teeth, but all teeth gave an acidic reaction when tested after six weeks.

Svanberg and Westgren(171), similar to Schaeken et al.(154), found that topical applications of 8% SnF_2 reduced significantly but did not eliminate S. mutans in both smooth surface and fissure plaques one day after treatment. However, four weeks after cessation of the topical applications colonisation by S. mutans had returned to pretreatment levels in the fissure plaque but was still significantly reduced in smooth surface plaque. In this study, thirteen of the eighteen teeth treated with 10% SnF_2 gave a non-acidic reaction to the BCP/sucrose indicator seven days after treatment but all eighteen teeth gave an acidic reaction six weeks after treatment.

Loesche et al.(110) had very promising results with APF gel containing 1.23% fluoride ion and reported a direct effect on plaque levels of S. mutans in pooled occlusal samples. The effect could be detected up to twelve weeks after treatment. However, in this study APF gel was eliminated after seven days, when an acidic reaction was obtained with the BCP/sucrose indicator in all eighteen teeth treated with APF gel.

The slow release preparations(AgF/AgI and AgI) tested in this study demonstrated some improved efficacy in inhibiting acid production in pits and fissures. Seven days after treatment fourteen of the eighteen teeth treated with AgF/AgI gave a non-acidic reaction to the BCP/sucrose indicator, while ten of the eighteen teeth treated with AgI gave a non-acidic reaction. In addition, twelve weeks after treatment six of the teeth treated with AgF/AgI still gave a non-acidic reaction and three of the

teeth treated with AgI. These effects may have been because of a more prolonged direct antibacterial action on acid producing organisms because of the retention of AgF/AgI or AgI compounds in the plaque. The very low solubility of AgI, permitting the slow release of silver over a period of time, may have further enhanced this antibacterial action. Nevertheless false non-acidic readings may have occurred because of the black staining of some fissures caused by the silver ions which made non-acidic readings(purple) very difficult to discern.

The penetration and diffusion of substances into fissures is severely limited and much slower than in smooth surface plaque. This is dramatically illustrated in a study by Newman and Wilson(131), where, in the fissure plaques of freshly extracted premolar teeth only the most superficial layers were labelled by the thymidine and thymine compounds after incubation at 37°C for varying periods of time ranging from 0.25 hours to 72 hours. Whereas, in the smooth surface plaques, distribution of the compounds was uneven but occurred throughout the plaques after some hours of incubation. Penetration of substances through smooth surface plaque and presumably through plaque in pits and fissures is largely by diffusion and its rate depends on the porosity of the plaque(121,178). This porosity is affected by the bacterial density, extracellular matrix and the amount of calcium bridging(121,178,207,208). The presence of plaque within the fissures will undoubtedly modify any capillary action which may occur in fissure systems(133). The calcifications and dead

cells which occur in the depths of fissure plaques may act as diffusion barriers(17).

The AgF/AgI preparation used in this study probably caused initial sterilisation of the superficial layers of the fissure but recolonisation occurred rapidly either from other areas of the mouth or from the deeper layers of the fissure which were not penetrated by the antimicrobial agent. It is interesting to speculate that with the death of the superficial bacteria there was an opportunity within the fissures for deeper bacteria to increase their numbers. In addition an antibacterial substance may kill or reduce the activity and levels of bacteria which inhibit organisms such as S. mutans and lactobacilli thereby permitting them to increase their numbers. Because of the small numbers of lactobacilli collected in this study and others(118,179,180) it is impossible to draw any definitive conclusions about the ecological importance of lactobacilli in the fissure. The observation that, within a short time after treatment with a highly bactericidal substance, the microbial flora of the fissure has returned to base levels supports the suggestion of Svanberg and Loesche(169) that a fissure forms a closed system and that after it has been colonised antimicrobial therapies may have little effect on the bacterial flora.

A more prolonged antibacterial effect could perhaps be obtained by better penetration of the fissure plaque by antimicrobial agents. However because of the difficulty in altering the fissure flora it appears that antibacterial treatments should be

directed against specific bacteria at the time of tooth eruption to prevent initial colonisation by cariogenic bacteria. If complete sterilisation of the fissure could be obtained and suppression of S. mutans or other unwanted bacteria in other areas of the mouth could be achieved, it might be possible to obtain recolonisation of a fissure with a non-cariogenic flora. In two studies(97,105), significantly higher levels of S. mutans were found six to twelve months prior to a clinical diagnosis of caries in a fissure. It is therefore possible that antibacterial treatments at regular time intervals could prevent rises in the level of this aciduric microorganism and the consequent creation of an environment suitable for the initiation of dental caries.

Further investigation of slow release chemical agents and/or delivery systems which would prolong antibacterial action against cariogenic bacteria may be of value. The successes in recent years of some slow-release delivery systems in dentistry may offer potential(126,151,158,194). Another possible mechanism of increasing the resistance of a fissure to caries could be to increase plaque mineralisation, providing not only a potential reservoir of fluoride ions and various cations which could aid in the protection of the enamel surface during periods of cariogenic challenge, but perhaps also providing a calcified 'plug' which would seal the fissure system. The dynamic ecosystem of the fissure is very poorly understood and studies need to be conducted on the relationships between bacterial types and the metabolic changes occurring within the fissures. In order that such experiments may be usefully carried out highly sophisticated

instruments and sampling techniques will have to be developed to provide data from the depths of the fissure.

SUMMARY AND CONCLUSIONS

This study was undertaken to evaluate some of the effects of a number of antimicrobial agents on the bacteria of the dental plaque in the pit and fissure systems of teeth. The pit and fissure systems of ninety permanent second molar teeth in forty-seven patients were treated with topical applications of either 0.5% chlorhexidine in alcohol, 40% AgF, a solution of 0.8% I_2 -KI, 5.2% $CuSO_4$ in 1.5% ethyl alcohol, acidulated phosphate fluoride gel(containing 1.23% fluoride ion) or 10% SnF_2 . Subsequently the teeth were tested with an acid-base colour indicator consisting of 0.37% BCP and 10% sucrose(BCP/sucrose), seven days, six weeks, and twelve weeks after treatment or until an acidic reaction reoccurred. Some of the teeth treated with agents containing heavy metals gave non-acidic reactions at seven days, however all the treated fissures gave an acidic reaction at six weeks.

In an endeavour to prolong the period of acid inhibition, the fissures of a further thirty-six permanent second molar teeth were treated with a sparingly soluble salt, AgI, either alone or in conjunction with AgF, and then tested with the BCP/sucrose indicator seven days, six weeks, and twelve weeks after treatment or until an acidic reaction reoccurred. Both preparations extended the inhibitory period, with nine of the thirty-six teeth still giving a non-acidic reaction at twelve weeks.

Consequently, a microbial study of the pit and fissure systems of another twenty pairs of contralateral teeth was undertaken to ascertain if the AgF/AgI preparation may have had an inhibitory effect on total recoverable organisms, total streptococci, Streptococcus mutans, and lactobacilli. One tooth of each pair of teeth was selected to receive the test agent and the other was used as control. Each fissure system was sampled with a twenty-six gauge hypodermic needle and then with a core sampling instrument, developed from an 06 endodontic K-file, especially for sampling the depths of pit and fissure systems. The samples were processed and grown anaerobically on selective and non-selective media.

No significant antibacterial effect from the AgF/AgI treatment was found either in the total bacterial counts on selective media or in the counts for specific bacteria on selective media.

The core sampling technique enabled the collection of substantial numbers of additional bacteria from the depths of the fissure after it had been initially sampled by the more widely used method of scraping a twenty-six gauge hypodermic needle across the fissure opening. Samples collected by either method showed enormous intersite variation.

APPENDICES

Appendix A. Sensitivity of Plaque Microbes to Bromcresol Purple(colony forming units/milligram[cfu/mg] of plaque, grown on 5% Sheep Blood Agar).

Test(T) (cfu/mg)	Control(C) (cfu/mg)	Difference (T - C)
6.2×10^7	4.6×10^7	$+1.6 \times 10^7$
1.0×10^7	2.6×10^7	-1.6×10^7
1.3×10^7	8.2×10^7	-6.9×10^7
1.6×10^7	1.4×10^7	$+0.2 \times 10^7$
1.6×10^7	1.7×10^7	-0.1×10^7
7.7×10^7	2.3×10^7	$+5.4 \times 10^7$
3.5×10^7	4.6×10^7	-1.1×10^7
1.3×10^7	1.5×10^7	-0.2×10^7
3.6×10^7	1.6×10^7	$+2.0 \times 10^7$
2.3×10^7	2.6×10^7	-0.3×10^7

Differences not significant at the 0.05 level(T=27.5, Wilcoxon's Signed Rank Test for paired observations).

Appendix B. Total Bacteria Recovered from Fissures on 5% Sheep Blood Agar(expressed as colony forming units--cfu).

Four Minute Treatments					Sixty Minute Treatments				
Subject	Needle Samples		K-file Samples		Subject	Needle Samples		K-file Samples	
	Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)		Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)
SC	7.8 x 10 ⁶	6.8 x 10 ⁵	5.2 x 10 ⁵	9.6 x 10 ⁴	TA	2.8 x 10 ⁶	2.6 x 10 ³	3.8 x 10 ⁵	4.0 x 10 ⁵
IC	2.2 x 10 ⁶	1.7 x 10 ⁵	8.0 x 10 ⁶	4.4 x 10 ⁴	RAB	3.6 x 10 ⁵	1.1 x 10 ⁵	2.0 x 10 ⁴	7.8 x 10 ³
CG	6.2 x 10 ⁴	4.2 x 10 ⁴	5.8 x 10 ⁴	1.2 x 10 ⁴	RJB	1.7 x 10 ⁶	7.2 x 10 ²	2.2 x 10 ⁵	4.0 x 10 ⁴
DJ	4.0 x 10 ⁶	1.4 x 10 ⁶	2.0 x 10 ⁵	2.8 x 10 ⁶	TD	3.8 x 10 ⁴	4.0 x 10 ²	2.2 x 10 ⁴	6.0 x 10 ⁴
MK	3.2 x 10 ⁵	5.6 x 10 ⁵	3.0 x 10 ⁵	4.4 x 10 ⁴	JH	2.2 x 10 ⁶	9.6 x 10 ⁶	4.0 x 10 ⁶	9.8 x 10 ⁵
DM	1.9 x 10 ⁵	6.8 x 10 ⁵	1.3 x 10 ⁶	1.6 x 10 ⁵	JN	2.8 x 10 ⁵	5.4 x 10 ⁶	3.2 x 10 ⁵	1.6 x 10 ⁵
AN	2.0 x 10 ⁶	3.4 x 10 ⁴	1.8 x 10 ⁶	1.7 x 10 ⁴	JOP	7.0 x 10 ⁴	1.7 x 10 ⁵	2.4 x 10 ⁵	9.6 x 10 ³
SP	9.4 x 10 ⁵	5.4 x 10 ⁴	2.0 x 10 ⁶	1.8 x 10 ⁵	GS	1.2 x 10 ⁵	4.0 x 10 ⁴	1.2 x 10 ⁴	7.6 x 10 ⁴
ST	4.6 x 10 ⁶	1.0 x 10 ⁷	3.8 x 10 ⁶	1.6 x 10 ⁶	HS	9.8 x 10 ⁵	1.0 x 10 ⁴	6.0 x 10 ⁵	1.5 x 10 ³
SW	1.6 x 10 ⁷	2.2 x 10 ⁶	1.9 x 10 ⁶	7.8 x 10 ⁶	JAP	5.4 x 10 ⁵	8.8 x 10 ⁶	8.4 x 10 ⁴	2.4 x 10 ⁶

Appendix C. Streptococci Recovered from Fissures on MSA Agar(expressed as colony forming units--cfu).

Four Minute Treatments					Sixty Minute Treatments				
Subject	Needle Samples		K-file Samples		Subject	Needle Samples		K-file Samples	
	Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)		Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)
SC	1.3 x 10 ⁵	2.4 x 10 ³	5.6 x 10 ³	1.6 x 10 ²	TA	1.4 x 10 ⁵	2.4 x 10 ²	4.8 x 10 ⁴	2.4 x 10 ⁴
IC	9.2 x 10 ⁴	2.6 x 10 ³	1.6 x 10 ⁵	2.2 x 10 ³	RAB	1.5 x 10 ³	1.5 x 10 ⁴	3.6 x 10 ³	3.4 x 10 ³
CG	1.9 x 10 ⁴	1.6 x 10 ⁴	1.2 x 10 ⁴	2.4 x 10 ³	RJB	1.3 x 10 ⁵	8.0 x 10	2.0 x 10 ⁴	1.4 x 10 ³
DJ	7.4 x 10 ³	1.6 x 10 ⁵	3.0 x 10 ³	1.6 x 10 ⁵	TD	1.3 x 10 ⁴	8.0 x 10	1.9 x 10 ³	1.2 x 10 ⁴
MK	1.4 x 10 ³	1.6 x 10 ⁵	5.8 x 10 ³	2.0 x 10 ⁴	JH	5.2 x 10 ⁴	2.4 x 10 ⁴	3.6 x 10 ⁴	1.0 x 10 ⁴
DM	5.4 x 10 ⁴	7.6 x 10 ⁴	5.0 x 10 ⁴	2.6 x 10 ³	JN	1.6 x 10 ⁴	1.6 x 10 ⁵	6.0 x 10 ⁴	2.2 x 10 ³
AN	7.2 x 10 ⁴	6.0 x 10 ²	5.4 x 10 ⁴	2.4 x 10 ²	JOP	7.8 x 10 ²	5.8 x 10 ³	3.4 x 10 ³	ND
SP	3.2 x 10 ⁴	2.8 x 10 ³	2.4 x 10 ⁴	3.2 x 10 ³	GS	2.0 x 10 ³	8.0 x 10 ³	ND	1.4 x 10 ⁴
ST	1.6 x 10 ⁵	1.6 x 10 ⁵	6.0 x 10 ⁴	6.4 x 10 ⁴	HS	4.0 x 10 ⁴	8.0 x 10	1.1 x 10 ⁵	ND
SW	1.6 x 10 ⁵	1.6 x 10 ⁵	1.6 x 10 ⁵	1.6 x 10 ⁵	JAP	1.6 x 10 ⁴	1.6 x 10 ⁵	3.2 x 10 ³	1.6 x 10 ⁵

ND No bacteria detected at the lowest dilution.

Appendix D. Streptococcus mutans Recovered from Fissures on MSB Agar(expressed as colony forming units--cfu).

Four Minute Treatments				Sixty Minute Treatments					
Subject	Needle Samples		K-file Samples		Subject	Needle Samples		K-file Samples	
	Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)		Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)
SC	3.2 x 10 ³	1.4 x 10 ³	1.2 x 10 ²	4.0 x 10 ²	TA	2.6 x 10 ³	2.0 x 10 ²	4.4 x 10 ²	7.6 x 10 ²
IC	1.5 x 10 ³	6.8 x 10 ²	1.4 x 10 ⁵	1.1 x 10 ³	RAB	ND	1.2 x 10 ²	ND	1.2 x 10 ²
CG	1.6 x 10 ²	1.2 x 10 ²	2.4 x 10 ²	1.6 x 10 ²	RJB	2.2 x 10 ⁴	4.0 x 10	2.0 x 10 ³	2.2 x 10 ³
DJ	9.4 x 10 ³	5.4 x 10 ³	1.2 x 10 ³	1.6 x 10 ⁵	TD	1.0 x 10 ³	4.0 x 10	3.0 x 10 ³	9.8 x 10 ³
MK	ND	1.7 x 10 ³	ND	8.0 x 10	JH	3.2 x 10 ²	8.0 x 10	1.2 x 10 ²	ND
DM	6.0 x 10 ³	1.9 x 10 ³	3.2 x 10 ³	4.8 x 10 ³	JN	2.0 x 10 ²	4.4 x 10 ⁴	2.4 x 10 ²	1.1 x 10 ³
AN	2.2 x 10 ⁴	4.0 x 10	1.2 x 10 ³	ND	JOP	6.0 x 10 ¹	ND	ND	ND
SP	1.3 x 10 ⁴	2.6 x 10 ³	2.8 x 10 ⁴	1.6 x 10 ³	GS	5.0 x 10 ³	7.0 x 10 ³	1.2 x 10 ³	1.4 x 10 ⁴
ST	3.6 x 10 ⁴	2.6 x 10 ⁴	7.0 x 10 ⁴	3.6 x 10 ³	HS	2.0 x 10 ⁴	ND	8.6 x 10 ⁴	ND
SW	5.0 x 10 ⁴	2.8 x 10 ³	2.4 x 10 ³	8.0 x 10 ³	JAP	2.4 x 10 ²	3.8 x 10 ⁴	4.0 x 10 ²	3.8 x 10 ³

ND No bacteria detected at the lowest dilution.

Appendix E. Lactobacilli Recovered from Fissures on LBS Agar(expressed as colony forming units--cfu).

Four Minute Treatments				Sixty Minute Treatments					
Subject	Needle Samples		K-file Samples		Subject	Needle Samples		K-file Samples	
	Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)		Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)
SC	1.3 x 10 ⁵	1.6 x 10 ²	7.2 x 10 ³	ND	TA	1.6 x 10 ²	ND	ND	ND
IC	2.0 x 10 ⁴	ND	2.4 x 10 ⁴	ND	RAB	ND	ND	ND	ND
CG	ND	ND	ND	ND	RJB	2.4 x 10 ²	ND	ND	ND
DJ	1.7 x 10 ³	4.4 x 10 ⁴	4.6 x 10 ⁴	1.8 x 10 ⁴	TD	ND	ND	2.4 x 10 ³	6.6 x 10 ³
MK	ND	ND	ND	ND	JH	2.6 x 10 ⁴	ND	8.4 x 10 ⁴	ND
DM	ND	ND	ND	ND	JN	ND	4.0 x 10	ND	ND
AN	ND	ND	6.0 x 10 ³	ND	JOP	ND	ND	2.4 x 10 ²	ND
SP	4.0 x 10	4.0 x 10	8.0 x 10	ND	GS	ND	ND	ND	ND
ST	2.0 x 10 ²	1.2 x 10 ²	8.8 x 10 ²	ND	HS	1.6 x 10 ²	ND	7.6 x 10 ²	ND
SW	ND	ND	ND	ND	JAP	ND	4.0 x 10 ³	4.0 x 10	6.6 x 10 ³

ND No bacteria detected at the lowest dilution.

Appendix F. Lacatobacilli Recovered from Fissures on LBS-TJ Agar(expressed as colony forming units--cfu).

Four Minute Treatments						Sixty Minute Treatments			
Subject	Needle Samples		K-file Samples		Subject	Needle Samples		K-file Samples	
	Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)		Test (cfu)	Control (cfu)	Test (cfu)	Control (cfu)
SC	1.5 x 10 ⁵	1.2 x 10 ²	8.4 x 10 ³	ND	TA	3.2 x 10 ²	ND	8.0 x 10	ND
IC	4.8 x 10 ³	ND	3.4 x 10 ⁴	ND	RAB	ND	6.0 x 10	ND	9.6 x 10 ²
CG	ND	ND	ND	ND	RJB	4.0 x 10 ³	ND	8.0 x 10	ND
DJ	5.0 x 10 ³	1.2 x 10 ⁵	1.1 x 10 ⁵	1.7 x 10 ⁴	TD	ND	ND	3.0 x 10 ³	6.2 x 10 ³
MK	ND	ND	ND	ND	JH	1.6 x 10 ⁵	ND	1.3 x 10 ⁵	ND
DM	ND	ND	ND	ND	JN	ND	ND	ND	ND
AN	ND	ND	1.1 x 10 ⁴	ND	JOP	ND	ND	ND	ND
SP	8.0 x 10	ND	2.0 x 10 ²	ND	GS	ND	ND	ND	ND
ST	8.0 x 10	4.0 x 10	1.2 x 10 ²	ND	HS	1.2 x 10 ²	ND	9.2 x 10 ²	ND
SW	ND	ND	ND	ND	JAP	ND	3.0 x 10 ³	ND	4.2 x 10 ³

ND No bacteria detected at the lowest dilution.

BIBLIOGRAPHY

1. AUSTRALIAN STANDARD 1411-1976. Dental Stainless Steel Root Canal Files and Reamers. Standards Association of Australia, 1976.
2. AUSTRALIAN STANDARD 1946-1984. Hypodermic Equipment-- Single-use Needles(Sterile for General Medical Use. Standards Association of Australia, 1984.
3. AWAZAWA Y. Electron Microscopic Study on the Hypomineralized Enamel Areas Descending from the Floors of Occlusal Fissures toward the Amelo-dentinal Junctions. J Nihon Univ Sch Dent 1966; 8:33-36.
4. AWAZAWA Y. Re-examination of the Morphology of Caries Susceptible Grooves in the Premolar and Molar. J Nihon Univ Sch Dent 1969; 11:1-15.
5. AWAZAWA Y. Values of Measurement of the Developmental Groove in the Third Molar. IRCS Medical Science: Dentistry and Oral Biology 1976; 4:223-224.
6. BAILEY N.T.J. Statistical Methods in Biology. 2nd Ed. London: Hodder & Stoughton, 1981.
7. BALENSFEIN J.W., MADONIA J.V. Study of Dental Plaque in Orthodontic Patients. J Dent Res 1970; 49:320-324.
8. BEIGHTON D., RIPPON H.R. The Distribution of Streptococcus mutans Serotypes and Dental Caries in a Group of 5- to 8-year-old Hampshire Schoolchildren. Br Dent J 1987; 162:103-106.
9. BEIRAGHI S., KATZ S., BEISWANGER B.B. In vitro Model for Pit and Fissure caries. Pediatr Dent 1987; 2:131-135.
10. BERGMAN G. The Plaque Test on Teeth Topically Treated with Sodium Fluoride. Acta Odontol Scand 1953; 10:111-117

11. BERRY B.W., HENRY C.A. The Effect of Adsorption on the Acid Production of Caries and Noncaries-producing Streptococci. J Dent Res 1977; 56:1193-1200
12. BLACK G.V. A Work on Operative Dentistry. Volume I. Chicago: Medico-Dental Publishing, 1908.
13. BLOCK S.S. Disinfection, Sterilization and Preservation. 3rd Ed. Philadelphia: Lea & Febiger, 1983.
14. BOHANNAN H.M. Caries Distribution and the Case for Sealants. J Public Health Dent 1983;43:200-204.
15. BOSSERT W.A. The Relationship Between the Shape of the Occlusal Surfaces of Molars and the Prevalence of Decay. J Dent Res 1933; 13:125-128.
16. BOWDEN G.H., HARDIE J.M., SLACK G.L. Microbial Variations in Approximal Dental Plaque. Caries Res 1975; 9:253-277.
17. BOWDEN G.H., NASH R., SPIERS R.L. The Localization and Retention of ^{32}P and ^{45}C with Surface Deposits of Streptococcus mutans and the Influence of such Deposits on the Release of these Isotopes from Enamel. Caries Res 1973; 7:185-200.
18. BOWEN W.H. Effect of Restricting Oral Intake to Invert Sugar or Casein on the Microbiology of Plaque in Macaca fascicularis(irus). Arch Oral Biol 1974; 19:231-235.
19. BRATTHALL D., SERINIRACH R., CARLSSON P., LEKFUANGFU S. Streptococcus mutans and Dental Caries in Urban and Rural Schoolchildren in Thailand. Community Dent Oral Epidemiol 1986; 14:274-276.
20. BROWN L.R., BILLINGS R.J., KASTER A.G. Quantitative Comparisons of Potentially Cariogenic Microorganisms Cultured from Noncarious and Carious Root and Coronal Tooth Surfaces. Infect Immun 1986; 51:765-770.
21. BURT B.A., LOESCHE W.J., EKLUND S.A., EARNEST R.W. Stability of Streptococcus mutans and Its Relationship to Caries in a Child Population over 2 Years. Caries Res 1983; 17:532-542.

22. CAREY C., GREGORY T., RUPP W., TATEVOSSIAN A., VOGEL G.L.
The Driving Forces in Human Dental Plaque Fluid for
Demineralisation and Remineralisation of Enamel Mineral.
In: Factors Relating to Demineralisation and
Remineralisation of Teeth. Ed. Leach, IRL Press,
1985:163-173.
23. CARLSSON J., FROLANDER F., SANDQUIST G. Oxygen Tolerance
of Anaerobic Bacteria Isolated from Necrotic Dental
Pulps. Acta Odont Scand 1977; 35:139-145.
24. CARLSSON J., GRIFFITHS C.J. Fermentation Products and
Bacterial Yields in Glucose-limited and Nitrogen-limited
Cultures of Streptococci. Arch Oral Biol 1974;
19:1105-1109.
25. CARLSSON P., GANDOUR I.A., OLSSON B., RICKARDSSON B.,
ABBAS K. High Prevalence of Mutans streptococci in a
Population with Extremely Low Prevalence of Dental
Caries. Oral Microbiol Immunol 1987; 2:121-124.
26. CAULFIELD P.W. Combined Effect of Iodine and Sodium
Fluoride on Dental caries in Rats and on Viability of
Streptococcus mutans in vitro. Caries Res 1981; 15:484-
491.
27. CAULFIELD P.W., GIBBONS, R.J. Suppression of
Streptococcus mutans in the Mouths of Humans by a Dental
Prophylaxis and Topically-applied Iodine. J Dent Res
1979; 58:1317-1326.
28. CAULFIELD P.W., NAVIA J.M. Antimicrobial Agents for
Management of Caries. In: The Biologic Basis of Dental
Caries. Ed. Menaker, U.S.A.: Harper & Row, 1980.
29. CHEMICAL ABSTRACTS: New Patents. High Purity Metal
Pyrophosphates. 1968; 69:98099m.
30. CRABB H.S.M. The Porous Outer Enamel of Unerupted Human
Premolars. Caries Res 1976; 10:1-7.
31. CRABB H.S.M. Fissures at Risk. Br Dent J 1976;
140:303-307.

32. CRAIG G.G. A Useful Method for the Topical Application of Fluorides. Dent Outlook 1975; 1:4-5.
33. CRAIG G.G., BURTON V.J. Pattern of Dental caries in 12-year-old Sydney Schoolchildren. Aust Dent J 1985; 30:128-130.
34. DAHL E., SILVERSTONE L.M. An in vitro Study of the Histopathology of Fissure Caries Using both Artificial Caries and Artificial Fissure Techniques. J Dent Res 1979; 58(Abs.No. 287):165.
35. DAVEY A.L., ROGERS A.H. Multiple Types of the Bacterium Streptococcus mutans in the Human Mouth and their Intra-family Transmission. Arch Oral Biol 1984; 29:453-460.
36. DAWES E.A. Endogenous Metabolism and The Survival of Starved Prokaryotes. In: The Survival of Vegetative Microbes. Eds. Gray, Postgate. Symp Soc Gen Micro 1976; 26:19-53.
37. DE LIEFDE B. A Study of the Chemical Treatment of Early Caries of Occlusal Pits and Fissures. NZ Dent J 1987; 83:10-12.
38. DISTLER W., KRONCKE A. The Acid Pattern in Human Dental Plaque. J Dent Res 1983; 62:87-91.
39. DUCHIN S., VAN HOUTE J. Colonization of Teeth in Humans by Streptococcus mutans as Related to Its Concentration in Saliva and Host Age. Infect Immun 1978; 20:120-125.
40. DUCHIN S., VAN HOUTE J. Relationship of Streptococcus mutans and Lactobacilli to Incipient Smooth Surface Dental Caries in Man. Arch Oral Biol 1978; 23:779-786.
41. EKLUND S.A., ISMAIL A.I. Time of Development of Occlusal and Proximal Lesions: Implications for Fissure Sealants. J Public Health Dent 1986; 46:114-121.
42. ELLEN R.P. Establishment and Distribution of Actinomyces viscosus and Actinomyces naeslundii in the Human Oral Cavity. Infect Immun 1976; 14:1119-1124.

43. ELLEN R.P., FILLERY E.D., BANTING D.W. Comparison of Selective Broth and Plating Methods for Isolation of Streptococcus mutans from Root Surface Dental Plaques. J Clin Microbiol 1980; 11:205-208.
44. FARDAL O., TURNBULL R.S. A Review of the Literature on Use of Chlorhexidine in Dentistry. JADA 1986; 112:863-869.
45. FEJERSKOV O., MELSEN B., KARRING T. Morphometric Analysis of Occlusal Fissures in Human Premolars. Scand J Dent Res 1973; 81:505-510.
46. FEJERSKOV O., SILNESS J., KARRING T., LOE H. The Occlusal Fissure of Unerupted Third Molars as an Experimental Caries Model in Man. Scand J Dent Res 1976; 84:142-149.
47. FIRESTONE A.R. Human Interdental Plaque-pH Data and Rat Caries Tests: Results with the Same Substances. J Dent Res 1982; 61:1130-1136.
48. FITZGERALD R.J., JORDAN H.V., ARCHARD H.O. Dental Caries in Gnotobiotic Rats Infected with a Variety of Lactobacillus acidophilus. Arch Oral Biol 1966; 11:473-476.
49. FITZGERALD R.J., KEYES P.H. Demonstration of the Etiologic Role of Streptococci in Experimental Caries in the Hamster. JADA 1960; 61:9-19.
50. FRANK R.M. Microscopie Electronique de la Carie des Sillons Chez L'Homme. Arch Oral Biol 1973; 18:9-25.
51. FUSYAMA T. Diagnosis and Distribution of Pits and Fissures. J Pros Dent 1964; 14:117-126.
52. GALIL K.A., GWINNETT A.J. Scanning Electron Microscopy: Observations on Occlusal Human Dental Plaque. J Can Dent Assoc 1973; 39:472-475.
53. GALIL K.A., GWINNETT A.J. Three-dimensional Replicas of Pits and Fissures in Human Teeth: Scanning Electron Microscopy Study. Arch Oral Biol 1975; 20:493-495.

54. GALIL K.A., GWINNETT A.J. Histology of Fissures in Human Unerupted Teeth. J Dent Res 1975; 54:960-964.
55. GALIL K.A., GWINNETT A.J. Human Tooth-fissure Contents and their Progressive Mineralization. Arch Oral Biol 1975; 20:559-562.
56. GASTRIN B., KALLINGS L.O., MARCETIC A. The Survival Time for Different Bacteria in Various Transport Media. Acta Pathol Microbiol Scand 1968; 74:371-379.
57. GAUNT W.A., OSBORN J.W., TEN CATE A.R. Advanced Dental Histology. 2nd Ed. Bristol: John Wright & Sons Ltd., 1971.
58. GEDDES D.A.M. Acids Produced by Human Dental Plaque Metabolism in situ. Caries Res 1975; 9:98-109.
59. GEDDES D.A.M. Current View of Plaque Acidogenicity. In: Cariology Today. Int Congr Zurich 1983:199-204.
60. GEDDES D.A.M., WEETMAN D.A. Acid Anion Profiles in Dental Plaque. Caries Res 1983; 17(Abs.No. 47):173.
61. GIBBONS R.J., DEPAOLA D.M., SPINELL D.M., SKOBE Z. Interdental Localization of Streptococcus mutans as Related to Dental Caries Experience. Infect Immun 1974; 9:481-488.
62. GIBBONS R.J., VAN HOUTE J. Bacterial adherence in Oral Microbiology. Ann Rev Microbiol 1975; 29:19-44.
63. GILLINGS B., BUONOCORE M. Thickness of Enamel at the Base of Pits and Fissures in Human Molars and Bicuspid. J Dent Res 1961; 40:119-133.
64. GJERMO P., BONESVOLD P., ROLLA G. Relationship Between Plaque-inhibiting Effect and Retention of Chlorhexidine in the Human Oral Cavity. Arch Oral Biol 1974; 10:1031-1034.
65. GOLD O.G., JORDAN H.V., VAN HOUTE J. A Selective Medium for Streptococcus mutans. Arch Oral Biol 1973; 18:1357-1364.

66. GROENEVELD A. Clinical Observations on Caries and Water Fluoridation. In: Tooth Development and Caries. Vol II. Eds. Driessens, Woltgens. Florida: CRC Press, 1986:147-183.
67. HAMADA S., SLADE H.D. Biology, Immunology, and Cariogenicity of Streptococcus mutans. Microbiol Rev 1980; 44:331-384.
68. HAMILTON I.R. Effects of Fluoride on Enzymatic Regulation of Bacterial Carbohydrate Metabolism. Caries Res 1977; 11(Suppl 1):262-278.
69. HANDLEMAN S.L. Effect of Sealant Placement on Occlusal Caries Progression. Clin Prevent Dent 1982; 4:11-16.
70. HANDLEMAN S.L., MILLS J.R., MEGGO I. A Medium for Differentiating Acidogenic Bacteria. Arch Oral Biol 1968; 13:1187-1196.
71. HANDLEMAN S.L., WASHBURN F., WOPPERER P. Two-year Report of Sealant Effect on Bacteria in Dental Caries. JADA 1976; 93:967-970.
72. HARDIE J.M., BOWDEN G.H. The Microbial Flora of Dental Plaque: Bacterial Succession and Isolation Considerations. In: Microbial Aspects of Dental Caries. Eds. Stiles, Loesche, O'Brien. Sp. Supp. Microbiology Abst 1976; Vol 1:63-87.
73. HARDWICK J.L. A Clinical Assessment of the Accuracy of the Methyl Red Test in Forecasting Caries. Br Dent J 1960; 108:255-259.
74. HARPER D.S., ABELSON D.S., JENSEN M.E. Human Plaque Acidity Models. J Dent Res 1986; 65(Spec Iss):1503-1510.
75. HARPER D.S., GRAY R., LENKE J.W., HEFFERREN J.J. Measurement of Human Plaque Acidity: Comparison of Interdental Touch and Indwelling Electrodes. Caries Res 1985; 19:536-546.

76. HAYES M.L., CARTER E.C., GRIFFITHS S.J. The Acidogenic Microbial Composition of Dental Plaque from Caries-free and Caries-prone People. Arch Oral Biol 1983; 28:381-386
77. HUXLEY H.G. The Histology of Rat Molar Tooth Fissure Plaque. Arch Oral Biol 1971; 16:1311-1328.
78. HUXLEY H.G. The Recovery of Microorganisms from the Fissures of Rat Molar Teeth. Arch Oral Biol 1972; 17:1481-1485.
79. HYATT T.P. A Statistical Study of the Location of Dental Caries Shows the Practical Value of Prophylactic Odontotomy. Dent Digest 1938; 34:235.
80. IKEDA T., SANDHAM H.J. Prevalence of Streptococcus mutans on Various Tooth Surfaces in Negro Children. Arch Oral Biol 1971; 16:1237-1240.
81. IKEDA T., SANDHAM H.J., BRADLEY E.L. Changes in Streptococcus mutans and Lactobacilli in Plaque in Relation to the Initiation of Dental Caries in Negro Children. Arch Oral Biol 1973; 18:555-556.
82. JENKINS G.N. The Physiology of the Mouth. 3rd Ed. Oxford: Blackwell, 1966.
83. JENKINS G.N. Recent Changes in Dental Caries. Br Med J 1985; 291:1297.
84. JENSEN M.E., SCHACHTAELE C.F. Plaque pH Measurement by Different Methods on the Buccal and Approximal Surfaces of Human Teeth after a Sucrose Rinse. J Dent Res 1983; 62:1058-1061.
85. JENSEN Ø.E., HANDLEMAN S.L. Effect of an Autopolymerizing Sealant on Viability of Microflora in Occlusal Dental Caries. Scand J Dent Res 1980; 88:382-388.
86. JUHL M. Localization of Carious Lesions in Occlusal Pits and Fissures of Human Premolars. Scand J Dent Res 1983; 91:251-255.

87. KALBERER P.U., SHROEDER H.E., GUGGENHEIM B.,
MUHLEMANN H.R. The Microbial Colonization in Fissures: A
Morphological and Morphometric Study in Rat Molars. *Helv
Odontol Acta* 1971; 15:1-14.
88. KARRING T., OESTERGAARD E., THEILADE J., LOE H.
Histochemical Study of the Formation of Dental Plaque in
Artificial Fissures. *Scand J Dent Res* 1974; 82:474-483
89. KATZ S., PARK K.K., STOOKEY G.K., SHEMEHORN B.R.
Development and Initial Testing of a Model for in vitro
Formation of Pit and Fissure Caries. *Caries Res* 1986;
20:424-428.
90. KEELE B.B., REEVES M.S., NAVIA J.M. An Improved Medium
for Differentiating Acidogenic Bacteria. *J Dent Res*
1973; 52:1054-1055.
91. KLIENBERG I., CHATTERJEE R., DENEPITIYA L. Effects of
Saliva and Dietary Eating Habits on the pH and
Demineralisation-remineralisation Potential of Dental
Plaque. In: *Demineralisation and Remineralisation of
Teeth*. Eds. Leach, Edgar. IRL Press, 1983:25-50.
92. KLEINBERG I., KANAPKE J.A., CRAW D. Effect of saliva and
Salivary Factors on the Metabolism of the Mixed Oral
Flora. In: *Microbial Aspects of Dental Caries*. Eds.
Stiles, Loesche, O'Brien. IRL Press, 1976; Vol II:433-
464.
93. KOHLER B., EMILSON C.G. Comparison between a Micromethod
and a Conventional Method for Estimation of Salivary
Streptococcus mutans. *Scand J Dent res* 1987;
95:132-135.
94. KONIG K.G. Dental Morphology in Relation to Caries
Resistance with Special Reference to Fissures as
Susceptible Areas. *J Dent Res* 1963; 42:461-476.
95. KORNMAN K.S. Antimicrobial Agents: State-of-the-science
Review. In: *Dental Plaque Control Measures and Oral
Hygiene Practices*. Eds. Loe, Kleinman. Oxford: IRL
Press, 1986:121-142.

96. KRASSE B. The Relationship between Lactobacilli, Candida and Streptococci and Dental Caries. *Odontol Revy* 1954; 5:241-261.
97. LANG N.P., HOTZ P.R., GUSBERTI F.A., JOSS A. Longitudinal Clinical and Microbiological Study on the Relationship between Infection with Streptococcus mutans and the Development of Caries in Humans. *Oral Microbiol Immunol* 1987; 2:39-47.
98. LAPAGE S.P., SHELTON J.E., MITCHELL T.G. Media for the Maintenance and Preservation of Bacteria. In: *Methods in Microbiology*, Vol III. Eds. Norris, Robbins. London: Academic Press, 1970.
99. LEADBETTER E.R., HOLT S.C. Influence of Sonification on Cultivable Flora. *J Dent Res* 1974; Abs. No. 208:106.
100. LINDHE J., AXELSSON P., TOLLSKOG G. Effect of Proper Oral Hygiene on Gingivitis and Dental Caries in Swedish Schoolchildren. *Community Dent Oral Epidemiol* 1975; 3:150-155.
101. LITTLE W.A., KORTS D.C., THOMSON L.A., BOWEN W.H. Comparative Recovery of Streptococcus mutans on Ten Isolation Media. *J Clin Microbiol* 1977; 5:578-583.
102. LOE H. A Review of the Prevention and Control of Plaque In: *Dental Plaque*. Ed. McHugh. Edinburgh: Livingstone, 1970;:259-270.
103. LOE H., KARRING T., THEILADE E. An in vivo Method for the Study of the Microbiology of Occlusal Fissures. *Caries Res* 1973; 7:120-129.
104. LOESCHE W.J. Chemotherapy of Dental Plaque Infections. *Oral Sc Rev* 1976; 9:65-107.
105. LOESCHE W.J., EKLUND S., EARNEST R., BURT B.A. Longitudinal Investigation of Bacteriology of Human Fissure Decay: Epidemiological Studies in Molars Shortly after Eruption. *Infect Immun* 1984; 46:765-772.

106. LOESCHE W.J., HOCKETT R.N., SYED S.A. The Predominant Cultivable Flora of Tooth Surface Plaque Removed from Institutionalized Subjects. Arch Oral Biol 1972; 17:1311-1325.
107. LOESCHE W.J., MURRAY R.J., MELLBERG J.R. The Effect of Topical Acidulated Fluoride on Percentage of Streptococcus mutans and Streptococcus sanguis in Interproximal Plaque Samples. Caries Res 1973; 7:283-296.
108. LOESCHE W.J., ROWAN J., STRAFFON I.H., LOOS P.J. Association of Streptococcus mutans with Human Dental Decay. Infect Immun 1975; 11:1252-1260.
109. LOESCHE W.J., STRAFFON L.H. Longitudinal Investigation of the Role of Streptococcus mutans in Human Fissure Decay. Infect Immun 1979; 26:498-507.
110. LOESCHE W.J., SYED S.A., MURRAY R.J., MELLBERG J.R. Effect of Topical Acidulated Phosphate Fluoride on Percentage of Streptococcus mutans and Streptococcus sanguis in Plaque. Caries Res 1975; 9:139-155.
111. McNAMARA T.F., FRIEDMAN B.K., KLEINBERG I. The Microbial Composition of Human Incisor Tooth Plaque. Arch Oral Biol 1979; 24:91-95.
112. McQUILLEN J.H. Microscopical Fissures in the Masticating Surface of Molars and Bicuspids. Dent Cosmos 1871; 13:300-302.
113. MARIGOLIS H.C., MORENO E.C., MURPHY B.J. Importance of High pKa Acids in Cariogenic Potential of Plaque. J Dent Res 1985; 64:786-792.
114. MARSH P.D., KEEVIL C.W. Environmental Regulation of Glycolysis in Dental Plaque. In: Factors Relating to Demineralisation and Remineralisation of Teeth. Ed. Leach. Oxford: IRL Press, 1986:51-59.
115. MARSH P.D., MARTIN M. Aspects of Microbiology: 1. Oral Microbiology. 2nd Ed. U.K.: Van Nostrand Reinhold, 1984.

116. MATEE M.I., MIKX F.H.M., FRENCKEN J.E.F.N., TRUIN G.J., RUIKEN H.M.H.M. Selection of a Micromethod and Its Use in the Estimation of Salivary Streptococcus mutans and Lactobacillus Counts in Relation to Dental Caries in Tanzanian Children. Caries Res 1985; 19:497-506.
117. MEIERS J.C., JENSEN M.E. Management of the Questionable Carious Fissure: Invasive vs Noninvasive Techniques. JADA 1984; 108:64-68.
118. MEIERS J.C., SCHACHTELE C.F. The Effect of an Antibacterial Solution on the Microflora of Human Incipient Fissure Caries. J Dent Res 1984; 63:47-51.
119. MEIERS J.C., SCHACHTELE C.F. Fissure Removal and Needle Scraping for Evaluation of the Bacteria in Occlusal Fissures of Human Teeth. J Dent Res 1984; 63:1051-1055.
120. MEIERS J.C., WRITHLIN M.R., SHKLAIR I.L. A Microbiological Analysis of Human Early Carious and Non-carious Fissures. J Dent Res 1982; 61:460-464.
121. MELSEN B., KAAE O., ROLLA G., FEJERSKOV O., KARRING T. Penetration of Ions in Human Dental Plaque. Arch Oral Biol 1979; 24:75-81.
122. THE MERCK INDEX. An Encyclopedia of Chemicals, Drugs, and Biologicals. 10th Ed. New Jersey: Merck & Co. Inc., 1983.
123. MINAH G.E., CHU N. Sucrose Metabolism in situ by Dental Plaque in Appliance-borne Bovine Enamel Tooth Fissure Inserts in Man. Arch Oral Biol 1984; 29:467-471.
124. MINAH G.E., KULA K.S., STRASSLER H.E. Microbiological Assessment of Dental Plaque on Bovine Enamel Implants Worn Intra-orally in Children. J Dent Res 1984; 63:1006-1009.
125. MIRTH D.B., SHERN R.J., EMILSON C.G., ADDERLY D.D., SHOU-HUA LI, GOMEZ I.M., BOWEN W.H. Clinical Evaluation of an Intraoral Device for the Controlled Release of Fluoride. JADA 1982; 105:791-797.

126. MODEER T., TWETMAN S., BERGSTRAND F. Three-year Study of the Effect of Fluoride Varnish(Duraphat) on Proximal Caries Progression in Teenagers. Scand J Dent Res 1984; 92:400-407.
127. MORTIMER K.V. Some Histological Features of Fissure Caries in Enamel. Adv Fluo Res Dent Car Prev 1964; 2:85-94.
128. NEWBRUN E. Cariology 2nd Ed. Baltimore: Williams & Wilkins, 1983.
129. NEWBRUN E. Chemical and Mechanical Removal of Plaque. Compend Contin Educ in Dent 1985; Supp.No. 6:S110-S116.
130. NEWMAN H.N. The Pre-eruptive Portion of the Human Enamel Integument. J Dent 1975; 3:110-120.
131. NEWMAN H.N., WILSON C.M. Thymidine and Thymine Uptake in Human Dental Plaque: an Autoradiographic Study. Caries Res 1975; 9:405-417.
132. NGANO T. The Form of Pits and Fissures and the Primary Lesion of Caries. Dent Abs 1961; 6:426.
133. O'BRIEN W.J. Capillary Action Around Dental Structures. J Dent Res 1973; 52:544-550.
134. OPPERMAN R.V. Effect of Chlorhexidine on Acidogenicity of Dental Plaque in vivo. Scand J Dent Res 1979; 87:302-308.
135. OPPERMAN R.V., JOHANSEN J.R. Effect of Fluoride and Non-fluoride Salts of Copper, Silver and Tin on the Acidogenicity of Dental Plaque in vivo. Scand J Dent Res 1980; 88:476-480.
136. OPPERMAN R.V., ROLLA G. Effect of Some Polyvalent Cations on the Acidogenicity of Dental Plaque in vivo. Caries Res 1980; 14:422-427.
137. PARFITT, G.J. Conditions Influencing the Incidence of Occlusal and Interstitial Caries in Children. J Dent Child 1956; 23:31-39.

138. PAYNTER K.J., GRAINGER R.M. Relationship of Morphology and Size of Teeth to Caries. Int Dent J 1962; 12:147-160.
139. PEATMAN J.C. Introduction to Applied Statistics. New York: Harper & Row, 1964.
140. PHARMACEUTICAL SOCIETY OF GREAT BRITAIN The Pharmaceutical Codex. 11th Ed. London: The Pharmaceutical Press, 1979:727.
141. PIPKIN F.B. Medical Statistics Made Easy. London: Churchill Livingstone, 1984.
142. POWELL K.R. An in vitro Study of the Penetrating and Sealing Properties of Bis-gma Resin Pit and Fissure Coatings. M.D.S. Thesis, Uni. of Syd. 1975.
143. RICKLES N.H. The Estimation of Dental Caries Activity by a New Colorimetric Laboratory Test. J Dent Res 1953; 32:3-17.
144. RIPA L.W., GWINNETT A.J., BUONOCORE M.G. The "Prismless" Outer Layer of Deciduous and Permanent Enamel. Arch Oral Biol 1966; 11:41-48.
145. RITZ H.L. Microbial Population Shifts in Developing Human Dental Plaque. Arch Oral Biol 1967; 12:1561-1568.
146. ROBERTSON W.A. A Practical Treatise on the Human Teeth. 1st Am. Ed. Philadelphia: Lea & Blanchard, 1841.
147. ROBRISH S.A., GROVE S.B., BERNSTEIN R.S., MARUCHA P.T., SOCRANSKY S.S., AMDUR B. Effects of Sonic Treatment on Pure Cultures and Aggregates of Bacteria. J Clin Microbiol 1976;3:474-479.
148. ROGOSA M., MITCHELL J.A., WISEMAN R.F. A Selective Medium for the Isolation and Enumeration of Oral Lactobacilli. J Dent Res 1951; 30:682-689.
149. RUNDELL B.B., THOMSON L.A., LOESCHE W.J., STILES H.M. Evaluation of a New Transport Medium for the Preservation of Oral Streptococci. Arch Oral Biol 1973; 18:871-878.

150. RUSSELL A.D., HUGO W.B., AYLIFFE G.A.J. Eds. Principles and Practice of Disinfection, Sterilization and Preservation. Oxford: Blackwells, 1982.
151. SANDHAM H.J., BROWN J., PHILLIPS, H.I., CHAN K.H. A Preliminary Report of Long-term Elimination of Detectable Mutans Streptococci in Man. J Dent Res 1988; 67:9-14.
152. SCHACHTELE C.F., JENSEN M.E. Comparison of Methods of Monitoring Changes in the pH of Human Dental Plaque. J Dent Res 1982; 61:1117-1125.
153. SCHAEKEN M.J.M., DE JONG M.H., FRANKEN J.S., VAN DER HOEVEN J.S. Effects of Chlorhexidine and Iodine on the Composition of the Human Dental Plaque Flora. Caries Res 1984; 18:401-407.
154. SCHAEKEN M.J.M., DE JONG M.H., FRANKEN J.S., VAN DER HOEVEN J.S. Effects of Highly Concentrated Stannous Fluoride and Chlorhexidine Regimes on Human Dental Plaque Flora. J Dent Res 1986; 65:57-61.
155. SCHAEKEN M.J.M., VAN DER HOEVEN J.S., FRANKEN H.C.M. Comparative Recovery of Streptococcus mutans on Five Isolation Media Including a New Simple Selective Medium. J Dent Res 1986; 65:906-908.
156. SCHEIE A.A., ARNEBERG P., KROGSTAD O. Effect of Orthodontic Treatment on Prevalence of Streptococcus mutans in Plaque and Saliva. Scand J Dent Res 1984; 92:211-217.
157. SCHROEDER H.E., DE BOEVER J. The Structure of Microbial Dental Plaque. In: Dental Plaque. Ed. McHugh. London: Livingstone, 1970:49-74.
158. SEPPA L., POLLANEN L. Caries Preventive Effect of Two Fluoride Varnishes and a Fluoride Mouthrinse. Caries Res 1987; 21:375-379.
159. SHUGAR G.J., SHUGAR R.A., BAUMAN L., BAUMAN R.S. Chemical Technicians Ready Reference Handbook. 2nd Ed. McGraw-Hill Book Co., 1981.

160. SMITS M.T., ARENDS J. In vitro Demineralization of Human Enamel in Artificial U-shaped Grooves. Caries Res 1986; 20:217-222.
161. SYNDER M.L., PORTER D.R., CLAYCOMB C.K., SIMS W. Evaluation of Laboratory Tests for Estimation of Caries Activity. JADA 1962; 65:30-45.
162. SOCRANSKY S.S. Caries Susceptibility Test. Ann NY Acad Sci 1968; 153:137-146.
163. SOCRANSKY S.S., MANGANIELLO A.D., PROPAS D., ORAM V., VAN HOUTE J. Bacteriological Studies of Developing Supragingival Dental Plaque. J Periodont Res 1977; 12:90-106.
164. STEPHAN R.M. Changes in the Hydrogen Ion Concentration on Tooth Surfaces and In Carious Lesions. JADA 1940; 27:718-723.
165. STEPHAN R.M. Intra-oral Hydrogen-ion Concentrations Associated with Dental Caries Activity. J Dent Res 1944; 23:257-266.
166. STRASSLER H.E., MINAH G.E., KULA K.S. Microbiological and Microhardness Evaluation of Artificial Enamel Fissures Worn Intraorally by Humans. J Clin Microbiol 1986; 23:6-10.
167. SUMMEY D.L., JORDAN H.V. Characterization of Bacteria Isolated from Human Root Surface Carious Lesions. J Dent Res 1974; 53:343-351.
168. SVANBERG M. Streptococcus mutans in Plaque after Mouthrinsing with Buffers of Varying pH Value. Scand J Dent Res 1980; 88:76-78.
169. SVANBERG M., LOESCHE W.J. The Salivary Concentration of Streptococci mutans and Streptococci sanguis and their Colonization of Artificial Tooth Fissures in Man. Arch Oral Biol 1977; 22:441-447.
170. SVAMBERG M., ROLLA G. Streptococcus mutans in Plaque and Saliva after Mouthrinsing with SnF₂. Scand J Dent Res 1982; 90:292-298.

171. SVANBERG M., WESTERGREN G. Effects of SnF₂, Administered as Mouthrinses or Topically Applied, on Streptococcus mutans, Streptococcus sanguis and Lactobacilli in Dental Plaque and Saliva. Scand J Dent Res 1983; 91:123-129.
172. SVATUN B., ATRAMADAL A. The Effect of Stannous Fluoride on Human Plaque Acidogenicity in situ (Stephan Curve). Acta Odontol Scand 1978; 36:211-218.
173. SVATUN B., ROLLA G. The Role of Stannous Pyrophosphate in the Plaque-inhibiting Effect of Dentrifices Containing Stannous Fluoride. In: Tooth Surface Interactions and Preventive Dentistry. Eds. Rolla, Sonju, Embery. London: IRL Press, 1981;33-37.
174. SVEEN O.B., FOLKE L., THOTT E. Ultrastructural Study of Plaque and Plaque formation in Natural Fissures. J Dent Res 1975;54(Abs.No. L86):L22.
175. SYED S.A., LOESCHE W.J. Survival of Human Dental Plaque Flora in Various Transport Media. Appl Microbiol 1972; 24:638-644.
176. SYED S.A., LOESCHE W.J. Efficiency of Various Growth Media in Recovering Oral Bacterial Flora from Human Dental Plaque. Appl Microbiol 1973; 26:459-465.
177. TANZER J.M. Essential Dependence of Smooth Surface Caries on and Augmentation of Fissure caries by, Sucrose and Streptococcus mutans Infection. Infect Immun 1979; 25:526-531.
178. TATEVOSSIAN A. Diffusion of Radiotracers in Human Dental Plaque. Caries Res 1979; 13:154-162.
179. THEILADE E., FEJERSKOV O., KARRING T., THEILADE J. A Microbiological Study of Old Plaque in Occlusal Fissures of Human Teeth. Caries Res 1978; 12:313-319.
180. THEILADE E., FEJERSKOV O., KARRING T., THEILADE J. Predominant Cultivable Microflora of Human Dental Fissure Plaque. Infect Immun 1982; 36:977-982.

181. THEILADE E., FEJERSKOV O., PRACHYABRUED W., KILIAN M. Microbiologic Study on Developing Plaque in Human Fissures. Scand J Dent LRes 1974; 82:420-427.
182. THEILADE E., LARSON R.H., KARRING T. Microbiological Studies of Plaque in Artificial Fissures Implanted in Human Teeth. Caries Res 1973; 7:130-138.
183. THEILADE E., THEILADE J. Formation and Ecology of Plaque at Different Locations in the Mouth. Scan J Dent Res 1985; 93:90-95.
184. THEILADE E., THEILADE J., MIKKELSE N.L. Microbiological Studies on Early Dento-gingival Plaque on Teeth and Mylar strips in Humans. J Periodont Res 1982; 17:12-25.
185. THEILADE J. Electron Microscope Study of Calculus Attachment to Smooth Surfaces. Acta Odontol Scand 1964; 22:379-387.
186. THEILADE J., FEJERSKOV O., HØRSTED M. Electron Microscopy of 7-day Old Plaque in Human Dental Fissures. J Dent Res 1975; 54(Abs.No. L88):L22.
187. THEILADE J., FEJERSKOV O., HØRSTED M. A Transmission Electron Microscopic Study of 7-day Old Bacterial Plaque in Human Tooth Fissures. Arch Oral Biol 1976; 21:587-598.
188. THEILADE J., KARRING T., ØSTERGAARD E., LOE H. Electron Microscope Study of the Formation of Dental Plaque in Artificial Fissures. J Biol Buccale 1974; 2:375-393.
189. THEUNS H.M., POT T.J. An in vivo Fissure Study, Its Implications on Conservative Treatment. Ned Tijdschr Geneesk 1978; 85(Suppl. 16):34-42.
190. THIBODEAU E.A., HANDELMAN S.L., MARQUIS R.E. Inhibition and Killing of Oral Bacteria by Silver Ions Generated with Low Intensity Direct Current. J Dent Res 1978; 57:922-926.

191. THOTT E.K., FOLKE L.E.A., SVEEN O.B. A Microbiologic Study of Human Fissure Plaque. Scand J Dent Res 1974; 82:428-436.
192. TINANOFF N. Stannous Fluoride in Clinical Dentistry. In: Clinical Uses of Fluorides. Ed. Wei. Philadelphia: Lea & Febiger, 1985.
193. TINANOFF N., KLOCK B., CAMOSCI D.A., MANWELL M.A. Microbiologic Effects of SnF₂ and NaF Mouthrinses in Subjects with High Caries Activity; Results after One Year. J Dent Res 1983; 62:907-911.
194. TINANOFF N., SIEGRIST B., LANG N.P. Safety and Antibacterial Properties of Controlled Release SnF₂. J Oral Rehabil 1986; 13:73-81.
195. TOGELIUS J., BRATTHALL D. Frequency of the Bacterium Streptococcus mutans in the saliva of selected Human Populations. Arch Oral Biol 1982; 27:113-116.
196. VANDERAS A.P. Bacteriologic and Non-bacteriologic Criteria for Identifying Individuals at High Risk of Developing Dental Caries: A Review. J Public Health Dent 1986; 46:106-113.
197. VAN DER FEHR F.R., KIRKEGAARD E., LOE H., SCHØTT C.R. Effect of Chlorhexidine Mouth Rinses on the Caries Incidence of Students. Caries Res 1975; 9:289-290.
198. VAN HOUTE J. Oral Bacterial Colonization: Mechanisms and Implications. In: Microbial Aspects of Dental Caries. Eds. Stiles, Loesche, O'Brien. 1976; Vol I:3-32.
199. VAN HOUTE J. Bacterial Specificity in the Etiology of Dental Caries. Int Dent J 1980; 30:305-326.
200. VAN HOUTE J., GIBBONS R.J., BANGHART S.B. Adherence as an Ecological Determinant of the Presence of Streptococcus salivarius and Streptococcus sanguis on the Human Tooth surface. Arch Oral Biol 1970; 15:1025-1034.
201. VAN HOUTE J., GREEN D.B. Relationship Between the Concentration of Bacteria in Saliva and the Colonization of Teeth in Humans. Infect Immun 1974; 9:624-630.

202. VAN HOUTE J., RUSSO J. Variable Colonization by Oral Streptococci in Molar Fissures of Monoinfected Gnotobiotic Rats. Infect Immun 1986; 52:620-622.
203. VAN PALENSTEIN HELDERMAN W.H., IJSSELDIJK M., HUIS IN'T VELD J.H.J. A Selective Medium for Two Major Subgroups of the Bacterium Streptococcus mutans Isolated from Human Dental Plaque and Saliva. Arch Oral Biol 1983; 28:599-603.
204. VOGEL'S Textbook of Quantitative Analysis. Eds. Bassett, Denney, Jeffrey, Mendham. 4th Ed. London: Longman, 1978.
205. VRATSANOS S.M., ABELSON D.C., MANDEL I.D. Plaque Acidogenesis and Caries Resistance. J Dent Res 1979; 58(Abst.No. 1340):425.
206. WESTERGREN G., KRASSE B. Evaluation of Micromethod for Determination of Streptococcus mutans and Lactobacillus Infections. J Clin Microbiol 1978; 7:82-83.
207. WILCOX M.D.P., DRUCKER D.B., GREEN R.M. Relative Cariogenicity and in vivo Plaque-forming Ability of the Bacterium Streptococcus oralis in Gnotobiotic WAG/RIJ Rats. Arch Oral Biol 1987; 32:455-457.
208. ZERO D.T., VAN HOUTE J., RUSSO J. The Intra-oral Effect on Enamel Demineralization of Extracellular Matrix Material Synthesized from Sucrose by Streptococcus mutans. J Dent Res 1986; 65:918-923.
209. ZOBELL C.E. The Effect of Solid Surfaces Upon Bacterial Activity. J Bacteriol 1943; 46:39-56.