JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 9 (1998) 141–146

Detection of mutagenic potential of some glass–ionomer cements through Ames testing

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The mutagenic potential of three commercially available glass–ionomer cements used in dentistry was examined. The cement components were mixed according to the manufacturers indications and set for two defined times: 1 h or, alternatively, 1 wk. Cements B and C set spontaneously; in the case of cement A, the manufacturer suggests the use of a lamp to trigger also a photopolymerization. Photopolymerization, however, was not used. Ames tests were performed on the dimethyl sulphoxide extracts of cements by using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 and TA 102. Cement A showed mutagenicity only against TA 1537 strain, either in the presence or absence of metabolic activation with microsomial fraction S9. The other two cements showed no mutagenic potential. We conclude that glass–ionomer cements are, on the whole, safe materials from the viewpoint of genotoxicity, and hypothesize that the mutagenicity observed in cement A could depend on its polymerization performed without light activation.

1. Introduction

Glass-ionomer cements (GICs) are bicomponent materials (powder/liquid) mixed just before use to produce a hard substance used for different purposes:

(a) to attach temporarily or permanently orthodontic devices and fixed prostheses on the tooth;

(b) to obtain a filling material for class III and V cavities, that chemically bonds to enamel and dentine, even if applied in moist conditions and without cavity preparation;

(c) to create linings for fillings in tooth restoration.

The various brands of glass–ionomer cement have been classified into Type I (luting cements), Type II (restorative materials either aesthetic and reinforced), and Type III (fast-setting lining materials and fissure sealants) [1]. They present many advantages, such as their chemical bonding with the tooth that is achieved in restoration, avoiding the need for undercutting cavities to obtain mechanical keying in. They provide a good seal with the tooth, thus preventing bacteria from infiltrating the margins of the restoration and causing secondary caries. They can also release fluoride for additional protection [2–4].

The hardening of GICs derives from an acid/basic chemical reaction between the calcium-fluoro-alumino-silicate powder and a polycarboxylic acid in aqueous solution, for example, homopolymers of acrylic acid or copolymers with, such as itaconic acid or malonic acid [5]. The diluent for them may be water or a solution of tartaric acid in water.

The reaction starts when powder and liquid are mixed and can be synthesized as follows. (i) Hydrogen ions released by the carboxylic groups of poycarboxylic acid first displace calcium ions and later aluminium ions from the surface of powder particles; these latter ions are assumed to be complexed in some way, for example with fluoride ions (AlF_2^+ and AlF^{2+}). (ii) Cations Ca^{2+} and Al^{3+} form saline bonds with the carboxylic groups of polycarboxylic acid. The resulting material was shown to be a composite consisting of a poly(acrylate) matrix with unreacted glass powder embedded in it [6, 7]. As polymers of such type are improperly named ionomers, and also vitreous particles occur in the material, these cements are called glass–ionomer cements.

The reaction is completed in 4–6 min. GICs then undergo other stabilization processes which cease after 24 h.

Recent development of the material has been the advent of light cured GICs. Although there are a number of varieties, the principle is the same. The water component is replaced by a water/hydroxyethylmethacrylate (HEMA) mixture. These formulations also contain an initiator/activator system. The resin–glass ionomer cements are mixed in the same way as the conventional materials and remain workable for at least 10 min. Two reactions take place: the ionomer acid–base reaction previously described and the photochemical polymerization of HEMA to poly-HEMA.

Recently, interest has been shown in the use of glass–ionomer cements not only in dentistry but also in orthopaedic surgery, due to the optimal adhesion of ionomeric cements to stainless steel alloys and to bone; they can offer a major advantage when compared with existing acrylic bone cements [8–11].

The glass–ionomer cements used in dentistry are class II medical devices according to the European directive 43/92 [12]. Like every medical device, they have to satisfy the biocompatibility requisite; standard EN 30993/1 establishes that the materials which are to be in contact with dentine for more than 30 d have also to undergo genotoxicity tests. The European viewpoint is shared also by document 41a–1982 ANSI-ADA (American National Standards Institute/American Dental Association), where it is stated that dental materials must also undergo the Ames test, even though they are not pharmacologically active.

Genotoxicity tests used in evaluating medical devices are screening tests, which can be performed *in vitro* without the use of animals. They have a good predictability on the capability of a substance of inducing tumor *in vivo* as there is a correlation between the capability of a substance to induce mutation or chromosomic damage and the development of a tumor. Some genotoxicity tests permit determination of whether the materials or devices induce genic mutations; others highlight alterations in DNA and others detect damage in the chromosomic structure. According to standard EN 30993-3 (Biological evaluation of medical devices–Part 3: Test for genotoxicity, carcinogenicity and reproductive toxicity), in order to assess the genotoxicity of a medical device three different tests have to be performed, one for each damage level [13]. At least two of these must be carried out on eucaryotes, preferably mammalian (European Committee for Standardization).

In order to highlight genic mutations, the Ames test was performed on six strains of *Salmonella typhimurium* (TA 98, TA 100, TA 102, TA 1535, TA 1537, TA 1538). The test evaluates the reversion from *his*− to *his* + induced by the examined substance, which can cause frame-shift or base-pair mutations. The bacteria are exposed to the substance with or without metabolic activation, and inoculated on to minimum agar plates. The trace amounts of histidine in the agar allows all the plated bacteria to undergo a few cell divisions, which is essential for mutagenesis to be fully expressed. The *his*+ revertants are readily discernable as colonies against the limited background growth of the *his* − cells.

Various dental materials, some of them at present on the market, turned out to be genotoxic [14–19], but to our knowledge very few results of studies of this type carried out on glass–ionomer cements have been published [20].

The aim of our work was to verify, by means of the Ames test, the capability of three glass–ionomer cements to induce gene mutations *in vitro*.

2. Materials and methods

The tests were performed on three commercially available dental glass–ionomer cements. Their commercial names and the production lot numbers are listed in Table I. The cement components were mixed according to the manufacturer's instructions. The photopolymerizing lamp was not used for cement A, even though it is a light-curing cement, that contains 10% by weight of HEMA monomer (2 hydroxyethylmethacrylate) and a photoinitiator (camphoroquinone). The products were set for 1 h or 1 wk, then they were weighed and extracted in dimethyl sulphoxide according to the standard ISO 10993 Part 12 (1 g/5 ml DMSO for 72 h at 37 °C). Knowing that the Ames test can yield false positive results when substances with a very low pH are tested, and knowing the chemical composition of the material, the extracts pH was controlled before storage; in no case was it necessary to correct the pH level as the range was acceptable: not lower than 6.5. The extract was aliquoted and frozen at −20 °C before being tested. On all specimens the plate incorporation test was carried out according to the method described by Ames 1975 [21], then revised by Maron and Ames in 1983 [22] following standard EN 30993/3. Five tester strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, TA 1537, TA 1538) were used to detect frame-shift and base-pair mutations. A sixth one was used to test only cement A. They were kindly provided by Dr Bruce Ames (University of California, Berkeley, CA, USA). Immediately after receipt, the strains were reisolated. Their genotypes were confirmed and the cultures were kept frozen at −80 °C. The characteristics of each strain are summarized in Table II.

Strains were chosen in order to detect mutagens causing either base-pair mutations (TA 1535, TA 100, TA 102) or frame-shift mutations (TA1537, TA1538 TA 98).

The test was carried out as follows: the overnight culture of the micro-organisms was performed in Oxoid Nutrient Broth N. 2. During the test, 0.1 ml broth was incorporated in 2 ml top agar previously added to a 0.5 mM *L*-histidine – 0.5 mM biotin solution. Extracts were added to top agar. Top agar, eventually enhanced with the above-mentioned components, was poured on minimal agar-glucosate plates. Petri dishes for cell cultures with a diameter of 9 cm, gamma-radiation sterilized (Costar, Cambridge, MA, USA), were used.

TABLE I Characteristics of the tested materials according to the manufacturer's description

Trademark and manufacturer	Identification	Characteristics	Lot
Vitrebond™, 3M, USA	A	Photopolymerizing	19950315
Fuji I™, GC Corporation, Japan	B	Non-photopolymerizing	180741
Ketac-Cem™, ESPE, Germany	C	Non-photopolymerizing	13164

TABLE II Battery of tester strains used in this study

Strain	*his* mutation[a]	LPS	Repair	pKM101[d]	Nature of mutation
TA 1535	*his* G46	rfa[b]	Δ uvr B[c]	−	AT → CG
TA 1537	*his* C3076	rfa	Δ uvr B	−	+ 1 near C...C
TA 1538	*his* D3052	rfa	Δ uvr B	−	− 1 near CG...CG
TA 98	*his* D3052	rfa	Δ uvr B	+	− 1 near C
TA 100	*his* G46	rfa	Δ uvr B	+	AT → CG
TA 102	*his* G428	rfa	Δ uvr B	+	CG → AT

[a]*his* mutation: mutation in the histidine operon.
[b]rfa: partial loss of the lipopolysaccaride barrier that coats the surface of *Salmonella*.
[c]Δ uvr B: deletion of a gene coding for the DNA excision repair system.
[d]pKM101: plasmide carrying the R-factor to ampicilin.

TABLE III Mutagenicity of the cements extracted 1 h after setting (experiments carried out without S9). Mut = mutagenic, Tox = toxic, Neg = non-mutagenic and non-toxic, Rever = revertants, Resp = response.

Extract	TA98		TA100		TA1535		TA1537		TA1538		TA102	
(μl/plate)	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.
Cement A												
100	5 ± 6	Tox	57 ± 60	Tox	2 ± 2	Tox	0	Tox	0	Tox	0	Tox
10	21 ± 5	Neg	137 ± 15	Neg	28 ± 10	Neg	39 ± 14	Mut	17 ± 2	Neg	245 ± 22	Neg
5	18 ± 7	Neg	131 ± 11	Neg	23 ± 5	Neg	26 ± 9	Neg	23 ± 10	Neg	295 ± 25	Neg
Cement B												
100	26 ± 4	Neg	142 ± 13	Neg	26 ± 3	Neg	19 ± 3	Neg	23 ± 4	Neg	/	
10	24 ± 7	Neg	115 ± 23	Neg	23 ± 21	Neg	15 ± 2	Neg	18 ± 3	Neg	/	
5	26 ± 5	Neg	123 ± 26	Neg	22 ± 3	Neg	14 ± 2	Neg	16 ± 4	Neg	/	
Cement C												
100	24 ± 6	Neg	125 ± 19	Neg	26 ± 5	Neg	15 ± 2	Neg	27 ± 8	Neg	/	
10	23 ± 6	Neg	143 ± 26	Neg	22 ± 2	Neg	11 ± 3	Neg	25 ± 9	Neg	/	
5	20 ± 6	Neg	125 ± 13	Neg	27 ± 3	Neg	10 ± 3	Neg	22 ± 5	Neg	/	
Negative control	21 ± 4		126 ± 12		23 ± 5		13 ± 2		18 ± 4		319 ± 15	
Positive control	1522 ± 308		1041 ± 107		1010 ± 26		1092 ± 79		2011 ± 195		1250 ± 86	

The experiments were carried out in the presence and absence of the microsomial fraction S9 (Moltox, Annapolis, MD, USA), at a concentration of 20 μl/plate.

The S9 mixture was prepared immediately before use, by adding 25 ml phosphate buffer Na/K, 0.2 M, pH 7.4, 2 ml NADP 0.1M, 0.25 ml glucose 6 phosphate 1 M, 1 ml $MgCl_2$ (0.4 M) + KCl (1.65 M), 19.75 ml distilled water to the lyophilized S9 reconstructed with 2.1 ml distilled water in a sterile flask maintained in ice bath. Any leftover S9 or S9 mix was discarded. In each experiment, positive mutagenesis controls were routinely included to confirm the reversion properties and specificity of each strain, as well as the efficacy of the S9 mix. Diagnostic mutagens were chosen as follows in the absence of S9 mix 4-nitro-ortophenylendiamine 20 μg/plate for strains TA 98 and TA 1538, sodium azide 1.5 μg/plate for strains TA 100 e TA 1535 and 9-aminoacridine 80 μg/plate for strain TA 1537, *t*-butyl hydroperoxide 100 μg/plate were used.

In the tests performed in the presence of S9, 2-antramine at the final concentration of 1 μg/plate was used with each strain except TA 102, where 1,8 dihydroxy anthrachinone (Danthron) 100 μg/plate was used. Counting of the revertant colonies was carried out after a 48 h incubation at 37 °C.

The quantities assayed for each tested material were 100, 10, 5 μl/plate. Lower concentrations were tested for cement A, whenever necessary. Strain 102 was used only for cement. A. The tests were performed in triplicate for each tester strain and material dosage. Moreover, each test compound was tested on at least two separate occasions. Scoring was performed without knowledge of treatment.

The test was considered positive when a statistically significant increase occurs ($p < 0.05$) in the number of revertant colonies with respect to control by using the Mann–Whitney non-parametric test.

3. Results

The results are shown in Tables III, IV, V and VI. In all tests the number of revertants for negative controls (DMSO) was within the range of historical data from this laboratory. Positive controls showed significant increases in the reversion of the tester bacteria when compared with negative controls. Values were comparable to those suggested in the literature [22].

TABLE IV Mutagenicity of the cements extracted 1 h after setting (experiments carried out with S9). Abbreviations as in Table III

Extract	TA98		TA100		TA1535		TA1537		TA1538		TA102	
(μl/plate)	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.
Cement A												
100	7 ± 4	Tox	0	Tox	0	Tox	0	Tox	0	Tox	0	Tox
20	/		70 ± 8	Tox	8 ± 1	Tox	78 ± 11	Mut	16 ± 11	Neg	–	
10	29 ± 8	Neg	134 ± 21	Neg	18 ± 3	Neg	51 ± 10	Mut	18 ± 7	Neg	264 ± 6	Neg.
5	43 ± 22	Neg	131 ± 14	Neg	18 ± 4	Neg	30 ± 5	Neg	22 ± 2	Neg	366 ± 18	Neg
2.5	35 ± 4	Neg	113 ± 15	Neg	14 ± 4	Neg	/	/	/	/	/	
Cement B												
100	30 ± 4	Neg	132 ± 6	Neg	30 ± 30	Neg	12 ± 1	Neg	25 ± 3	Neg		
10	25 ± 5	Neg	121 ± 16	Neg	19 ± 3	Neg	12 ± 3	Neg	21 ± 2	Neg		
5	31 ± 6	Neg	127 ± 15	Neg	19 ± 3	Neg	11 ± 1	Neg	18 ± 1	Neg		
Cement C												
100	30 ± 2	Neg	119 ± 13	Neg	20 ± 4	Neg	12 ± 2	Neg	23 ± 2	Neg		
10	30 ± 9	Neg	124 ± 11	Neg	17 ± 4	Neg	13 ± 2	Neg	22 ± 3	Neg		
5	27 ± 5	Neg	125 ± 9	Neg	18 ± 6	Neg	12 ± 3	Neg	21 ± 3	Neg		
Negative control	24 ± 4		126 ± 18		14 ± 3		16 ± 2		20 ± 4		272 ± 20	
Positive control	1924 ± 128		934 ± 101		958 ± 58		1146 ± 60		1777 ± 217		1123 ± 23	

TABLE V Mutagenicity of the cements extracted 1 wk after setting (experiments carried out with S9). Abbreviations as in Table III

Extract	TA98		TA100		TA1535		TA1537		TA1538		TA102	
(μl/plate)	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.
Cement A												
100	2 ± 3	Tox	0	Tox	0	Tox	0	Tox	0	Tox	0	Tox
10	24 ± 4	Neg	105 ± 11	Neg	23 ± 2	Neg	55 ± 3	Mut	18 ± 1	Tox	302 ± 18	Neg
5	20 ± 5	Neg	119 ± 3	Neg	15 ± 1	Neg	37 ± 3	Mut	17 ± 2	Neg	329 ± 7	Neg
Cement B												
100	21 ± 3	Neg	128 ± 10	Neg	16 ± 1	Neg	10 ± 1	Neg	21 ± 1	Neg	/	
Cement C												
100	20 ± 3	Neg	111 ± 1	Neg	18 ± 3	Neg	10 ± 2	Neg	19 ± 5	Neg	/	
Negative control	21 ± 3		109 ± 7		14 ± 4		9 ± 1		19 ± 2		341 ± 4	
Positive control	1611 ± 384		1033 ± 49		1037 ± 42		1266 ± 274		2011 ± 195		1330 ± 1 51	

Colonies appearing in the absence of a background lawn are survivors of the killing effect of the test chemical, and were not counted as revertants. This happens when most of the bacteria on the plate are killed because of the toxic effect of the test chemical, allowing the survivors to grow into small colonies by using up the available histidine in the top agar.

The statistical analysis of the results shows that the cement A extract is toxic at high doses (100 μl/plate); at lower doses (20, 10 μl/plate) it induces a statistically significant, even though not very high, increase in the number of revertant colonies of the strain TA 1537, both when it is extracted 1 h after polymerization, and when extracted after 1 wk. This result is obtained both in the tests performed in the presence and in the absence of S9. None of the other five *Salmonella* strains were affected by the action of cement A extract.

The extracts of cements B and C show no toxicity on bacteria up to a concentration of 100 μl/plate, nor induce in any case an increase in revertants; therefore, they do not have any mutagenic activity.

4. Discussion

Evidence of cytotoxic agents leaching from glass–ionomer cements has previously been shown [23–25]. Recently, evidence for genotoxicity of some dental materials, and among them glass–ionomer cement, has been shown. The authors provide several lines of evidence that the extracts of Cement A, even if light-cured, elicited clear concentration-related genotoxic responses in three different tests. [20].

The Ames test, which evaluates the capability of a substance to induce retromutations in histidine-dependent *Salmonella* strains, was chosen above all for its high sensitivity (0.84%) [26] and its universally known validity. By choosing to test different *Salmonella* strains, each one sensitive to different

TABLE VI Mutagenicity of the cements extracted 1 wk after setting (experiments carried out with S9). Abbreviations as in Table III

Extract	TA98		TA100		TA1535		TA1537		TA1538		TA102	
(μl/plate)	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.	Rever.	Resp.
Cement A												
100	0	Tox	0	Tox	0	Tox	0	Tox	2 ± 1	Tox	0	Tox
10	22 ± 3	Neg	119 ± 8	Neg	17 ± 2	Neg	52 ± 4	Mut	17 ± 4	Neg	256 ± 7	Neg
5	23 ± 1	Neg	119 ± 10	Neg	18 ± 2	Neg	43 ± 6	Mut	22 ± 2	Neg	292 ± 13	Neg
Cement B												
100	27 ± 6	Neg	130 ± 7	Neg	14 ± 1	Neg	15 ± 6	Neg	20 ± 5	Neg	/	
Cement C												
100	26 ± 2	Neg	130 ± 9	Neg	13 ± 2	Neg	14 ± 4	Neg	14 ± 5	Neg	/	
Negative control	26 ± 3		119 ± 9		16 ± 3		10 ± 1		14 ± 4		272 ± 20	
Positive control	2005 ± 105		1368 ± 73		194 ± 45		188 ± 2 6		1489 ± 107		1123 ± 25	

chemicals, it is possible to detect mutagens. Moreover, if the test is carried out in the absence of metabolic activator S9, the presence of a direct mutagen can be demonstrated. If the test is performed in the presence of the activator, the presence of indirect mutagen is detected, which have to be metabolized *in vivo* at the hepatic level to perform their activity.

In the experiments described, positive results were obtained both in the tests performed with and without S9. This demonstrates that at least a direct mutagen and an indirect one occur in cement A extract. The former, unlike the latter, acts without being metabolized.

The substances responsible for retromutations, which occurred only in strain TA1537 *Salmonellae*, are present both in the extract of the just polymerized cement and in the cement extract 1 wk after polymerization, when the setting process is completed. On the basis of the response of the strain TA1537 it can be inferred that the mutagenic substance or substances occurring in cement A extract are able to induce a frame-shift-type mutation near a sequence C...C.

However it can be hypothesized that the choice of allowing cement A to polymerize without light activation could have affected the result. According to the manufacturer's information, the cement Vitrebond™ has the capability of polymerizing in two different ways: the classical acid–base way and, additionally, photopolymerization induced by halogen light with a wave-length of 460 nm. The light supplies the energy needed for the formation of free radicals which react with acrylic groups, triggering the process. The material, even without being exposed to light, as sometimes happens in clinical applications, should polymerize in 4–6 min following the acid–base reaction, without any influence on the final result.

The delayed auto-setting mechanism of the Vitrebond™ will ensure an eventual cure of material shielded from light polymerization such as in undercut areas. By following only this route, it is, however, possible that substances with slight genotoxic substances remain. This could also be the reason for the toxicity of the material [27, 28]. Completely acceptable results were, on the contrary, obtained with cements Fuji I™ and Ketac-Cem™, which showed neither toxic nor mutagenic response.

As established in the standards on the genotoxicity determination on medical devices, the aim is that of completing the test panel by performing tests of sister chromatide exchanges to highlight the damage on DNA and to evaluate the chromosomic aberrations induced *in vitro*, to assess the damage on the chromosomic structure.

Acknowledgement

Grants from Ministero della Sanità, Ricerca finalizzata 'Citocompatibilità quantitativa..' are gratefully acknowledged.

References

1. J. McLEAN, *Clin. Mater.* **7** (1991) 283.
2. M. H. REISBICK, *J. Prosthet. Dent.* **46** (1981) 525.
3. M. J. TYAS, *J. Dent. Res.* **62** (1983) 646.
4. A. D. WILSON, *Clin. Mater.* **7** (1991) 275.
5. J. W. McLEAN, *J.A.D.A.* **96** (1978) 785.
6. B. E. KENT, B. G. LEWIS and A. D. WILSON *J. Dent. Res.* **58** (1979) 1607.
7. E. A. WASSON and J. W. NICHOLSON, *Clin. Mater.* **7** (1991) 289.
8. U. MEYER, D. H. SZULCZEWSKI, R. H. BARCKHAUS, M. ATKINSON and D. B. JONES, *Biomaterials* **14** (1993) 917.
9. E. WASSON and J. NICHOLSON, *Clin. Mater.* **8** (1991) 125.
10. J. W. McLEAN, *ibid.* **7** (1991) 283.
11. I. M. BROOK, G. T. CRAING and D. J. LAMB, *ibid.* **7** (1991) 295.
12. Directive 93/42/CEE, Council 14 June 1993 on medical devices, published 12 July 1993.
13. EN 30993-3: "Biological evaluation of medical devices – Part 3: Test for genotoxicity, carcinogenicity and reproductive toxicity" (European Committee for Standardization, 1993).
14. Y. LI, T. W. NOBLITT, A. J. DUNIPACE and G. K. STOOKEY, *J. Dent. Res.* **69** (1990) 1188.
15. H. SCHWEIKL and G. SCHMALZ, *J. Mater. Sci. Mater. Med.* **2** (1991) 181.
16. N. KANEMATSU and H. KAWAHARA in "Transactions of the Fourth World Biomaterials Congress". 24–28 April 1992, Berlin, Abstract p. 374.

17. D. ORSTAVIK and J. K. HONGSLO, *Biomaterials* **6** (1985) 129.
18. N. G. CROSS and R. F. TAYLOR, *Am. J. Orthod.* **84** (1983) 344.
19. S. STEA, L. SAVARINO, G. CIAPETTI, E. CENNI, S. STEA, F. TROTTA, G. MOROZZI and A. PIZZOFERRATO, *J. Biomed. Mater. Res.* **28** (1994) 319.
20. J. HEIL, G. REIFFERSCHIEID, P. WALDMAN, G. LEYHAUSEN and W. GEURSTEN, *Mutat. Res.* **368** (1996) 181.
21. B. AMES, J. MC CANN and E. YAMASAKI, *Mutat. Res.* **34** (1975) 317.
22. D. MARON and B. AMES, *ibid.* **113** (1983) 173.
23. P. J. DOHERTY, *Clin. Mater.* **7** (1991) 335.
24. A. D. WILSON, D. R. GROFMAN and A. T. KUHN, *Biomaterials* **6** (1985) 431.
25. P. SASANALUCKIT, P. J. ALBUSTANY, P. J. DOHERTY and D. F. WILLIAMS, *ibid.* **14** (1993) 906.
26. J. ASHBY and R. TENNANT, *Mutat. Res.* **257** (1991) 229.
27. B. S. CHONG, I. D. OWADALLY, T. R. PITT-FORD and R. F. WILSON, *Endod. Dent. Traumatol* **10(3)** (1994) 129.
28. M. A. GOMEZ CAMARILLO and C. MASCRES, *J. Dent. Que* **28** (1991) 201.

Received 11 December 1996
and accepted 1 September 1997