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Pulp response to collagen and glutaraldehyde in pulpotomized primary teeth of baboons

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Abstract

This investigation assessed histologically the pulp tissue reaction to glutaraldehyde (GA) and to a commercial collagen preparation in pulpotomized primary teeth of baboons. One hundred and eighty-eight primary teeth were pulpotomized; in half of them inflammation was induced prior to the treatment. The teeth were divided into five groups: in three of them GA was used as a pulp dressing and applied for 1 min (group 1), 5 min (group 2), or mixed into the paste (group 3); collagen was used in group 4 and in group 5 (control) IRM was placed directly over the pulp stumps. Follow-up times were two, eight, and 24 weeks. Total necrosis was observed only in the collagen group. Partial necrosis and severe inflammation also were seen mainly in this group, and when the GA was incorporated into the paste. Slight to moderate inflammation was evident in all groups two and eight weeks postoperatively; however, 78% of the teeth of group 2 (GA 5 min) were inflammation-free after 24 weeks. Partial dentin bridges were seen in 92% of the teeth of the control group, in 82% of group 2, and 50% each of groups 1 and 3 eight weeks postoperatively. Dentin bridges were present in only 4% of the collagen group. After 24 weeks, all the teeth in group 2 and 83% of group 1 had dentin bridges. We conclude that Zyderm® (Colagen Corp. Palo Alto, CA) led to unacceptable results, 5 min application of GA presented the best healing response, and GA 1 min and IRM also were satisfactory.

Introduction

Formocresol is still the pulp dressing most frequently utilized in clinical practice. A recent survey demonstrated that the vast majority of pediatric dental practitioners in Canada (92.4%) and worldwide (76.8%) utilize either the full strength or the 1:5 dilution of formocresol as the preferred pulpotomy medicament for vital primary teeth (Avram and Pulver 1989). Despite these facts, following several reports demonstrating its toxic and other deleterious effects, finding a substitute to formocresol has been looked upon as imperative by many investigators (Block et al. 1977, 1978; Magnusson 1978; Myers et al. 1981; Fuks et al. 1983; Myers et al. 1983).

It would be ideal to use a biologic material that would lead to healing of the pulpotomy wound. Previous reports in which an enriched collagen solution was used demonstrated favorable results (Bimstein and Shoshan 1981; Fuks et al. 1984), and proliferation of connective tissue cells and blood vessels were observed coronal to the newly formed dentin bridges. The enriched collagen preparations employed in these studies consisted of native collagen extracted from the animals' skins, and is therefore, impractical for clinical use.

Glutaraldehyde (GA), a mild fixative, has been suggested as a possible alternative to formocresol, mainly for being less toxic; its positive effect, particularly in a 2% concentration, has been demonstrated in several studies (Davis et al. 1982; Ranly 1983; Tagger and Tagger 1984; Garcia-Godoy 1986; Fuks et al. 1986). Most animal studies utilizing GA as a pulpotomy agent report short-term results, no longer than two months (Davis et al. 1982; Tagger and Tagger 1984; Fuks et al. 1986a). Rölling and Thylstrup (1975) demonstrated that the success rate of primary molars treated with formocresol pulpotomies decreased as the follow-up time increased. A similar type of tissue reaction could occur with GA after a protracted postoperative time.

Another point to be considered is that the effects of different materials on the pulp were tested on healthy teeth (Myers et al. 1981, 1983). Conversely, pulpotomies in a clinical situation are performed, in most cases, in teeth with eventual coronal pulp inflammation, caused by caries. Trying to mimic the clinic situation in an animal model could lead to more meaningful results.

Before adopting the GA technique and recommending it for routine clinical practice, several questions still remain to be answered:

1. What is the ideal time and mode of application of GA?
2. Would the effect of GA on healthy pulps be

similar to those with localized, induced coronal pulp inflammation?
3. Would long-term results be favorable and similar to those with short-term follow up?

In the present study, we will attempt to find the answers to these questions. Therefore, the objectives of this investigation were to assess histologically the pulp tissue response of pulpotomized primary teeth of baboons to the following:

1. A commercial collagen preparation (Zyderm), with and without induced inflammation.
2. Different times and modes of application of GA in similar conditions (with and without inflammation).
3. A commercial Zinc Oxide Eugenol preparation (IRM) also with and without inflammation.

Materials and Methods

The study sample was 188 primary teeth (96 incisors, 48 cuspids and 44 mandibular molars) of 12 baboons, whose age ranged between 1–1/2 and 2 years. In half of these teeth, inflammation was induced by inserting human carious dentin into buccal or occlusal cavities for a period of three to five days prior to performing the pulpotomy (Lervik and Mjör 1977). This technique has been utilized previously by the authors in a pilot study, and inflammation, limited to the cavity area, was seen in all the teeth tested (Fuks et al. 1986a). The sampling of the teeth was done in such a way that all treatment groups were represented in every animal. The distribution of the teeth by group and follow-up time is presented in Table 1.

The animals were immobilized with ketamine (10 mg/kg body weight) and preoperative radiographs were exposed to evaluate the degree of root resorption of the teeth. Only teeth with full roots and without signs of pathosis and/or advanced resorption were included in the experiment.

Pulpotomy Technique

All treatments were done with the animals anesthetized with sodium pentobarbitone titrated to effect and endotracheally intubated. The teeth were isolated with rubber dam, and cleaned with a 2% chlorhexidine solution. Access to the pulp chamber was gained using a #330 tungsten bur, mounted on a high-speed handpiece under water coolant. Coronal pulp resection was performed with a sterile round bur in a slow-speed engine followed by saline rinses. Hemostasis was attained by placing a cotton pellet moistened in sterile saline with slight pressure. Following hemostasis the following materials were applied over the pulp stumps:

1. A cotton pellet moistened with a freshly prepared 2% buffered GA solution was placed for 1 min (groups 1a and b) and for 5 min (groups 2a and b)
2. A collagen solution covered with a layer of sterile wax (groups 4a and b)
3. A layer of IRM mixed with a drop of GA (groups 3a and b)
4. A layer of IRM (groups 5a and b).

In groups 1 and 2 (a + b), the cotton pellet was removed and a layer of IRM was placed over the pulp stumps. All teeth were sealed with a thick mix of IRM.

At the various time intervals listed in Table 1, the baboons were given an IV overdose of sodium pentobarbitone and perfused with saline followed by a phosphate-buffered GA solution, according to the technique described by Cox et al. (1987). The mandibulae and maxillae were removed en block and cut vertically in three blocks each, one anterior and two posterior. The blocks were fixed in Bouin Hollande solution (Lillie 1965), demineralized in 10% EDTA, and separated into individual teeth. These were then trimmed, embedded in paraplast, and cut longitudinally to obtain serial 6µm thin sections. Every fifth section was stained with H & E and examined under a light microscope. The results were assessed by "blind testing" of the different preparations and ranked according the criteria by Horsted et al. (1981) modified by Fuks et al. (1986a):

1. Degree of inflammation
   a. None — vital pulp, absence of inflammation

Table 1. Distribution of teeth by pulp dressing agent and follow-up time

Experimental Groups	*2% Buffered (GA) Glutaraldehyde*			*Collagen Preparation*			*IRM (Control)*			*Total*
GA 1 min*	5	5	5	-	-	-	-	-	-	15
GA 1 min	5	5	5	-	-	-	-	-	-	15
GA 5 min*	5	5	5	-	-	-	-	-	-	15
GA 5 min	5	5	5	-	-	-	-	-	-	15
GA in IRM*	5	5	5	-	-	-	-	-	-	15
GA in IRM	5	5	5	-	-	-	-	-	-	15
Collagen *	-	-	-	12	12	10	-	-	-	34
Collagen	-	-	-	12	12	10	-	-	-	34
IRM*	-	-	-	-	-	-	5	5	5	15
IRM	-	-	-	-	-	-	5	5	5	15
Totals	30	30	30	24	24	20	10	10	10	188

* Inflammation induced prior to pulpotomy.

(Fig 1B)
   b. Slight — a few inflammatory cells limited to the pulpotomy site (Fig 2, next page)
   c. Moderate — inflammation evident below the pulpotomy site, but limited to the coronal third of the radicular pulp
   d. Severe — inflammation and circulatory disturbances affecting most of the pulp. Under this criterion were included teeth with partial necrosis (Fig 4 and 5, next page)
   e. Necrosis
2. Presence of a dentin bridge (Fig 1A and 6A, next page). Calcified tissue band formed across the pulp adjacent to the pulpotomy site
3. Presence of reparative dentin along the canal, below the dentin bridge area (Fig 6A)
4. Presence and regularity of an odontoblastic layer
   a. Regular — present all along the root canal (Figs 1B and 1C)
   b. Irregular — disrupted or existing in only part of the pulp canal (Fig 3, next page)
   c. Absence of the odontoblastic layer (Fig 5, next page).
5. Presence of calcifications in the pulp, not related to the bridge.

The data were analyzed with the Kruskal-Wallis one-way analysis of variance (ANOVA), using the Siegel S. 1956 Statistical Package for the Social Sciences (SPSS), version 9.0, (1984).

In addition to these criteria, scores were attributed to the various parameters in order to assess the best histologic response by group. The following scores were given to the individual teeth:

Vital pulp, no inflammation = 4
Vital pulp, slight inflammation = 3
Vital pulp, moderate inflammation = 2
Vital pulp, severe inflammation = 1
Presence of dentin bridge = 1
Regular odontoblastic layer = 2
Irregular odontoblastic layer = 1
Partial necrosis, severe inflammation = 0.5

A higher score represented a better tissue response.

Results

One hundred and sixty-five teeth were evaluated histologically; 13 teeth had to be discarded due to filling loss or to faulty histologic preparation. No differences were found between teeth in which pretreatment inflammation was induced when compared to those without it; therefore the findings were pooled, and are presented together. In addition, since the differences between the collagen and the other groups were extreme, it was decided to exclude it from the ANOVA.

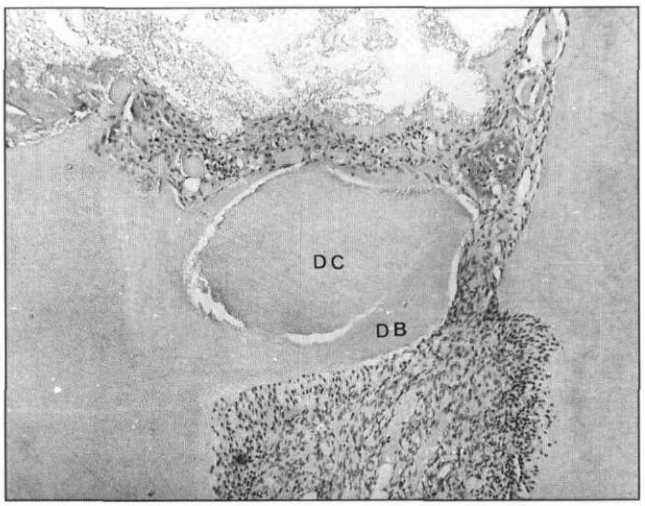
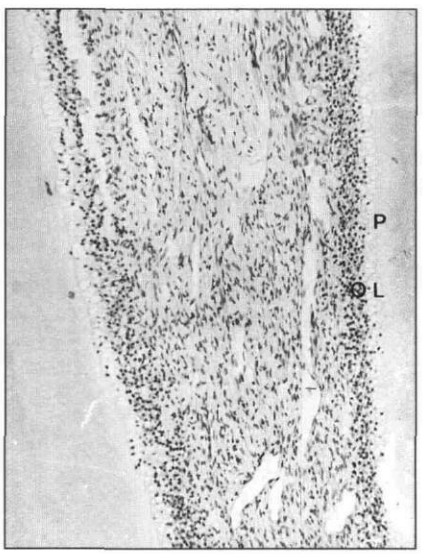
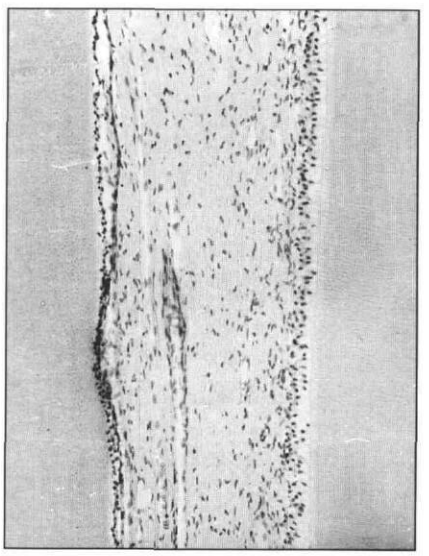


Fig 1. A. Maxillary central incisor treated with 5 min GA (Group 2) eight weeks post-pulpotomy. H&E 75x.



B. (left) Coronal third of the radicular pulp. Notice the partial dentin bridge (DB) formed around a dentin chip (DC) and proliferation of cells coronal to it.

C. (right) Middle third of the radicular pulp showing a regular odontoblastic layer (OL), predentin (P) and no inflammation.



Two Weeks Postpulpotomy

Considerable difference was observed in the degree of inflammation two weeks after treatment. None of the teeth treated with GA presented severe inflammation or necrosis as opposed to those treated with collagen, of which 76% either were inflamed severely with partial necrosis or completely necrotic. Moderate inflammation was evident in 44% of the teeth in which GA was incorporated in the dressing paste, whereas in the other GA groups or at the control (IRM) moderate inflammation was seen in 11 and 9% respectively (Table 2).

The presence and regularity of the odontoblastic layer of the teeth two weeks postpulpotomy are presented in Table 3. The pulp tissue response was superior in the teeth where the GA was placed over the pulp stumps for 5 min; a regular odontoblastic layer was evident in 89% of these teeth as opposed to less than 15% in the other GA and collagen groups. In the control group, the odontoblastic layer was regular in 64% of the teeth. The odontoblastic layer was absent in 57 and 22% of the teeth of the collagen group and when the GA was incorporated in the paste, respectively.

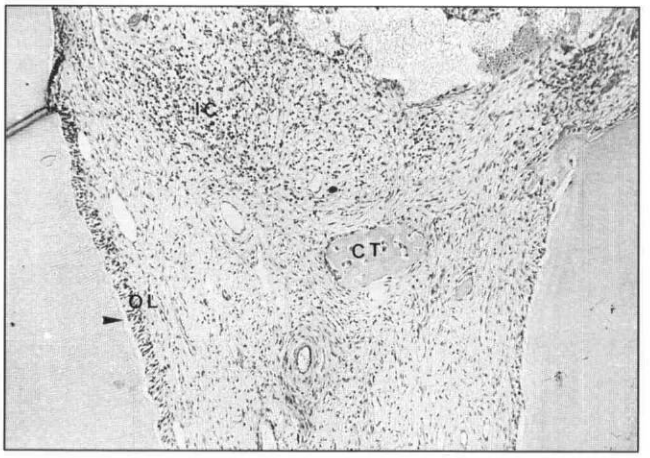


Fig 2. Maxillary cuspid of group 1 (1 min GA) with slight inflammation (two weeks postoperative). Scattered inflammatory cells (IC) are evident just below the pulpotomy site. Note the presence of calcified tissue (CT) with cell inclusions and a regular odontoblastic layer (OL) adjacent to predentin (arrow). H&E 75x.

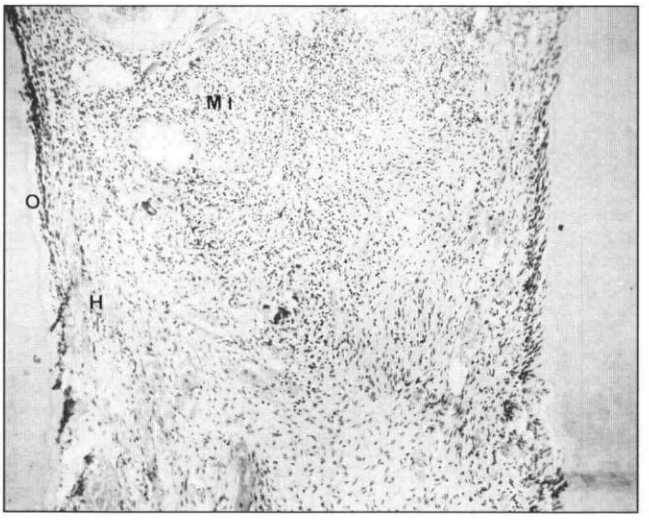


Fig 3. Mandibular cuspid of group 3 (GA incorporated into the paste) eight weeks postpulpotomy. Moderate inflammation (MI) with hemorrage (H) is present in the coronal third of the pulp and the odontoblastic layer (OL) is irregular. H&E 75x.

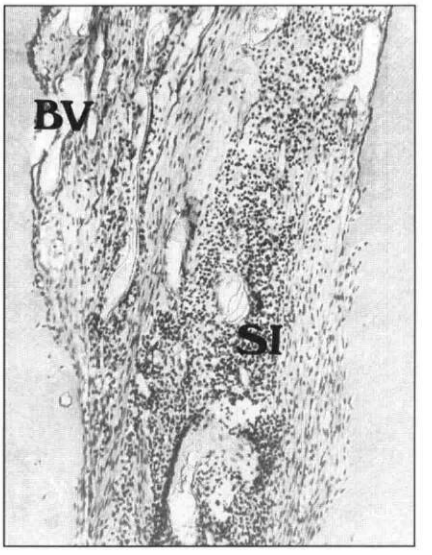


Fig 4. Mandibular central incisor of group 3, 24 weeks after treatment (middle third). Note a severe inflammatory infiltrate (51) with an increased number of blood vessels (BV). H&E 75x.

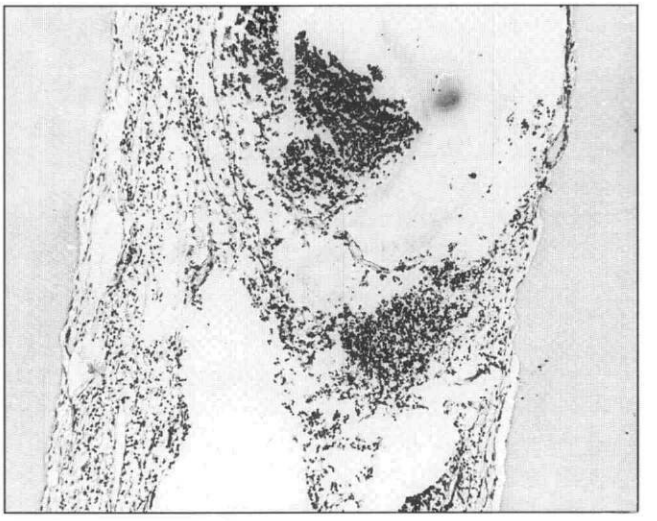


Fig 5. Maxillary cuspid eight weeks after pulpotomy with Zyderm (group 4). Severe inflammation and partial necrosis are evident. H&E 75x.

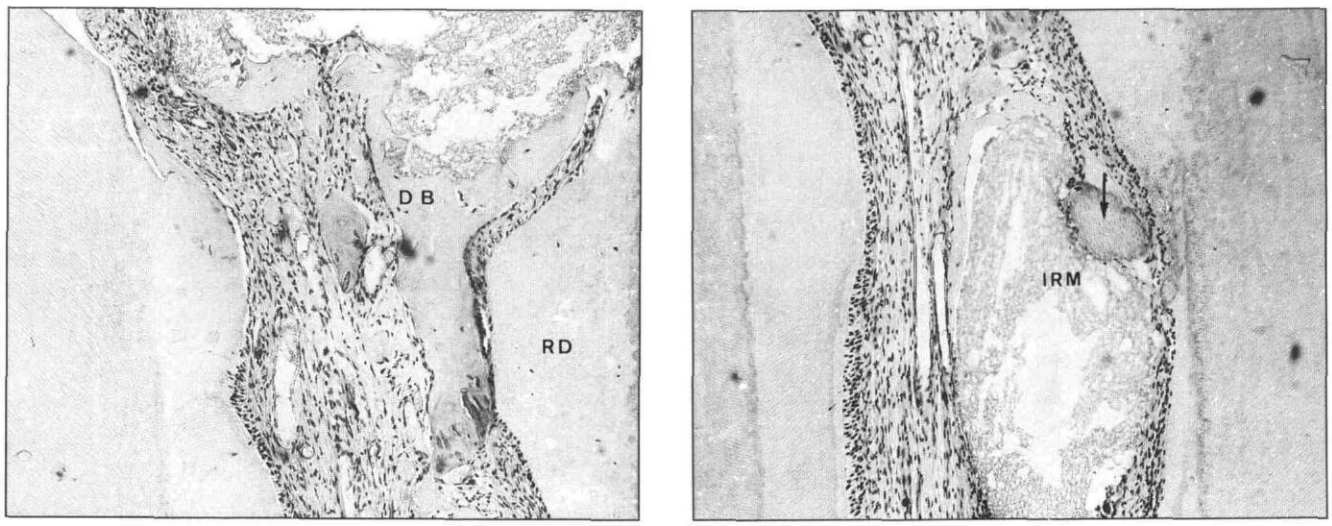


Fig 6. Mandibular cuspid of the control group (IRM, eight weeks postoperative). **A.** (left) Coronal third of the radicular pulp. A partial dentin bridge with cell inclusions (DB) is evident below the amputation site and a considerable amount of reparative dentin (RD) has been layed down along the root canal. H&E 75x. **B.** (right) A normal pulp around a fragment of IRM and a dentin chip (arrow) that has been pushed inadvertently into the pulp is seen in the middle third. H&E 75x.

Eight Weeks Postpulpotomy

The differences between the groups were even more striking eight weeks postpulpotomy. More than 95% of the teeth in the collagen group were either completely necrotic or severely inflamed, whereas all the teeth in the other groups were vital and had inflammation to a lesser degree. Approximately 1/3 of the control teeth and those in which GA was not included in the paste were free of inflammation; moderate inflammation was seen in 20% of the teeth of group 1 (GA 1 min) and in 50% of those when the GA was incorporated in the paste (Table 4, next page). These differences were not statistically significant when the GA groups and the control were compared. However, the difference in inflammation among the GA groups was significant ($P < 0.05$), being more pronounced between groups 2 and 3 ($P < 0.02$).

Table 2. Degree of inflammation 2 weeks postpulpotomy

	Degree of Inflammation											
	None		*Slight*		*Moderate*		*Severe*		*Necrosis*		*Totals*	
Experimental group	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
1 - GA 1 min	-	-	8	89	1	11	-	-	-	-	9	100
2 - GA 5 min	-	-	8	89	1	11	-	-	-	-	9	100
3 - GA in paste	1	12	4	44	4	44	-	-	-	-	9	100
4 - Collagen	-	-	3	14	2	10	8*	38	8	38	21	100
5 - IRM	-	-	10	91	1	9	-	-	-	-	11	100

* Includes 7 teeth with partial necrosis

Table 3. Presence and regularity of the odontoblastic layer 2 weeks postpulpotomy

	Odontoblastic Layer							
	Regular		*Irregular*		*Absent*		*Total*	
Experimental Group	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
1-GA 1 min	1	11	8	89	-	-	9	100
2-GA 5 min	8	89	1	11	-	-	9	100
3-GA in paste	1	11	6	67	2	22	9	100
4-Collagen	3	14	6	29	12	57	21	100
5-IRM	7	64	4	36	-	-	11	100

The odontoblastic layer was absent in 78% of the teeth in the collagen group. In contrast, this layer was regular in 50% of the IRM teeth, in 60 and 82% of the teeth groups 1 (GA 1 min) and 2 (GA 5 min) respectively. An irregular odontoblastic layer was mostly seen (75%) when the GA was incorporated in the paste (Table 5). No statistically significant differences were found between the groups when the collagen was excluded. A significant difference was found when only the GA groups were compared ($P = 0.05$), mainly be-

tween groups 2 and 3 ($P \leq 0.02$).

Most of the control (92%) and those in group 2 (82%) presented partial dentin bridges; in only 1 tooth (4%) of the collagen group was a rudimentary dentin bridge observed (Table 6). Dentin bridges were seen in 50% each in groups 1 and 3.

Table 4. Degree of inflammation 8 weeks postpulpotomy

	Degree of Inflammation Post-pulpotomy											
	None		Slight		Moderate		Severe*		Necrosis		Totals	
Experimental group	N	%	N	%	N	%	N	%	N	%	N	%
1 - GA 1 min	3	30	5	50	2	20	-	-	-	-	10	100
2 - GA 5 min	4	36	7	64	-	-	-	-	-	-	11	100
3 - GA in paste	-	-	4	50	4	50	-	-	-	-	8	100
4 - Collagen*	0	-	1	4	-	-	8*	35	14	61	23	100
5 - IRM	4	33	5	42	3	25	-	-	-	-	12	100

* Includes 4 teeth which had partial necrosis.

24 Weeks Postpulpotomy

The teeth of group 2 (GA 5 min) presented the best response when compared to the other groups. Of the 21 teeth treated with collagen, 5 showed signs of severely inflamed pulp, while the other 16 were completely necrotic. Severe inflammation was observed in 17% of the teeth treated with 1 min GA and in approximately 20% of those where the GA was incorporated into the paste. Conversely, the majority of the teeth (78%) of group 2 (GA 5 min) were inflammation-free as opposed to none in group 1 (GA 1 min), 11% in group 3 (GA incorporated into the paste), and in 29% of the controls (IRM) (Table 7). These differences were not statistically significant. When the GA groups only were compared, a significant difference was observed ($P < 0.03$). In addition, a significant difference was found between groups 2 and 3 ($P < 0.01$) and between group 2 and the control ($P < 0.01$).

The odontoblastic layer was regular in all the teeth of group 2 (GA 5 min) as compared to 67% in group 1 (GA 1 min), 44% in group 3 (GA incorporated into the paste) and in 29% of the controls (IRM) (Table 8). A statistically significant difference was found between the groups ($P < 0.02$).

Dentin bridges were evident in all the teeth treated with 5 min GA, in 83% of those treated with 1 min GA, in 57% of the IRM group, and in 44% when the GA was incorporated into the paste (Table 6).

Fig 7 is a graphic representation of the total scores summarizing the tissue response to the various parameters analyzed eight and 24 weeks postoperatively. When the parameters assessed were scored to obtain an overall tissue response, the highest value was observed in group 2 (score 6) after eight weeks; similar scores were evident in groups 1 (GA 1 min) and 5 (IRM), 5.2 and 5.5 respectively, and the lowest score (4.2) was presented by group 3 (GA incorporated in the paste). The score of the collagen group was so low that it was not even taken into consideration for comparison.

At 24 weeks postoperatively the tissue response in group 2 was considerably better than in the other group. Thus the score in this group increased from 6 after eight weeks to 6.6; the scores in the other groups remained similar and in group 5 it decreased.

Discussion

The main objective of the pulpotomy procedure in primary teeth is to preserve the tooth until normal exfoliation. It is known that the dental pulp has the potential for recovery from injury under favorable conditions (Seltzer and Bender 1975).

The surgical removal of the coronal pulp causes

Table 5. Presence and regularity of the odontoblastic layer 8 weeks postpulpotomy

	Odontoblastic Layer							
	Regular		Irregular		Absent		Total	
Experimental Group	N	%	N	%	N	%	N	%
1-GA 1 min	6	60	4	40	-	-	10	100
2-GA 5 min	9	82	2	18	-	-	11	100
3-GA in paste	2	25	6	75	-	-	8	100
4-Collagen*	-	-	5	22	18*	78	23	100
5-IRM	6	50	6	50	-	-	12	100

* 14 teeth had total necrosis.

Table 6. Dentin bridge formation 8 and 24 weeks postpulpotomy

	Dentin Bridge Formation			
	8 weeks		24 weeks	
Experimental Group	N	%	N	%
1-GA 1 min	5	50	5	83
2-GA 5 min	9	82	9	100
3-GA in paste	4	50	4	44
4-Collagen	1	4	-	-
5-IRM	11	92	4	57

injury, and it would be ideal to utilize a dressing material that would lead to healing of the pulpotomy wound and allow for the continuity of the normal pulp physiology. In the present study, the healing potential of a commercial collagen preparation was tested. Since previous reports utilizing an enriched collagen solution (ECS) revealed pulp healing in most of the teeth treated utilizing the same technique (Bimstein and Shoshan 1981, Fuks et al. 1984), the results obtained with this collagen preparation were extremely disappointing. One contributing factor to these findings could be related to the use of a hot instrument to adapt the sterile wax. This procedure could have a deleterious effect on the underlying tissue. However, healing of the pulpotomy wound followed by proliferation of connective tissue cells located coronally to the dentin bridge has been observed in monkey teeth treated with the same technique but utilizing a native enriched collagen solution (ECS). The figures in the present study resemble those of the control group (wax and IRM) in the mentioned study, where only 20% of the teeth presented vital pulp two months after treatment (Fuks et al. 1984). Another reason for the high failure rate could be related to the material itself. Similar results were obtained when Zyderm was utilized in a pilot study (unpublished data). It is possible that the industrialized collagen does not maintain the healing properties of the native collagen.

Marginal leakage also could be involved in the low success rate with Zyderm. Adaptation of the IRM over sterile wax was done gently to prevent dislodging of the material, and could have inadvertently resulted in an imperfect seal. This might have contributed to the difference between the collagen and other groups, since the same sealing material (IRM) was utilized in all of them. Möller et al. (1983) demonstrated that the sealing capacity of IRM, widely utilized in pediatric dentistry practice, is superior to conventional ZnOE cements. Conversely, Pashley et al. (1988) claimed that the clinical success of IRM and ZnOE may be a result of their antimicrobial or anodyne properties instead of their sealing qualities, since microleakage was observed with time in teeth that had good initial seal. Since the overall tissue response was good in all groups, whether due to good sealing or to bactericidal properties of the IRM, it seems improbable that microleakage is responsible for the poor results obtained with the collagen preparation in the present study. The tissue response to IRM was good and similar to that observed when GA was used for 1 min. Thus, one could question the need of utilizing a fixative, even one with minor deleterious effects as has been attributed to GA (Davis et al. 1982; Ranly 1983). Even when the IRM was accidentally pushed into the coronal third of the radicular pulp in one tooth, it did not cause irritation, and the pulp appeared normal (Fig 6b, shown previously). Magnusson et al. (1981) reported that healing failed to occur in every root treated

Table 7. Degree of inflammation 24 weeks postpulpotomy

Experimental group	Degree of Inflammation: None N	None %	Slight N	Slight %	Moderate N	Moderate %	Severe* N	Severe* %	Necrosis N	Necrosis %	Totals N	Totals %
1 - GA 1 min	-	-	5	83	-	-	1	17	-	-	6	100
2 - GA 5 min	7	78	1	11	1	11	-	-	-	-	9	100
3 - GA in paste	1	11	5	56	1	11	2	22	-	-	9	100
4 - Collagen	-	-	-	-	-	-	5*	24	16	76	21	100
5 - IRM	2	29	3	42	2	29	-	-	-	-	7	100

* Includes partial necrosis.

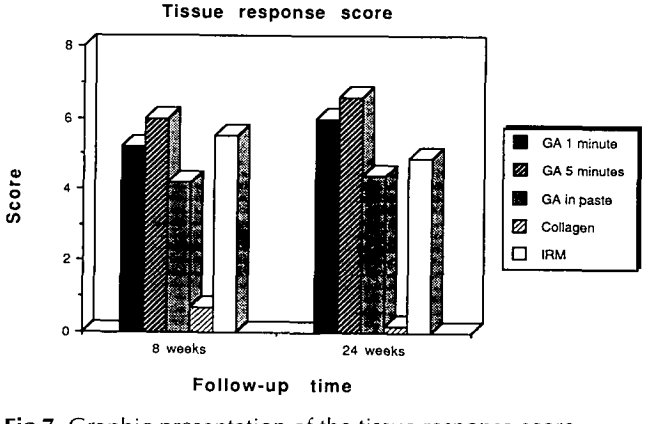


Fig 7. Graphic presentation of the tissue response score.

Table 8. Presence and regularity of the odontoblastic layer 24 weeks postpulpotomy

Experimental Group	Odontoblastic Layer: Regular N	Regular %	Irregular N	Irregular %	Absent N	Absent %	Total N	Total %
1-GA 1 min	4	67	2	33	-	-	6	100
2-GA 5 min	9	100	-	-	-	-	9	100
3-GA in paste	4	44	5	56	-	-	9	100
4-Collagen	-	-	-	-	21	100	21	100
5-IRM	2	29	5	71	-	-	7	100

when ZnOE was used as a wound dressing after pulpotomy, and chronic inflammation was observed histologically. They claimed that the absence of internal resorption with this material as opposed to what is seen when $Ca(OH)_2$ is utilized in pulpotomized primary teeth, is not the expression of good tissue response, but the result of severe pulp damage leading to the loss of the resorbing capacity of the cells. Since the tissue response to IRM in the present study was good, and inflammation, when present, was mild or moderate even after 24 weeks, one could wonder if the poor results described by Magnusson (1981) would not be the expression of preexisting chronic inflammation of the radicular pulp. The appearance, texture, and color of the pulp tissue, as well as the cessation of bleeding after amputation have been used in pediatric dentistry as indicators of the status of the radicular pulp. Since more precise diagnostic tools are not available in a clinical situation, pulpotomies might have been performed in teeth where this treatment would be histologically contraindicated.

Lloyd et al. (1988) suggest that the reaction of the pulp tissue to GA is related to the concentration and the time of application, since they observed that the depth of tissue fixation increased with the concentration and application time. The authors also observed aggressive internal resorption in teeth treated with low concentrations of GA and for lesser application times. Internal resorption was a rare finding in the present study, and was disclosed in only two teeth eight weeks postpulpotomy, but in none of the long-term (24 weeks) groups.

It seems to be that a longer exposure time to GA (5 min) leads to a better tissue response (Fig 7). This reaction pattern is similar to that observed in a previous study utilizing the same GA concentration and application time (Fuks et al. 1986). Dentin bridges were evident to a greater extent in the present study with 5 min GA, particularly 24 weeks postpulpotomy, when they were observed in all the treated teeth. It is interesting to notice that bridges were seen less frequently in the Fuks et al. 1986 article. One could speculate whether this difference would be due to the use of a soft mix of ZnOE over the pulp stumps followed by IRM sealing, as opposed to IRM only as in the present study. The presence of free eugenol as found in a soft, freshly prepared ZnOE mix could have a significant irritating effect to the tissue (Magnusson et al. 1981). Since the IRM was prepared following the manufacturer's instructions, and its setting time is short, one could assume that free eugenol would be present in a smaller amount than in the arbitrary mix of ZnOE. Cox et al. (1987) observed no signs of hard tissue repair when pulp exposures were capped with ZnOE, despite the fact that chronic inflammation was evident only at the exposure site, and "the deeper pulp was invariably free of inflammation." These authors, quoting Hanks et al. (1983), state that a low-grade irritation is needed to induce hard tissue repair. They reported that pulpal healing had occurred with ZnOE surface-sealed amalgam fillings without the association of new hard tissue formation. They believe that when no leakage is present amalgam is so inert to the pulp tissue that matrix formation and new hard tissue repair is not induced. The intermediate restorative material (IRM) is a modified ZnOE cement to which a resin has been added to improve its scaling and retentive properties. The deleterious effects of a resin to rat dental pulps have been demonstrated previously (Sela et al. 1973). Even if part of this effect would be related to microleakage and not to the resin per se, its mild irritation could function as a trigger to hard tissue formation.

Tagger and Tagger (1984) reported good tissue response to 5% GA just incorporated into the ZOE in monkey teeth. Conversely, Garcia-Godoy and Ranly (1987) reported a failure rate of 48.6% in 35 pulpotomized human primary molars utilizing a similar technique. They assumed that these results could be due either to 1) the 2% GA solution being inappropriate, or 2) because the eugenol diffuses from the paste faster than the GA. In the present study, incorporation of the GA into the paste resulted in the poorest tissue response, leading us again to the assumption that the poor results previously reported (Garcia-Godoy and Ranly 1987) could be the expression of the effect of GA over a preexisting inflamed pulp, or to a different immunological response of humans as compared to baboons. Additional clinical studies are necessary to disclose whether higher concentrations of GA and/or prolonged application times could lead to a decrease in the discrepancy between the human and animal studies.

Analyzing the individual parameters for the different groups did not express the general tissue response to each treatment modality. Therefore, attributing a score based on the criteria evaluated allowed us to have an overall assessment of the tissue reaction in every group. Although at eight weeks postoperatively the general tissue response was similar to both GA 1 min and GA 5 min and for IRM, the best reaction pattern was observed when GA was used for 5 min. This pattern improved at the 24 weeks examination, demonstrating that the healing potential of a 2% GA solution used for 5 min led to an excellent result in laboratory animals. Internal resorption was described in a clinical study in primary teeth (Fuks et al. 1986b) utilizing a 5-min application of 2% GA, as opposed to excellent results observed in the present and in a previous study, employing the same concentration and time of application

in laboratory animals. Further research is necessary to disclose the reasons for the discrepancy between the human and animals studies.

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