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EFFECT OF FLUORIDES ON ROOT RESORPTION IN REPLANTED RAT MOLARS

by

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INTRODUCTION

Reimplantation of teeth following accidental avulsion is usually followed by a process of root resorption. The amount of resorption depends on the developmental stage of the tooth, the time between extraction and replantation, the storage of the tooth, etc. (*Loe & Waerhaug*, 1961; *Andreasen & Hjørting-Hansen*, 1966; *Anderson, Sharav & Massler*, 1968, *Kaqueler & Massler*, 1969). This resorption may be intermittant and reparative, progressive and destructive, and may or may not be accompanied by ankylosis.

There is some indication that fluoride could reduce the amount of bone resorption (*Roholm*, 1937; *Epker*, 1966; *Baylink & Bernstein*, 1967; *Goldhaber*, 1967). The hypothesis that resorption of cementum and dentin could also be inhibited by fluoride is supported by a study by Shulman *et al.* (1968). They showed an inhibition of root resorption in tooth replants of Cebus monkeys after local application of sodium fluoride. *Bjorvatn* and *Weiss* (1971) found local application of stannous fluoride to be an effective means of preventing root resorption in replanted rat molars. (The teeth were replanted after having been soaked in a 10 % solution of SnF_2 for fifteen minutes).

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The following experiment was performed to test the influence of F on tooth replants where the resorptive processes had been greatly enhanced by drying out of the periodontium.

MATERIAL AND METHODS

Experimental group. Eighteen rats of the Sprague-Dawley strain, approximately forty days of age, were anesthetized (gaseous ether plus intraperitoneal injection of 0.1 % nembutal, 0.3 cc. per 100 gr. body weight). The first maxillary molars were extracted using specially designed forceps. The teeth were air dried on the laboratory table for 30 to 60 minutes. No special precautions were taken to keep the teeth under sterile conditions. Just before replantation the teeth were placed in a freshly prepared, saturated solution of SnF_2 (10 g SnF_2 in 90 cc. aqua dest., pH 2.2) for five minutes and then re-inserted in their original sockets.

The rats were sacrificed after one, three, and six weeks (Table 1.) The heads were skinned, hemisectioned, and placed in 10 % neutral, buffered formalin for fixation. Specimens were decalcified in formic acid/sodium citrate solution, and embedded in paraffin. Buccal-lingual sections were made at five microns through the main (mesial) root of the maxillary first molar. Sections were stained by hematoxylin and eosin, Mallory's triple connective stain or van Gieson silver nitrate stain.

Reference-group. The »normal» reaction to extraction/replantation of rat molars has been studied and described in detail by *Bendeich* (1969). As she was working under the same conditions, using rats of the same strain and age, her findings are taken as a reference (Table 1).

Controls. As controls for the three weeks series, and the six weeks series another group was used, consisting of 8 airdried teeth where no medication was made before replantation.

RESULTS

One week post operative, reference group

Immediate replants. No F applied. According to *Bendeich*, after one week immediate replants showed a good reattachment of gingival epithelium and periodontal ligament, i.e. the epithelial cuff was in close contact with the surface of the tooth, in most cases no pocket formation was evidenced. New fibres were formed, in a few specimens already crossing the torn area of the periodontium.

Table I.

Number of replanted teeth in the various groups

Length of observation Type of treatment:	1 week	3 weeks	6 weeks
Drying + saline (controls)		6	2
Drying + 10 % SnF_2	3	8	7
Immediate replantation Ref. series (Bendeich)	12	9	9 (5 weeks)
Drying + 2 % NaF		7	
Drying + 1 % SnF_2		9	

A slight resorption was seen in the alveolar bone. The coronal pulp showed regressive changes, and some venous stasis. Otherwise the pulpal tissue appeared normal. A large number of small resorption lacunae were found along the surface of the root (Fig. 1).

One week survival, experimental group

Teeth air-dried for 60 minutes and then immersed in SnF_2 10 % solution.

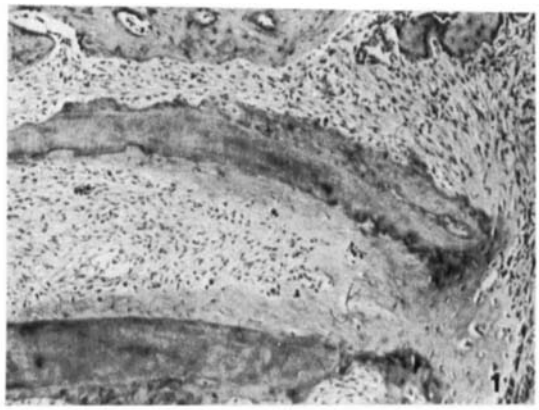
The three animals in this group demonstrated similar histopathologic pictures. The epithelial reattachment was poor, with some downgrowth of epithelial cells. The periodontal ligament showed slight to moderate inflammatory changes. Some blood clot was still present. The periodontal fibres were disintegrating, and few new fibres could be found. The pulp had undergone severe regressive changes, and the pulp chamber was filled with vacuoles surrounded by fibrotic tissue. Dilated blood vessels were seen in the root canal. The apical one-fourth of the root was filled with blood clot, which was undergoing organization from the periapical osseous tissue (Fig. 2).

Bone resorption, originating in the apical area, worked its way towards the alveolar crest and the basal bone. Some new bone formation was seen near the apex. There was no root resorption.

It was interesting to note, however, that a small root fragment, which broke off during extraction and remained in the jaw, demonstrated resorption lacunae, while the extracted, dried, and SnF_2 treated replant was intact (Fig. 3).

Three weeks survival

Reference series. Three weeks after extraction with immediate replantation (no medication) the histologic picture showed good to perfect epithelial



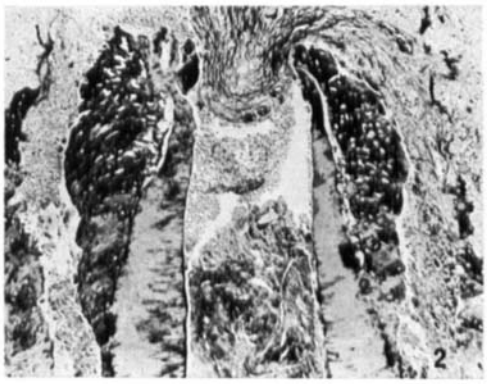


Fig. 1. Immediate replantation. No medication. 1 week survival. H & E, × 100. Shallow root resorption. Vital pulp.
Fig. 2. Delayed replantation (1 hour air drying). 10 % SnF_2. 1 week survival. Mallory, × 100. No root resorption. Almost necrotic pulp.
Fig. 3. Delayed replantation (1 hour air drying). 10 % SnF_2. 1 week survival. van Gieson. × 100. Resorption lacunae only in unextracted (fractured) part of the root.

reattachment with no pocket formation. The periodontal fibres were well re-established, with a functional orientation. Initial bone resorption had been more than compensated for, in some specimens resulting in localized ankylosis. When ankylosis was present, functional orientation of the periodontal ligament was absent.

In some of the teeth, the coronal part of the pulp was necrotic. Otherwise the pulpchambers were filled with vital, though slightly fibrotic, tissue. These three-week immediate replants showed internal and external root resorption of a moderate degree.

Control series. In a special three-week control series six teeth were kept dry for 30 minutes after extraction, and subsequently immersed in saline

for five minutes before being replanted in their original sockets. Histologic examination of the replants revealed a healing of much poorer quality than what was found in the immediate replant series.

The epithelial attachment seemed to be the least affected, even though one specimen demonstrated a marked downgrowth of epithelial cells. The periodontal ligament (PDL) showed very little repair. The remaining periodontal fibres were most often arranged parallel to the root surface, and as a moderate degree of bone resorption had widened the periodontal space, the teeth were slightly loose.

The pulp tissue was necrotic, with a varying degree of vital cells invading the pulp chamber through apex. Root resorption was seen in all specimens, ranging from a general, rather superficial resorption to an almost total loss of the root. (Figs. 4 and 5).

Experimental series. SnF_2 10 % treated group, eight teeth. The gingival (epithelial) cuff was fairly well reattached to the root surface. However, epithelial downgrowth had proceded in a few instances on the »deep» side of sequestrated parts of the alveolar crest.

The process of bone resorption was generally replaced by bone deposition. Considerable osteoclastic activity was evident apical to the alveolar crest. This part of the bone often appeared to be totally detached from the remainder of the bone, and, being partially surrounded by epithelium, was in the process of being exfoliated.

The root surface showed no signs of resorption. The greater part of the pulp was necrotic, but vital pulp tissue could be seen at the apex where invagination of periapical tissue occurred in some of the specimens (Fig. 6).

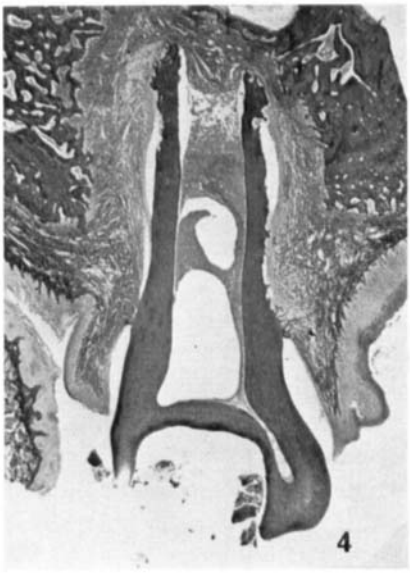
Six weeks survival

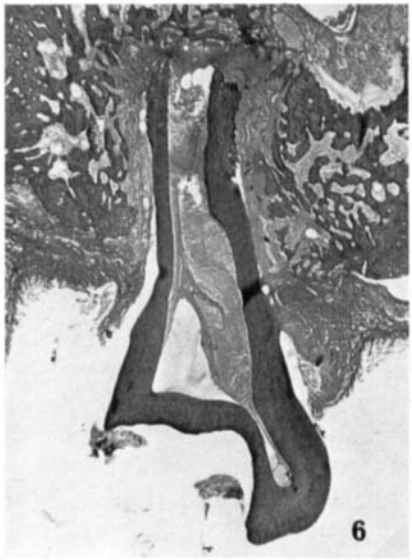
Reference series. Reattachment of the epithelial cuff at the cementoenamel junction was observed in all specimens.

The transseptal fibers were joined and functionally oriented. The periodontal space was of normal width with cellularity as well as fiber orientation normal. However, there were areas of root resorption where fibers were not attached. Other areas of resorption showed repair, i.e., deposition of a thin layer of cementum and, in these areas, fiber reattachment was clearly evident, even though half the thickness of the dentin had been resorbed.

Root resorptions were found in all these specimens, particularly in the apical regions.

The coronal pulp in most specimens contained some vital tissue. The fibrous stroma was rather coarse, but well supplied with blood vessels. In some specimens calcified deposits were seen within the pulp, while others





Figs. 4 and 5. Delayed replantation (30 min. air drying). No medication. 3 weeks survival. van Gieson, × 25. Moderate to great root resorption. Necrotic pulps.

Fig. 6. Delayed replantation (30 min. air drying). 10 % SnF_2. 3 weeks survival. van Gieson. × 25. No root resorption. Necrotic pulp.

Fig. 7. Immediate replantation. No medication. 6 weeks survival. van Gieson, × 25. Moderate root resorption. Vital pulp with osteodentin.

had a wide border of irregular tubular dentin along the dentin walls, lined by odontoblast-like cells. (Fig. 7).

Controls. A couple of special controls were left to bench-dry for one hour, then moistened in saline for five minutes before being replaced in their original sockets. These controls confirmed the observation that drying out of the tooth reduces the reparative ability of a replant. The general picture of the controls resembled that of the similarly treated specimens from the 3-week series. Root resorption had progressed still further, while the process of bone resorption had been replaced by bone formation (Fig. 8).

Experimental group. 7 specimens, air-dried 60 minutes, soaked in SnF_2 10 % for five minutes and replanted.

Epithelial attachment was moderately good, with a varying degree of pocket formation. In one specimen the epithelium extended practically around the apex of the tooth.

Some reattachment could be seen in the periodontium, but much less than in the series of immediate replants without medication. The orientation of the fibers was to a great extent parallel to the surface, rather than bridging the span between bone and dentin. Two teeth were lost in this series, probably due to lack of periodontal reattachment and subsequent increased epithelial downgrowth.

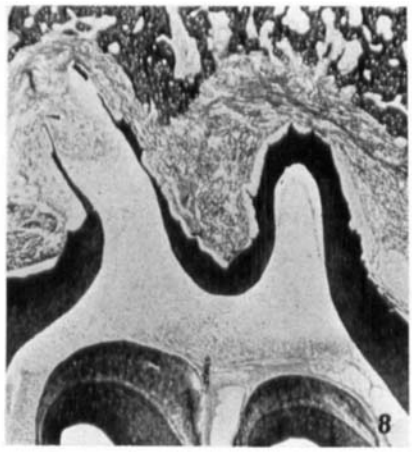
Active bone resorption seemed to have subsided to bone apposition. The new bone, however, had not completely compensated for the resorptive loss.

Five specimens were processed in this group, three of which had a considerable amount of vital tissue in the pulp. The root surface was practically without resorption, in striking contrast to the dried but non-fluoride treated control teeth. This fact is demonstrated in Figures 9 and 10, showing antimeres taken from the same animal.

Some of the fluoride treated replanted teeth showed the brownish stain of SnF_2 on the root surfaces. This suggests that the 10 % fluoride solution was too strong.

In order to determine the effect of weaker fluoride solutions an additional series was studied using 2 % NaF and 1 % SnF_2 applied to the extracted teeth for 5 minutes before replantation. 16 rats, approximately 40 days old, were subjected to the extraction/replantation procedure as described above. The rats were sacrificed three weeks after operation and processed as previously described.

The seven specimens treated by NaF 2 % solution showed a good epithelial reattachment, with occasional downgrowth of epithelial cells. The periodontal reattachment was also good, even though orientation of the fibers was less than perfect. The teeth were all well fixed within a rebuilt bony



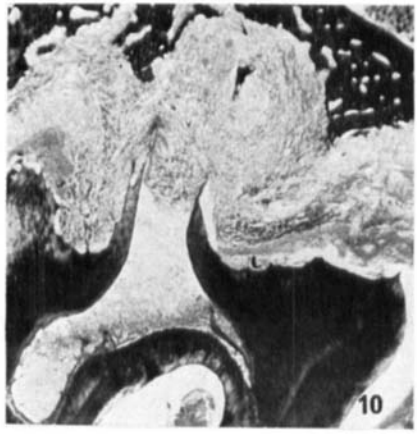


Fig. 8. Delayed replantation (1 hour air drying). No medication. 6 weeks survival. van Gieson. × 40. (Sagittal sectioning). General root resorption. Necrotic pulp.

Fig. 9. Delayed replantation (1 hour air drying) 10 % SnF_2. 6 weeks survival. Mallory, × 40. (Sagittal sectioning). No root resorption. Partly vital pulp.

Fig. 10. (Same animal as Fig. 9.) Delayed replantation (1 hour air drying). No medication. 6 weeks survival. van Gieson, × 40. (Sagittal sectioning). Great root resorption. Necrotic pulp.

socket. The pulp was sometimes necrotic, and areas of external root resorption were found. Some specimens showed localized areas of ankylosis. Fig. 11 gives the typical picture of a molar replant treated with a NaF 2 % solution.

Nine specimens treated with a 1 % solution of SnF_2 showed a definite increase in healing as compared with the SnF_2 10 % treated teeth. The epithelial cuff was well preserved, the broken periodontal fibers had to

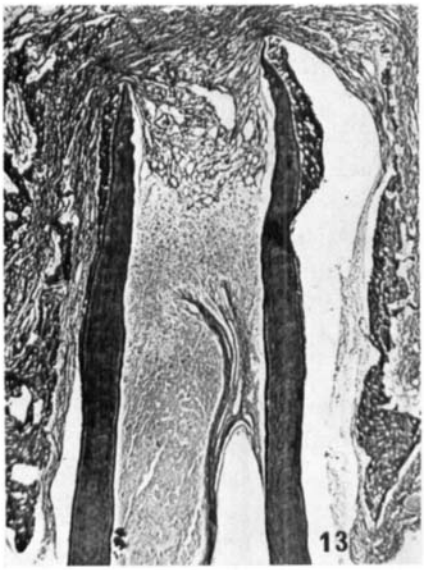


Fig. 11. Delayed replantation (30 min. air drying). 2 % NaF. 3 weeks survival. van Gieson, × 25. Some root resorption. Partly vital pulp.

Fig. 12. Delayed replantation (30 min. air drying). 1 % SnF_2. 3 weeks survival. van Gieson. × 25. No external root resorption. Vital pulp. Fairly good gingival reattachment.

Fig. 13. Delayed replantation (30 min. air drying). 10 % SnF_2. 3 weeks survival. van Gieson, × 100. No root resorption. Necrotic pulp. Poor gingival reattachment.

Fig. 14. (Same animal as Fig. 13.) Delayed replantation (30 min. air drying). 1 % SnF_2. 3 weeks survival. H & E, × 100. No root resorption. Partly vital pulp. Gingival reattachment.

some degree been repaired, and most of the pulps remained vital. Practically no root resorption was found (Fig. 12).

The difference in one animal between a tooth treated by 1 % SnF_2 and the contralateral treated by 10 % SnF_2 can be seen in Figures 13 and 14.

DISCUSSION

Local application of fluorides especially in the form of SnF_2 reduced root resorption in reimplanted rat molars. It was apparent that a 10 % solution of stannous fluoride had a detrimental effect on the healing process of the periodontal membrane, dental pulp, etc. By diluting the SnF_2 to a 1 % solution, these adverse reactions were at least partly avoided. The effect of locally applied NaF was less dramatic as far as root resorption is concerned.

The mechanism of stannous fluoride's influence on resorption of hard tissue is not known. Theories most often encountered are:

1. Direct action of fluoride on bone, cementum and dentin to change hydroxyapatite into fluorapatite, which is more resistant to resorptive processes (*Likins et al.*, 1963).
2. Specific inhibitory influence on the formation of the highly specialized cells responsible for the resorption: Osteoclasts, cementoclasts, etc. (*Roholm*, 1937, *Hudson*, 1961).
3. A combination of 1. and 2. might be considered a possibility.

The concentration of F^- ions is considerably higher in a 2 % NaF solution than in a solution of 1 % SnF_2. (0.48 and 0.13 molar resp.). The greater effect of 1 % stannous fluoride may be due to some special action of the tin (*Stookey et al.*, 1967, *Myers*, 1968) or to the difference in the pH between the two solutions, freshly made 1 % SnF_2 having pH 3.0, and 24 hours old 2 % NaF pH 7.5.

In teeth subjected to air drying before replantation, survival of the pulp was poor. The vital tissue found in the pulp chambers of some of the experimental specimens and also in the controls often seemed to represent a secondary invagination of periapical connective tissue.

Whether this should actually be called »pulp-tissue» is a question of semantics. Although the majority of the experimental teeth showed necrosis of the pulp, no apical abscesses were found. The functional »survival» of the tooth, therefore, does not seem to depend on vital pulp.

SUMMARY

Eighteen young rats, aged 30—40 days, were subjected to extraction and replantation of first maxillary molars. The teeth were airdried for at least

30 minutes before being replaced in their proper sockets to increase the amount of root resorption. The experimental teeth were soaked in 10 % aqueous solution of SnF_2 for five minutes. The rats were sacrificed after one week (3 rats), three weeks (8 rats) and six weeks (7 rats).

In another experimental series, 16 rats of the same age were treated similarly, with final soaking of the airdried teeth in 2 % NaF or 1 % SnF_2. These animals were sacrificed after three weeks.

These two experimental groups were compared to a series of similar rats, subjected to extraction of first maxillary molars, but with immediate replantation without medication.

For further comparison a control group was included consisting of airdried teeth without F application.

Local application of SnF_2 solution for 5 minutes prior to replantation greatly reduced the amount of root resorption in these extracted/reimplanted rat molars. In a 10 % solution the stannous fluoride had a detrimental effect on the healing of PDL, pulp and alveolar bone. If a 2 % NaF solution was used for soaking the dried teeth, root resorption was not totally prevented but the pulpal condition and periodontal situation was superior to those in the 10 % SnF_2 series. Best results were found in the group treated by 1 % SnF_2 for five minutes prior to replantation. These specimens showed a minimal amount of root resorption, good reattachment of epithelial cuff and PDL, and a remarkably good survival of the pulp, taking into consideration that the specimens had been airdried for at least half an hour.

Local application of fluorides to the roots of avulsed teeth may prove to be of clinical value.

RÉSUMÉ

ACTION DES FLUORURES SUR LA RÉSORPTION RADICULAIRE DANS LES MOLAIRES DE RATS APRÈS RÉIMPLANTATION

Dix-huit jeunes rats, âgés de 30—40 jours, ont subi l'extraction et la réimplantation des premières molaires supérieures. Ces dents ont été séchées à l'air pendant au moins 30 minutes avant d'être replacées dans leur propre alvéole, afin d'augmenter le degré de la résorption radiculaire. Les dents de l'expérience ont été plongées pendant cinq minutes dans une solution de 10 % de SnF_2. Les rats ont été sacrifiés au bout d'une semaine (3 rats), trois semaines (8 rats) et six semaines (7 rats).

Dans une autre série expérimentale, seize rats du même âge ont été traités de la même manière, mais les dents séchées à l'air ont à la fin été plongées

dans une solution de 2 % de NaF ou de 1 % de SnF_2. Ces animaux ont été sacrifiés au bout de trois semaines.

Ces deux groupes expérimentaux ont été comparés à une série de rats semblables, ayant subi l'extraction des premières molaires supérieures, mais avec réimplantation immédiate, sans traitement médicamenteux.

De plus, une comparaison a été faite avec un groupe témoin dans lequel les dents séchées à l'air n'ont pas subi d'application de F.

L'application locale de solution de SnF_2 pendant 5 minutes avant réimplantation a fortement réduit le degré de résorption radiculaire dans ces molaires de rats extraites et réimplantées. En solution à 10 %, le fluorure stanneux avait un effet nuisible sur la cicatrisation du desmodonte, de la pulpe et de l'os alvéolaire. Lorsqu'on plongeait les dents séchées dans une solution de NaF à 2 %, la résorption radiculaire n'était pas entièrement empêchée, mais l'état de la pulpe et du parodonte était supérieur à celui de la série utilisant le SnF_2 à 10 %. Les meilleurs résultats ont été trouvés dans le groupe traité par le SnF_2 à 1 % pendant cinq minutes avant la réimplantation. Ces dents présentaient un degré minime de résorption radiculaire, un bon réattachement du manchon épithélial et du desmodonte, et une survie pulpaire remarquablement bonne, lorsqu'on prend en considération le fait que les dents ont été séchées à l'air pendant au moins ½ heure.

Il est possible que l'application locale de flourures aux racines des dents après avulsion se révèle avoir une valeur clinique.

ZUSAMMENFASSUNG

TIEREXPERIMENTELLE ZAHN-REPLANTATIONEN. DIE WIRKUNG VON LOKAL APPLIZIERTEN FLUORIDEN AUF DIE WURZELRESORPTION AN RATTENMOLAREN

In zwei Versuchsgruppen von je 18 und 16 Sprague-Dawley Ratten wurden die oberen, ersten Molaren extrahiert und wieder replantiert, nachdem die Zähne 30 bis 60 Min. getrocknet, und danach 5 Min. teils in 10 % oder 1 % SnF_2, teils in 2 % NaF getränckt wurden.

Die lichtmikroskopischen Untersuchungen des Fluorideneffektes wurde nach einem Versuchdauer von einer, drei und sechs Wochen durchgeführt.

Die beiden Konzentrationen der SnF_2 verhinderten die Wurzelresorption; 2 % NaF nicht im gleichen Grade. Allerdings wurde eine Heilungsstörung des Parodontiums und der Pulpa bei der 10 % SnF_2 beobachtet.

Die Autoren halten es für möglich, dass lokale Applikation von niedrigkonzentrierten Fluoriden auf replantierte Zähne von klinischer Bedeutung sein kann.

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