The Influence of Extra-Oral Time upon Healing after Tooth Replantation

—A histological study in rat incisors—

by

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Abstract

The authors studied, through replantation of rat incisors, the effect of extra-alveolar time upon postoperative events. Root resorption and ankylosis seemed to be particularly affected by this factor.

Keywords: Replantation, Extra-alveolar permanence, Root-resorption.

Introduction

Among the changes observed after dental replantation, cement-dentin resorption was the most significant pathological alteration, not only for its constancy [1, 2] but also for being by itself one of the causes of failure.

All observations indicate that these resorptions are related to the inflammation of the periodontal ligament, as a consequence of surgical trauma [3, 4, 5]. The periodontal ligament, by the way, seems to play an important role in replanted teeth [5, 6, 7, 8, 9, 10].

Löe and Waerhaug [7], after replantations in dogs and monkeys, verified the absence of ankylosis when the surgical act was performed with preservation of the vitality of the periodontal ligament. On the other hand, when the periodontal ligament was removed or dehydrated before replantation, resorption and ankylosis were a common feature.

Butcher and Vidair [11], through immersion of the teeth to be replanted in 10% alcohol or in water at 60°C, observed resorption and ankylosis areas on all root surfaces, as well as inflammatory areas, restricted especially to the apex and gingival sulcus. Ankylosis was more frequent in the teeth treated with formalin or alcohol, while resorption was greater in the teeth treated with formalin.

In an attempt to avoid root resorption, Shulman et al. [12] kept the teeth immersed in a 1 M solution of sodium fluoride for 18–54 hours before replantation. In accordance with the preliminary results, they suggested that such a treatment is a viable clinical procedure to inhibit resorption.

On the other hand, Barbakow et al. [9] could not reduce the degree of resorption and ankylosis by immersing the teeth in 2% acid sodium fluoride, pH 5.5, for 30 minutes before replantation. More recently, Hardy et al. [13] used polilatic acid to cover the tooth root before replantation and, according to their results, were...
only able to inhibit ankylosis, not resorption.

It seems probable that the occurrence of cement and dentin resorption after replantation would be directly related to the period of permanence of the tooth out of the dental socket [1, 10].

Those results agree with the opinion of Heithersay [2] in that the more resorption and/or ankylosis occurs the longer the tooth remains out of the alveolus. This same view is shared by Barbakow et al. [9], who reported that resorption occurs in 10% of replanted teeth after 30 minutes and becomes severe when this period exceeds 120 minutes.

When the tooth remains out of the alveolus for about 15 minutes, periodontal reattachment is possible [14, 15].

Data obtained in the review of literature indicate the probability of several factors being associated with the development of root resorptions, as well as other changes after tooth replantation. One of the most significant factors seems to be the extra-alveolar period. Therefore we thought it opportune to study the influence of that factor in the development of alterations after dental replantations, by means of methods already standardized in other experiments [5, 16, 17].

So, the purpose of the present paper is to study the influence of extra-alveolar time upon cement and dentin resorption and other alterations, through the replantation of rat incisors.

Materials and Methods

In the present study, 108 male rats (Rattus norvegicus albinus, Wistar), weighing between 100 and 110 grams, were used.

All the animals were fed solid rations,* before and after surgery and water was given “ad libitum.”

After general anesthesia with sodium pentobarbital,** the upper right incisors of all the animals were extracted with adequate instruments [18].

The 108 rats were separated into 3 groups of 36 animals, which received the following procedures:

Group I — The incisors were immediately replanted in their respective sockets.

Group II — The incisors were kept in gauze soaked with physiological saline for 30 minutes, and then replanted in their respective sockets.

Group III — Same procedures as for group II, except that the extra oral time was 120 minutes.

The upper right incisors of all the groups were replanted with the help of clinical nippers (cotton forceps).

After replantation, the teeth were immobilized by a 4–0 silk ligature splint, at the level of the gingival free margin and also involving the opposite incisors. The splints were removed 24 hours after surgery.

Right after replantation, all animals were intraperitoneally injected with 15,000 IU penicillin G benzatin*** by means of a hypodermic syringe.

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Six rats in each group were killed by sulphuric ether inhalation 2, 5, 10, 20, 30, and 60 days after surgery. The maxillae were incised and separated by means of a scalpel with a fixed blade at the median sagittal plane; straight scissors were used posteriorly to the distal surface of the molars in order to obtain a piece with the replanted tooth, removing the excess tissue.

The pieces were then fixed in 10% formalin and decalcified in a sodium citrate and formic acid solution. After decalcification, the pieces were embedded in paraffin, cut in 6 µm thick semi-serial sections and stained with hematoxilin-eosin for histological study.

Results

The pulp and the dental papilla, as well as the enamel organ in the animals of groups II and III, presented necrosis 5 days after the operation. From the 10th day to the end of the experimental period, they remained without significant changes. Therefore, no reference will be made to the succeeding stages.

2 days

Group I

Cement and dentin seem intact, but dentin, at the vesticular side, however, undergoes deflection near the apex.

The periodontal ligament shows good blood supply in the apical third and part of the medium third (Fig. 1). In the remaining areas it is less evident and the space between the cement and alveolar bone is filled by a blood clot, with a few fibroblasts which display hyperchromatic nuclei.

The pulp, in the apical third, shows signs of vitality, presenting dilated blood vessels. In some areas, odontoblasts lose their characteristic alignment. At the apex, the pulp maintains its embryonic characteristics.

In some areas of the enamel organ, ameloblasts exhibit alterations in their

Fig. 1 Group I. Periodontal ligament at the level of the medium third with good vascularization. (2 days, Hematoxylin and eosin stain. Original magnification \( \times 100 \)).

Fig. 2 Group II. Periodontal ligament showing the fibroblasts with signs of degeneration. (2 days, Hematoxylin and eosin stain. Original magnification \( \times 100 \)).
arrangement.

The alveolar bone is apparently unchanged.

Group II

Cement and dentin seem to be thoroughly normal on the vestibular side, dentin is deflected at the apex.

The periodontal ligament generally shows little blood supply and the fibroblasts, when present, have pycnotic nuclei (Fig. 2). Blood clots are seen in many areas of the ligament.

The pulp exhibits degenerative signs as the pycnotic nuclei of fibroblasts and odontoblasts. The same occurs with the dental papilla and enamel organ, while the ameloblasts entirely lose their typical arrangement.

In one of the specimens, part of the enamel organ and the dental papilla seemed vital.

The alveolar bone is apparently normal.

Group III

Cement and dentin are apparently unchanged.

Small portions of the periodontal ligament remain around the apical third, adjacent to the alveolar bone (Fig. 3), and show a mild neutrophilic infiltration.

Rests of degenerate tissues and blood clot are seen in a few points of other areas.

The pulp as well as the dental papilla are degenerate. The enamel organ is absent in all specimens.

The alveolar bone wall is apparently intact on both sides.

5 days

Group I

Cement and dentin are apparently normal. Dentin, however, shows deflection at the apex both at the vestibular and lingual sides. At this level and more intensely on the vestibular side, this dentin maintains its mineralized structure, resembling bone.

The periodontal ligament is entirely vital and shows good blood supply. At the cervical third, a well-marked fibroblastic proliferation is seen at the level of the rupture of the periodontal ligament. In some cases, hyaline bulks are observed at the medium third with fibroblast resembling cells near them.

The dental pulp shows signs of vitality in the medium and apical thirds, and dilated blood vessels in other areas. At the apex, it keeps its embryonic characteristics.

In the enamel organ, at the apical third, the ameloblastic layer covers a mineralized structure with osseous tissue characteristics. This organ is apparently normal, however, towards the bottom of the alveolus.

The alveolar bone exhibits resorption areas on either side, especially on the vestibular wall.

Group II

Cement and dentin are apparently intact. Dentin exhibits deflection at the apex, especially on the vestibular side. At the cervical and medium thirds, the peri-
odontal ligament adjacent to the bone tissue shows a rich blood supply, but with a moderate number of neutrophils. The region next to the cement exhibits signs of degeneration. In some cases, a band is seen between such regions, in which the quantity of young fibroblasts and newly formed capillaries is remarkable. At the apical third, such connective tissue is well vascularized and rich in fibroblasts. Sometimes, these two portions are separated by rests of blood clot, without any organization.

The pulp as well as the dental papilla is entirely degenerated and exhibit pycnotic nuclei. The enamel organ is absent in all the cases.

The alveolar bone tissues, both at the vestibular and lingual sides, show a more intense resorption than in the anterior group.

Group III

Cement and dentin are apparently intact throughout. Dentin is deflected at the apex level at the vestibular side.

Next to the cement, the periodontal ligament exhibits degeneration at the cervical and medium thirds. On the other hand, the region inserted into the bone tissue shows vitality, with a moderate neutrophilic infiltrate with the exception of small areas at the cervical third. At the apical third level, no limit was seen between the two portions, although it was possible to see fibroblastic and capillary proliferation in the medium region of the periodontal ligament.

The pulp, dental papilla and enamel organ have totally degenerated.

The alveolar bone tissue exhibits many areas of resorptions on both sides and osteoclasts are numerous. In most cases, resorption is more intense at the lingual side.

10 days

Group I

In some areas, cement and dentin show incipient resorption (Fig. 4). In the
apical third, and especially at the vestibular side, dentin is deflected.

The periodontal ligament presents vitality almost all over. In some areas, from the alveolar bone, newly-formed bone spines invade the periodontal ligament without reaching the cement surface, however.

The dental pulp, from the medium third towards the apex, presents good vascularization.

In the enamel organ over the deflected dentin, the ameloblast layer is interrupted, being, however, unchanged towards the apex.

The alveolar bone tissue presents moderate resorption on both sides and numerous osteoclasts. This resorption region is generally located at the alveolar cervical and medium thirds. At some areas of the lingual side, little newly-formed bone spines invade the periodontal ligament.

Group II

Cement and dentin resorption appear in many areas, and it is more extensive than it was for the anterior group (Fig. 5). The dentin on the vestibular side shows the same aspects as seem for the 5 day period for the same group.

In two specimens, the periodontal ligament exhibits vitality throughout its extension. In others, connective tissue with signs of degeneration was evident at the cervical third. In all cases, however, the periodontal ligament space was partially occupied by newly-formed bone tissue.

The alveolar bone tissue undergoes intense resorption, particularly at the lingual side. At the cervical third, a great part of the alveolar wall is often resorbed. Sometimes, newly-formed bone tissue is observed on the periodontal ligament side, producing a narrowing of the space.

Group III

Cement and dentin undergo little resorption at many points, with the presence of dentinoclasts. Such resorption is approximately comparable with the one seen for the anterior group and the same period of time.

In two specimens, the periodontal ligament shows degenerative signs at the cervical and medium thirds. In the other cases, it displays vitality, although with little vascularization and a moderate number of neutrophils. In many areas, the periodontal space was occupied by newly-formed bone trabeculae.

The alveolar bone tissue shows resorption on both sides, in a degree approximately comparable with the one observed for the anterior group and the same period of time. Newly-formed bone is also seen at the periodontal space, often entirely occupying such space.

20 days

Group I

Cement and dentin exhibit 2 or 3 resorption areas which are generally superficial and small. The dentin at the vestibular and lingual sides no longer presents its morphological characteristics between the medium and apical thirds. At this region, mineralized tissue resembling bone for dentin is observed, and frequently occupies the pulp cavity. Such structure advances towards the apex without definite limits, as apparently normal dentin.
The dental pulp is vital at the medium and apical thirds. At many areas, however, mineralized masses are present and resemble either bone or irregular dentin (Fig. 6).

The dental papilla as well as the enamel organ is apparently normal.

The resorbed alveolar bone wall is generally substituted by cancellous bone, especially at the cervical third.

**Group II**

Cement and dentin undergo resorption in many areas. At some regions the resorption is extensive and practically involves the pulp cavity (Fig. 7). Sometimes the resorption area is filled by newly-formed bone.

In one of the specimens, there is a small portion of vital connective tissue at the apex, exhibiting morphologically indefinite mineralized masses in its interior, which present characteristics of either bone or irregular dentin. In others, an intense neutrophilic infiltrate is seen at the apex.

In the alveolar bone, the resorbed portion is substituted by trabeculated bone, which causes a narrowing of the periodontal space.

**Group III**

Cement and dentin present a degree of resorption which may be compared, in extension and depthness, to the one observed for the anterior group at the same stage.

At many areas with a poor blood supply, the periodontal ligament shows vitality and the fibers do not present a definite orientation.

At the periodontal space there is an increase in bone tissue neoformation. Such bone tissue often partially fills the gaps created by cement and dentin resorption (Fig. 8).

In two specimens, a dense neutrophilic infiltrate is observed at the apex, part of it in degeneration.

The resorbed alveolar bone wall is substituted by trabeculated bone, which
frequently fills the gaps formed by cement and dentin resorption, promoting ankylosis.

30 days

Group I

At the cervical third, cement and dentin exhibit resorption areas, which are similar to those seen after 20 days for the same group. At the medium third, the dentin on both sides loses its characteristic morphology turning into osseous tissue and/or irregular dentin, which often occupies the pulp cavity. Towards the apex, the dentin is apparently normal.

The vital periodontal ligament is replaced by newly-formed bone tissue at some areas, which occurs in a greater quantity than it was observed for the anterior stage of the same group. Fibroblasts and collagen fibers have a characteristic arrangement at several areas (Fig. 9).

The dental pulp exhibits vitality at the medium and apical thirds. In most cases, however, in a great part of the medium third, the dental pulp is replaced by mineralized tissue resembling bone and/or dentin.

The dental papilla as well as the enamel organ is apparently normal. The resorbed bone wall is replaced by trabeculated bone tissue, which narrows the periodontal space.

Group II

At many regions, cement and dentin undergo resorption, which in most cases may be comparable with the one observed at 20 days for this same group. In some cases, however, a greater quantity of bone tissue was evident, occupying the spaces produced by cement and dentin resorption.

The vital periodontal ligament exhibits fibroblasts and collagen fibers, without a definite arrangement. In all the cases, a greater quantity of bone tissue in the periodontal space is observed when compared with the anterior stage of the same group.
At the apex level of two specimens an intense polymorphonuclear neutrophilic infiltrate is seen, with many neutrophils undergoing degeneration.

At the resorbed alveolar bone wall, there is a greater quantity of trabeculated bone tissue.

Group III

At various points cement and dentin resorption is much more extensive and deeper than the one observed in the previous stages. Sometimes resorption brings about a communication between the periodontal ligament and the pulp cavity.

In other instances the resorbed cement and dentin are replaced by bone tissue.

The periodontal ligament, although vital, presents fibroblasts and collagen fibers without a definite arrangement.

Furthermore, the ligament thickness is frequently reduced due to bone neoformation at that region. The newly-formed bone substitutes the resorbed cement and dentin at some areas.

At the apex of 3 cases, there is a great infiltration of polymorphonuclear neutrophils, some of which are undergoing degeneration.

The resorbed alveolar bone wall is replaced by trabeculated bone tissue, which often partially fills the gaps left by root resorption.

60 days

Group I

In one case, the dentin shows resorption areas at the cervical and medium thirds, which may be comparable with the one from the anterior stage. In the other cases, both cement and dentin are apparently unchanged.

The vital periodontal ligament is replaced by newly-formed bone tissue at some areas, which caused an almost thorough narrowing of the periodontal space (Fig. 10). Fibroblasts and collagen fibers, however, have a characteristic arrangement.

The dental pulp is vital, except in one case, and exhibits good vascularization. The odontoblasts are typically arranged.

The dental papilla and the enamel organ are apparently normal, except in one
The resorbed bone wall is replaced by trabeculated bone tissue.

Group II

In many sites, cement and dentin show deep areas of resorption, which frequently reached the pulp cavity. The resorbed cement and dentin are often partially replaced by bone. In two specimens, a small band of newly formed cement is observed at the level of the cervical third (Fig. 11).

In most cases, an acute inflammatory infiltrate is seen at the apex, with many degenerate cells. In one case tooth germ structures were seen next to the alveolar fundus (Fig. 12).

The resorbed bone wall is replaced by trabeculated osseous tissue, which, at some areas, occupies the lacunae left after root resorption.

Group III

Cement and dentin exhibit deep and wide resorption areas which, in most cases, reach the pulp cavity. The lacunae are, however, filled by newly-formed bone tissue (Fig. 13).

In some cases, the dentin is partially resorbed at the level of the medium
third on the lingual side.

The periodontal ligament is present in small areas, either at the cervical third or at the apical third, since a great deal of it has been replaced by bone tissue.

In most cases, an intense neutrophilic infiltrate is seen at the apex.

The resorbed alveolar bone wall is replaced by trabeculated bone, which even occupies a great part of the periodontal ligament space as well as the spaces left after root resorption.

**Discussion**

In the present work, root resorption was observed in the three groups studied after the tenth postoperative day. Such resorption was slight for the immediately replanted teeth, and rather marked in those which remained for 120 minutes out of their sockets.

The resorption of cement and dentin, which is considered the main cause of failure in dental replantation, is related to operative trauma [3, 5, 16, 19, 20] and to the inflammatory process of the periodontal ligament [1, 21, 22].

According to OKAMOTO et al. [17], root resorptions in replanted teeth ought to be related to traumatic, inflammatory and immunological phenomena.

Whatever may be the causes of such resorptions, the permanence of the tooth out of its alveolus for more than 30 minutes undoubtedly aggravates the occurrence of these post-operative alterations.

These results agree with those reported by ANDREASEN & HJORTING-HANSEN [1], HEITHERSAY [2], BARBAKOW et al. [9] and KOUMARAS & FIELDING [10].

On the other hand, we may consider the "surgical trauma" factor as one that may have little influence on the different degrees of root resorption in the three experimental groups. The reason is that although the tooth has been kept out of the alveolus for different periods of time, the intensity of trauma was the same in all operations, except for a slight curettage and irrigation with saline for the removal of blood clots in group III. Nevertheless, we believe this curettage does not present any influence once it is carried out only to partially remove the blood clots.

Our results also confirm the important role of the periodontal ligament in the outbreak of root resorption [5, 7, 17]. This seems to be confirmed by the teeth replanted soon after extraction, which exhibited rapid revascularization and proliferation of new fibroblasts in the periodontal ligament. In addition, in this case, fibroblasts and collagen fibers showed a typical arrangement in the final stages. In the other groups, however, the part of the ligament which was inserted in the cement undergoes total degeneration and its substitution by new connective tissue is rather delayed.

It is greatly probable that the permanence of degenerate periodontal ligaments on the cement has caused the inflammation at the initial stages, thus contributing to the outbreak of root resorption.

On the other hand, the simple fact of the replanted incisors in groups II and III remaining without growing means they have undergone severe trauma during mastication, which could contribute to a more intense resorption in those teeth [5].

It is, however, accepted that other factors may be involved in the etiology of
such resorption, including immunological factors [17].

Some authors admit that the replanted tooth may act as a foreign body [23].

It is interesting to point out that bone trabeculae formation in the periodontal ligament occurred more intensively and faster in groups II and III.

The appearance of these osseous trabeculae at the initial stages ought to be a metaplastic process, as a consequence of local metabolic changes.

In the more advance stages, however, it may be a healing process of the resorbed bone cortex. Starting at the cortex, osseous neoformation is progressive and, in groups II and III, fills up the gaps caused by root resorption, thus bringing about ankylosis.

Dento-alveolar ankylosis, which may be one of the causes of failure in such operations, was also observed elsewhere [5, 16, 17, 24].

This post-operative alteration seems to be closely related to root resorption, since the more intense the resorption is, the greater is the area of ankylosis.

Alveolar bone cortex resorption, more intense in groups II and III, seems to be in close relation to the inflammatory process of the periodontal ligament [5].

In the more advanced stages, this resorption causes a more marked bone neoformation in the respective groups, as mentioned above.

In the dental pulp, not only in replants, but also as a consequence of other kinds of trauma, the presence of alterations, such as mineralized masses of varying characteristics, have been noticed.

In our work, the pulp and the dental papilla in groups II and III underwent degeneration since the initial stage.

In group I, however, the dental papilla and, initially, part of the pulp at the apical third, exhibited vitality.

The maintenance of vitality is due, as evidences indicate, to revascularization at the apex level [5, 16].

Still in group I, at the apical third, mineralized formations appear adjacent to the dentin on the vestibular side. They have characteristics of both bone tissue and apical dentin, without any definite limits between them. These mineralizations of mesenchymal origin seem to occur due to metabolic changes caused by trauma [5, 16, 25, 26].

As the post-operative time goes by, it was noticed that those masses grow progressively and invade the dental pulp. As the teeth grow, however, such atypical formations end up by being located, more and more, towards the cervical third. So much so that the pulp was vital and free of such masses at the last stage at which it was examined.

Pulpal revascularization was also evidenced by Skoglund et al. [27], through microangiographic studies, and supports the opinion of Heithersay [2] in that teeth with incomplete rhizogenesis have a tendency towards revascularization, although pulpal calcifications may occur in a few cases.

The enamel organ, present in all the specimens of group I, presented changes or gaps in the ameloblastic arrangement at the beginning stages.

These changes, which coincide with the point of dentin deflexion, may be caused by the trauma during operative procedures [5].
The maintenance of enamel organ vitality at the different stages is due to the good blood supply in the connective tissue between bone and enamel, responsible for the nutrition of this organ [5].

The formation of tooth germs at the level of the apex has been reported to have occurred not only after dental replantation, but also following operations which may displace the enamel organ [5, 16, 28, 29].

In our work, we observed structures with tooth-germ characteristics at the alveolar fundus, in one of the specimens.

The presence of such tooth germs was caused by the remaining of parts of the dental papilla and enamel organ, which kept their potentiality and vitality in the alveolus after extraction. This viewpoint is based on the fact of such an alteration having occurred in one animal of group II, where the dental papilla and the enamel organ underwent degeneration in all the other cases. This result supports those reported by Carvalho et al. [26] and Carvalho & Okamoto [30].

An occurrence related to pulpal necrosis was the formation of periapical abscesses, in the last stages, in the animals of groups II and III.

These results support those found by Nasileti et al. [31], in which replanted teeth with untreated root canals are susceptible to periapical alterations after long periods of post-operative time.

Finally, it is necessary to point out that the data obtained in this experimental study should not be directly extrapolated to man, especially if we take into account the metabolic rate in rats and the peculiar characteristics of continuous-growth teeth. Nevertheless, many of the observed facts clearly showed the influence of the time of permanence of the tooth out of the dental alveolus, after dental replantation. So, these results confirm, at the microscopic level, the clinical observations pertinent to the time spent before replantation. The alterations such as root resorption and dento-alveolar ankylosis as well as alveolar wall resorption, found in groups II and III, may serve as a warning sign to those who perform dental replantations.

Summary and Conclusions

In the present work, a study was effected on rat upper incisor replants and the influence of the extra alveolar time upon root resorption and other alterations.

Therefore, 108 rats were used, separated into 3 groups of 36 animals, which received the following procedures.

In group I, the extracted incisors were immediately replanted in their respective alveoli.

In group II, the extracted incisors were kept in saline-soaked gauze for 30 minutes and then replanted in their respective alveoli.

In group III, the procedure was the same as for group II, except for the extra-alveolar time which was 120 minutes.

In the 3 groups, 6 rats of each group were sacrificed at 2, 5, 10, 20, 30 and 60 days after the operation. The obtained pieces were fixed, decalcified and embedded in paraffin.

Six micrometer thick semi-serial sections were stained by hematoxylin-eosin for histological study.
Within the conditions established for the present study, we can conclude that:

1. In the replants where the teeth remained out of the alveoli for 30 or 120 minutes:
   a) cement and dentin resorption was deeper and more extensive;
   b) there was dento-alveolar ankylosis and degeneration of the enamel organ, pulp and dental papilla.

2. In the teeth replanted immediately after extraction:
   a) there was regeneration of the periodontal ligament and maintenance of enamel organ vitality.

References


