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TISSUE REACTIONS IN THE PERIODONTAL MEMBRANE INCIDENT TO EXTRACTION OF THE NEIGHBOURING TOOTH

A HISTOLOGIC AND AUTORADIOGRAPHIC STUDY IN GUINEA PIGS

by

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INTRODUCTION

It is generally accepted that a tooth and its supporting tissues may be injured if used as fulcrum for an elevator when an adjacent tooth is to be extracted. However, to the best of our knowledge, no reports have been presented to date concerning the tissue reactions in the periodontal membrane of such a tooth following this particular type of injury.

The healing of the periodontal mebrane following mechanical injuries has been reported in several studies reviewed by *Solt & Glickman* (1968). The majority of these studies dealt with the result of a constant force applied over various periods of time.

The present investigation has been directed towards the tissue reactions occurring in the periodontal membrane of the mandibular guinea pig incisor following the elevation of the neighbouring mandibular incisor which was subsequently extracted.

Like other rodents, the guinea pig has two long rooted, continously erupting mandibular incisors, seprated by a fibro-cartilaginous symphysis between the left and right parts of the mandible. Any serious trauma to the periodontal membrane of the tooth used as fulcrum is not expected to occur due to the elasticity of the symphyseal region. Therefore, it is anticipated that the tissue

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reactions observed in the periodontal membrane of the animals in the present study represent those occurring after a minor trauma of short duration.

MATERIAL AND METHODS

Twenty young, male, albino guinea pigs were used in this investigation. The animals are identical to those described by us in a preceeding paper (*Johansen & Gilhuus-Moe*, 1969).

Under intraperitoneal barbiturate anesthesia a mandibular incisor was elevated by careful rotation of a small scalpel between the teeth. The tooth to be extracted was then removed by means of a hemostat with jaws which had been recontoured ot fit the tooth. Great care was exercised to prevent excessive movements of the tooth used as fulcrum during the elevation procedure.

Pairs of animals were sacrificed at various intervals from six hours to twenty-one days after the surgical procedure. The animals were given an injection of tritiated thymidine (H3TDR) (1μc/g. body weight) sixty minutes prior to sacrifice. Two animals served as controls.

The mandibles were fixed in a modified Lavdowsky's solution, decalcified in 10 % formic acid with Winn 3,000®, embedded in paraffin, oriented and sectioned sagittally and frontally. Slides were then prepared for histologic and autoradiographic examinations as described earlier (*Johansen & Gilhuus-Moe*, 1969).

OBSERVATIONS

Control animals

The periodontal membrane of the continuously erupting guinea pig incisor reveals special features:

a. At the lingual and lateral portions of the socket *a periodontal membrane proper* connects the tooth to the alveolar bone. This periodontal membrane is composed of three well defined zones (Fig. 1):

An *inner* cell rich zone adjacent to the cementum, the cells are cementoblasts (Figure 1:I).

An *intermediate* zone with less cells and the collagen fibers oriented obliquely towards the tooth surface. Mesenchymal cells are observed between the fiber bundles (Figure 1:II).

An *outer* zone towards the compact bony plate of the alveolus. This zone is highly vascularized, the number of vessels increases towards the fundal part of the periodontal membrane. The compact bony plate is lined by osteogenic cells and penetrated by vascular canals (Figure 1:III).

In the autoradiographs (Figs. 2 & 3) scattered H3TDR labelled cells, mainly cementoblasts are observed in the inner zone. In the intermediate zone scat-

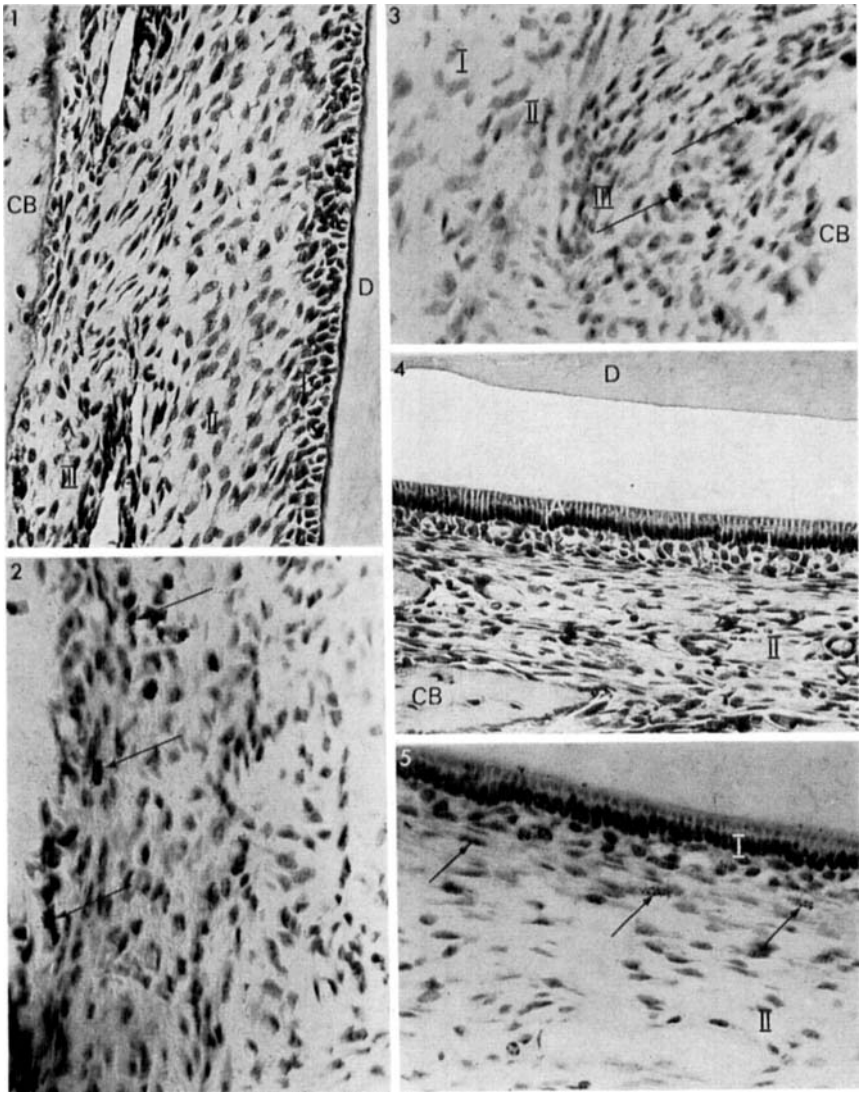

Fig. 1. Periodontal membrane proper (H+E, ×250).
I: *inner, cell-rich zone.*
II: intermediate, fibrous zone.
III: outer, vascular zone.
D: dentin, CB: compact bone of alveolus.

Fig. 2. Periodontal membrane proper (autoradiograph H. ×400).
Arrows: labeled cells of the inner, cell-rich zone.

Fig. 3. Periodontal membrane proper. (Autoradiograph H. ×400).
Arrows: labeled preosteoblats in the outer zone (III) of the periodontal membrane, line the compact bone plate of the alveolus. I: inner cell-rich and II: intermediate zones of the periodontal membrane proper. CB compact bone plate.

Fig. 4. Enamel epithelium and buccal periodontal membrane. (Masson ×250).
A: ameloblasts, SI: stratum intermedium of the enamel epithelium (I). II: loose, connective tissue of the periodontal membrane. CB: compact bone plate of alveolus.

Fig. 5. Same area as Fig. 4. (Autoradiograph ×400).
I: enamel epithelium remains nonlabeled.
II: arrows indicate labeled mesenchymal cells in the periodontal tissue.

tered mesenchymal cells are also labeled. H3TDR labeled preosteoblasts lining the compact bony plate of the alveolus is a constant feature.

b. In the labial portion the periodontal membrane reveals a different morphology due to the presence of an enamel epithelium in this area (*Wollbach & Howe*, 1933). The labial periodontal membrane can according to this be separated in two zones (Fig. 4):

An *inner* zone comprising the enamel epithelium. This zone consists of a singly layer of cuboidal ameloblasts, gradually becoming cylindrical towards the fundal, germinal area. Beneath the ameloblasts a layer of two to three rows of cuboidal to flat cells, representing the stratum intermedium of the enamel organ, is observed (Fig. 4: A, SI). The *outer* zone — representing the mesenchymal periodontal tissue — is composed of loose connective tissue with a network of collagen fibers oriented parallel to the tooth surface. This zone is cell rich and is vascularized towards the alveolar bone (Fig. 4:II).

In the autoradioraphs (Fig. 5) labeled fibroblasts are observed in the outer zone. The cells of the enamel epithelium, except in the fundal, germinal portion, remain unlabeled.

Experimental animals

Following the elevation and extraction of one mandibular incisor, the following observations were made in the periodontal membrane of the remaining incisor:

a. Periodontal membrane proper.

Prominent histologic changes were observed in all three zones (Fig. 6, 7, 8) at the early intervals following the trauma (six hours to three days). In the outer zone marked vasodilatation and hyperemia were present six hours after the surgical procedure. At these early times the tooth gradually became mobile, and a frontal section through the alveolus (Fig. 6) demonstrated that the intense vasodilation of the outer zone induced a tooth wich was almost »floating» in its socket. This hyperemia and mobility decreased gradually. After twenty-one days no difference between control animals and experimental animals was noted. In the inner and intermediate zones, interstitial tissue edema and hemorrhages were marked features (Figs. 7 & 8). Remnants of the extravasated blood in these zones could be discerned fourteen to eighteen days after the trauma. Degenerative changes or necrosis of the mesenchymal cells or the cementoblasts were not prominent features at any observation.

The compact bony plate of the alveolus revealed bone resorption through the first seven to ten days after the trauma, while in the later part of the experimental period bone apposition was prominent. At the end of the experimental

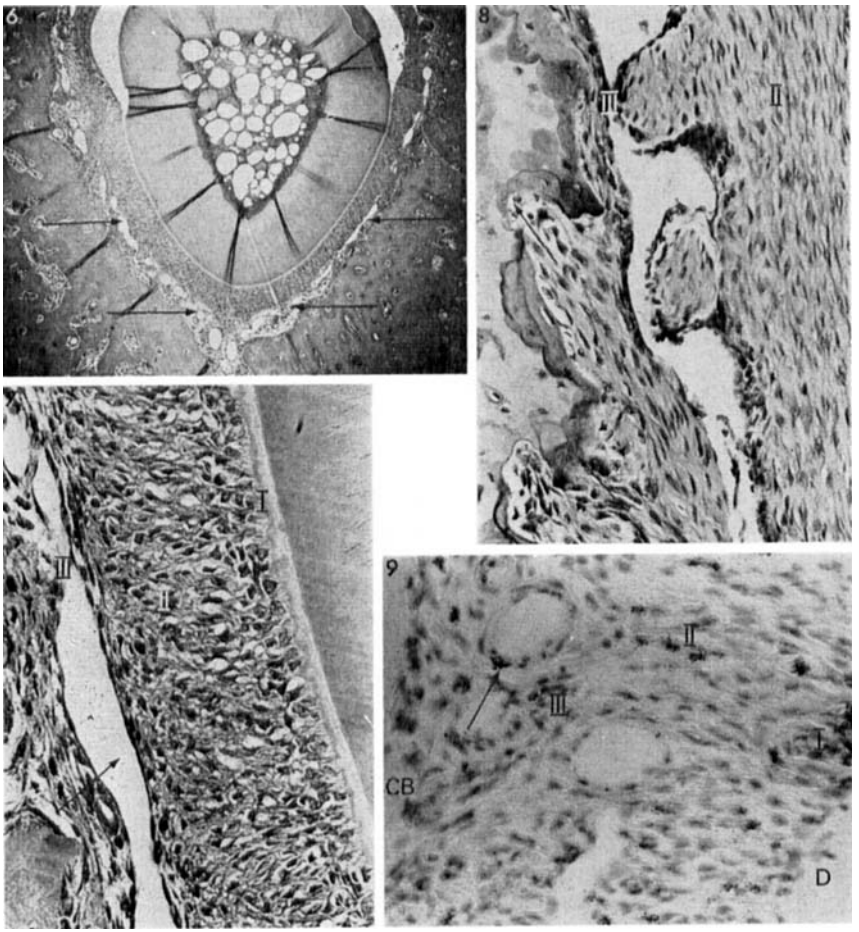

Fig. 6. Frontal section through the midportion of the incisor right mandibular socket 72 hours after extraction of the left incisor. (Masson ×40).

Arrows: Marked vasodilation of vessels in the outer zone of the periodontal membrane. Tooth clinically mobile.

Fig. 7. Periodontal membrane proper 72 hours after extraction of the adjacent incisor. (Masson ×250).

Marked interstitial edema in the inner zone (I) and the intermediate zone (II). Arrow: vasodilatation in the outer (III) zone.

Fig. 8. Periodontal membrane proper 24 hours after extraction of adjacent incisor (H+E. ×250).

Note intense vasodilation in the outer zone (III) of periodontal membrane. Arrow: osteclast at compact bone plate of the alveolus.

Fig. 9. Periodontal membrane proper, 48 hours after extraction of adjacent incisor (Autoradiograph H. ×400).

Observe intense labeling of mesenchymal cells of the intermediate zone of the periodontal membrane (II). Further, labeled sementoblasts are observed in the inner zone (I). Arrow: labeled endothelial cell in the outer, vascular zone (III). D dentine, CB compact bone plate of alveolus.

period the periodontal membrane demonstrated a histologic appearance close to that observed in the untreated animals.

The autoradiographs demonstrated that within six hours to three days after the surgical procedure an increase in the number of labeled cells in all three zones of the periodontal membrane proper (Fig. 9). H3TDR labeled cementoblasts and mesenchymal cells of the intermediate zone were observed frequently. In the later zone the concentration of H3TDR labeled cells was found in its middle part (Fig. 9). In the outer, vascular zone labeled mesenchymal cells, labeled endothelial cells and labeled preosteoblasts were found. At later observations (seven to twenty-one days after the surgical procedure) the number of labeled cells revealed a gradual decrease in all zones of the periodontal membrane proper.

b. Labial periodontal membrane and enamel epithelium.

At six hours to three days after the trauma the cells of the enamel epithelium underwent marked morphological changes. These features were mainly localized to the middle and orificial portions of the socket and did not involve the fundal progenitive portion. These features were (Fig. 10), interstitial edema of the ameloblasts and a pronounced vasodilatation of the stratum intermedium.

In the outer vascular zone, interstitial edema, vasodilation and extravasation of blood, similar to the observations in the periodontal membrane proper, were marked. Remnants of hemorrhage, indicated by hemosiderin deposits between the collagenous connective tissue fibers in this zone (Fig. 11), were noticed throughout the entire experimental period. At observation intervals of five to twelve days after the trauma, ameloblasts of the middle portion of the socket underwent progressive degenerative changes which finally led to disintegration of these cells (Figs. 12, 13). In some areas the stratum intermedium remained a short period after the disintegration of the ameloblasts, but in other areas this cell layer underwent disintegration and was replaced by connective tissue from the outer zone. Due to the continous eruption of the tooth, these changes moved towards the orificial part of the alvelous at the end of the experimental period.

The buccal alveolar plate underwent rapid osteoclastic resorption. This initial resorption was not later replaced by bone apposition, as observed in the lingual and lateral parts of the socket.

The autoradiographs (Fig. 14) revealed that the cells of the enamel epithelium remained unlabeled, except in the germinative areas. In the outer zone a high number of labeled mesenchymal cells and cells of the endothelial lining of the vessels were found. There was a gradual decrease in the number of these cells at the later time intervals. The compact bony plate of the labial

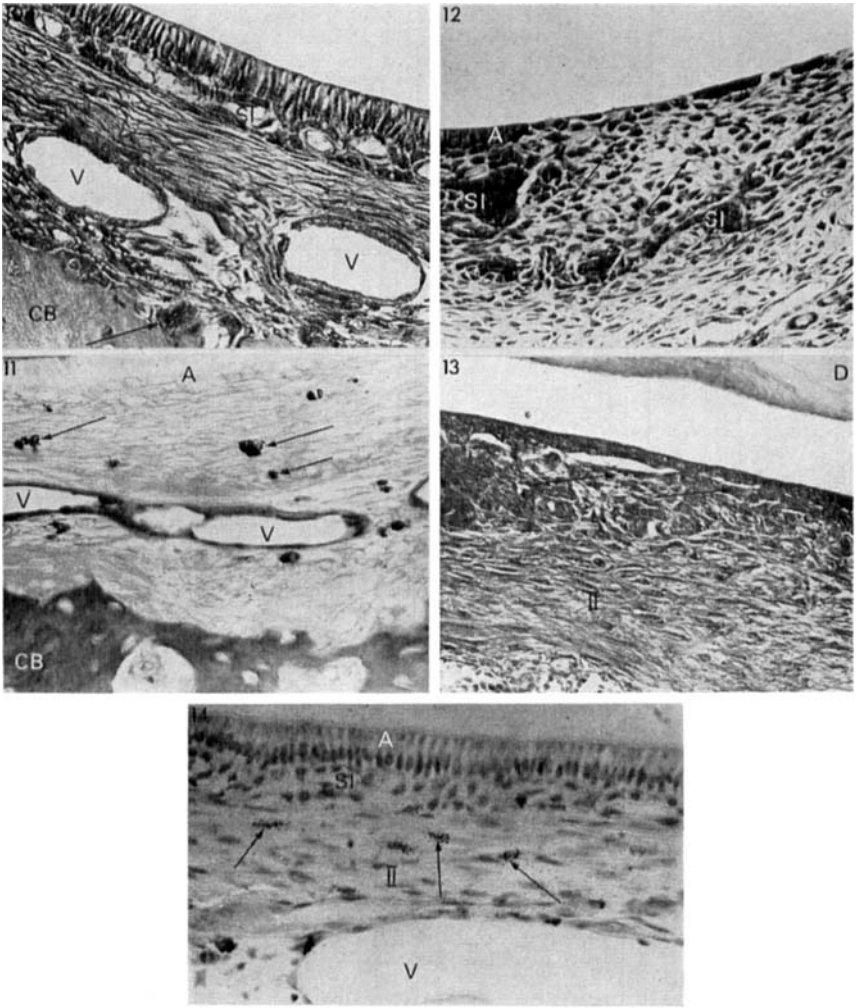

Fig. 10. Observations 72 hours after extraction of adjacent incisor (Masson ×400).
Observe: interstitial edema of the ameloblasts (A). Further note vacuolization in the stratum intermedium (SI): Dilated vessels (V) are observed in the loose mesenchymal tissue of the periodontal membrane. Arrow: osteoblast line the compact bone plate (CB) of the alveolus.

Fig. 11. Buccal periodontal membrane 72 hours after trauma (AB-PAS ×400).
Arrows: hemosiderin deposits are observed in the loose connective tissue of the periodontal membrane. V dilated vessels. A ameloblasts CB compact bone.

Fig. 12. Buccal periodontal membrane, midportion of socket, 10 days after trauma. (H+E. ×250).
Ameloblasts (A) are disappeared over a major part of the area observed. The stratum intermedium (SI) is disrupted and replaced by fibrous tissue.
Arrows: strands of fibrous tissue replacing the enamel epithelium.

Fig. 13. Buccal periodontal membrane 14 days after trauma. (H+E. ×250).
The enamel epithelium is disappeared completely and is replaced by fibrous tissue (arrow) of the loose connective tissue (II) of the periodontal membrane. D: dentin.

Fig. 14. Buccal periodontal membrane 72 hours after extraction of the adjacent incisor. (Autoradiograph H. ×400).
Arrows: labeled mesenchymal cells are observed frequently in the loose connective tissue of the periodontal membrane (II): Both ameloblasts (A) and cells of the stratum intermedium (SI) remain nonlabeled. V dilated vessel.

alveolar wall did not at any observation demonstrate an increased number of labeled preosteoblasts; the heavy concentration of osteoclasts remained unlabeled.

c. Alveolar bone and mandibular periosteum.

The cancellous bone of the mandible demonstrated scattered areas of hemorrhage while later bone apposition on the trabeculae was prominent. The mandibular periosteum of the alveolar region demonstrated an increased number of labeled osteogenic cells compared to the control animals. However, this increase in number of H3TDR labeled preosteoblasts was a less prominent feature compared to that of the adjacent postextration alveolus.

DISCUSSION

The guinea pig incisor is characterized by continuous growth and eruption maintained by the progenitive tissues at the fundal portion of the alveolus. In the present study the germinal, apical area has been exluded from further examination because of the problems implicated in a differentiation of pathologic patterns in this highly proliferative area.

On the other hand the central and orificial portions of the periodontal membrane demonstrated both a homogenous morphology and distribution of H3TDR labeled cells, enabling the analysis of the proliferative rate of the various cell compartments. The distribution of labeled cells of the periodontal membrane of the guinea pig is in agreement with those described in mice by *Hwang & Tonna* (1965), *Grewe & Felts* (1968), and in rats by *Lavelle* (1968). It appears that the proliferative rate of the periodontal membrane of rodent incisors remains in a steady state after the eruption through the gingiva (*Lavelle 1968*).

The present observations tend to demonstrate that a minor, short acting trauma interferes with this steady state of the periodontal tissues. In the periodontal membrane proper the observed tissue reactions to these surgical procedure are reversible. In this way the present observations are in agreement with those of *Solt & Glickman* (1968), who observed that sixty minutes interdental wedging initiated a rapid increase in proliferation with a complete return to the normal situation within twenty-one days.

The autoradiographic observations showing a pronounced concentration of H3TDR labeled mesenchymal cells in the intermediate zone of the periodontal membrane proper, initiates a renewal of the problems concerning the existence of an intermediate plexus in this structure (*Sicher*, 1959; *Johansen*, 1960; *Goldman*, 1962, *Stallard*, 1967; *Ramos & Hunt*, 1967; and *Melcher*, 1967).

The observations within the labial portion of the periodontal membrane have revealed that *irreversible* tissue reactions followed the surgical procedure in this area. The progressive degenerative changes in the enamel epithelium resembled those observed by *Wollbach & Howe* (1933) in hypovitaminosis A in the guinea pig mandibular incisor. It appears that the present findings represent the reaction of a highly differentiated epithelium to trauma and subsequent circulatory idsturbances. The primary damage to the enamel epithelium was observed in the mid-portion of the socket, and it might be anticipated that the fulcrum effect during elevation reached a maximum in this area. Due to the continuous eruption of the incisor, the area of degenerative changes moved towards the orificial portion of the socket.

It is of importance to observe that mature ameloblasts did not take up H3TDR suggesting that these cells have lost their, reproducibility. In the same areas stratum intermedium, (in the fundal portion producer of the cells of the stellate reticulum (*Hunt & Paynter* 1963)), appeared also to have lost their ability of proliferation. In the longer experimental periods these cells were completely replaced by mesenchymal tissues. Consequently, the mild trauma produced by the elevation procedure resulted in a complete disintegration of the enamel organ, in the mid-portion of the socket.

The loss of the buccal alveolar plate may be a result of the increased functional requirements to the remaining incisor in the guinea pig. As emphasized previously (*Johansen & Gilhuus-Moe* 1969) the extraction of one mandibular incisor severely interfered with the masticatory ability of the experimental animals, which may explain the marked resorption of the buccal alveolar bone.

The reactions in the mandibular periosteum have been interpreted as a distant effect by the increased proliferative rate in the periosteum of the post-extraction alveolus, as originally demonstrated in bone injuries in mice by *Tonna & Gronkite* (1961).

SUMMARY

The periodontal tissues of the guinea pig mandibular incisor normally remain in a steady state. The results of this study indicate that the elevation and extraction of a neighbouring tooth interfered with this steady state.

The *periodontal membrane proper* (i. e. the lingual and lateral portions) reacted to the trauma by *reversible* tissue reactions, demonstrated by circulatory disturbances and mesenchymal cell proliferation, reaching a maximum six hours to three days after the trauma. Thereafter, there was a gradual return to the normal situation over a fourteen day period. Twenty-one days

after the trauma the findings did not differ from those observed in the control animals.

The *buccal periodontal membrane*, *buccal compact alveolar bone* and *the enamel epithelium* revealed *irreversible* tissue reactions. The cells of the enamel epithelium underwent degeneration and disintegration outside the fundal, germinative areas. The buccal compact bony plate was rapidly resorbed and not replaced through the twenty-one days observation period.

RÉSUMÉ

RÉACTIONS TISSULAIRES DANS LE DESMODONTE APRÈS EXTRACTION DE LA DENT VOISINE

ÉTUDE HISTOLOGIQUE ET AUTORADIOGRAPHIQUE SUR LE COBAYE

Les tissus parodontaires de l'incisive inférieure du cobaye se trouvent normalement en état d'équilibre. Les résultats de la présente étude indiquent que la luxation et l'avulsion d'une dent voisine portent atteinte à cette équilibre.

Le *périodonte proprement dit* (c.à.d. les parties linguales et latérales) répondaient au traumatisme par des réactions tissulaires réversibles, révélées par des altérations circulatoires et une prolifération des cellules mésenchymateuses, atteignant un maximum de six heures à trois jours après l'action traumatique. Il se produisait ensuite un retour progressif à la normale au cours d'une période de 14 jours. La comparaison avec les animaux témoins 21 jours après l'action traumatique ne révélait pas de différence.

Le *périodonte vestibulaire*, *l'os alvéolaire compact du côté vestibulaire* et *l'épithélium adamantin* présentaient des réactions tissulaires irréversibles. Les cellules de l'épithélium adamantin subissaient une dégénération et une désintégration en dehors des zones germinatives du fond de l'alvéole. La lame osseuse compacte vestibulaire se résorbait rapidement et n'était pas remplacée à la fin de la période d'observation de 21 jours.

ZUSAMMENFASSUNG

GEWEBSREAKTIONEN IN DER PERIODONTALMEMBRAN INFOLGE EXTRAKTION DES NACHBARZAHNES

EINE HISTOLOGISCHE UND AUTORADIOGRAPHISCHE UNTERSUCHUNG MIT MEERSCHWEINCHEN

Die periodontalen Gewebe der Schneidezähne im Unterkiefer des Meerschweinchens verbleiben normalerweise im Gleichgewicht. Die Resultate

dieser Studie deuteten darauf hin, das Luxation und Extraktion des Nachbarzahnes dieses Gleichgewicht stören. Auf das Trauma reagierte die periodontale Membran (d.h. linguales und laterales Segment) mit reversiblen Gewebsreaktionen festgestellt durch Kreislaufstörungen und Proliferation der Mesenchymzellen. Diese Reaktion (Proliferation) war am deutlichsten sechs Stunden bis drei Tage nach dem Trauma. Danach normalisierte sich allmählich der Zustand innerhalb vierzehn Tage. Observationen 21 Tage nach dem Trauma unterschieden sich nicht von denen gemacht über Kontrolltiere.

Bukkale Periodontalmembran, kompakter bukkaler Alveolarknochen und Schmelzepithel demonstrierten irreversible Gewebsreaktionen. Die Zellen des Schmelzepithels ausserhalb der tiefsten germinativen Gebieten zeigten Degeneration und Auflösung.

Die bukkale kompakte Knochenlamelle wurde schnell resorbiert und nicht innerhalb der 21-tägigen Observationszeit regeneriert.

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