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REPAIR OF THE POST-EXTRACTION ALVEOLUS IN THE GUINEA PIG

A HISTOLOGICAL AND AUTORADIOGRAPHIC STUDY

by

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OLE GILHUUS-MOE

INTRODUCTION

The healing of extraction wounds have been investigated in humans (*Schram*, 1929; *Mangos*, 1941; *Christopher*, 1942; *Amler et al.*, 1960, 1964; *Boyne*, 1966) as well as in animals, (*Euler*, 1923; *Claflin*, 1936; *Simpson*, 1960, 1961; *Boyne et al.*, 1962) and techniques, such as histology, radiology, histochemistry, fluoroscence microscopy and clinical evaluation have been employed. There are diverging opinions on the sequence of events in the healing process. Thus *Hüebsch et al.* (1952) postulated the following steps:

»1. Formation of a blood clot in the socket.
2. Organization of the clot by the formation of granulation tissue.
3. Replacement of the granulation tissue by connective tissue.
4. Formation of osteoid tissue, which gradually is converted to new bone, filling out the socket.
5. Remodelling processes in the socket and in the areas adjacent of the alveolar process.
6. Epithelization of the wound surface parallel with the reparative processes in the socket.«

However, *Boyne et al.* (1962) and *Boyne* (1966) found that the initial changes took place in the marrow spaces and the cortical bone surrounding the alveolus. Within the post-extraction alveolus, bone formation appeared

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first in its lateral portion rather than in its fundus. *Boyne* (1966) stressed that the post-extraction wound heals by a complex mechanism similar to that of a compound wound and not like a simple surgical wound.

The proliferative changes in the post-extraction alveolus has not previously been dealt with in detail although the sources of cellular proliferation appears to be a crucial point in any pattern of wound healing. There is reason to believe that autoradiography with tritiated thymidine (H^3TDR) may give valuable information as to the proliferative capacity of the cells lining the bony socket as well as cells of the surrounding structures. Therefore, the present study has been performed as a combined histologic-autoradiographic investigation. The present study aims at answering the following questions:

Does the oral epithelium, the cuff epithelium, or both of them participate in closing the wound? What happens to the enamel epithelium and the enamel organ? To what extent do remnants of the periodontal membrane participate in the healing of the socket? Which are the patterns of osseous repair in the marrow spaces and at the jaw surface?

MATERIAL AND METHODS

For economical reasons when using H^3TDR autoradiography a small laboratory animal had to be used. Extraction of molars in rodents is a complicated procedure (*Hüebsch et al.* 1952, *Smith*, 1958). In order to reduce surgical trauma a single rooted tooth would be more convenient; and preliminary experiments on extraction showed the guinea pig mandibular incisor to be the easiest to extract in toto. The main advantage of choosing an incisor is that on extraction it leaves a long alveolus (Fig. 1), which makes it possible to distinguish clearly between an orificial, a middle and a fundal portion of the socket.

The incisors of the guinea pig are covered with enamel on the labial surfaces and with cementum on the lingual surfaces, and this has profound effect on the appearance of the periodontal membrane in corresponding areas. Adjacent to the enamel there will be enamel epithelium in various phases of differentiation, whereas, attached to the cementum there will be the usual type of periodontal fibers. The fundus of the alveolus is medial to the roots of the second molar, (Figs. 1 and 2).

Twenty young, male albino guinea pigs were used in this experiment. The animals were thirty days of age upon delivering and they were caged (2 in each cage) for seven days prior to the extraction of the incisors. They were fed standard pig pellets (*Erichsen*, 1966) and drinking water, supplemented with ascorbic acid (25 mg per 100 ml), *ad libitum*.

Under intraperitoneal barbiturate anesthesia (3 mg Nembutal® per 100 g body weight) one of the mandibular incisors in each animal was extracted. A conventional mosquito hemostat was modified to fit the curved incisor and the tooth was extracted after careful rotation by means of a small scalpel between the teeth. In two animals the tooth fractured, but the remaining fragments could be removed without additional trauma to the surrounding tissues.

The extracted teeth were fixed in 10 % buffered formalin, decalcified in 5 % nitric acid, sectioned at 5 microns and stained with hematoxylin and eosin.

The guinea pigs were sacrified by exsanguination in Nembutal® anaesthesia after varying observation periods (Table I). One hour prior to sacrifice each one of the animals received an intraperitoneal injection of 1 μci. H^3-thymidine per g. body weight. The lower jaws were dissected out, fixed in Lawdovsky's solution, and decalcified in 5 % nitric acid and embedded in paraffin. For each observation period there were two animals (Table I); the lower jaw from one of them was orientated for sectioning in longitudinal direction, while the other lower jaw was orientated and sectioned in a transversal plane.

Sections were cut at 5 microns and stained with hematoxylin and eosin, Masson's trichrome stain and according to the Alcian blue—PAS techniques (*Mowry*, 1963). Sections were prepared for autoradiography following the dipping techniques (*Joftes*, 1963). Kodak NTB 2 emulsion was used. The slides, after dipping and drying, were exposed for 21 days, developed, fixed and stained with Harris hematoxylin. Some of the sections were stained prior to autoradiography.

Table I.

Distribution of material according to observation time following extraction

Observation time	No. of animals
6 hours	2
24 »	2
2 days	2
3 »	2
5 »	2
7 »	2
10 »	2
14 »	2
18 »	2
21 »	2
	Total 20

OBSERVATIONS

All animals survived the experimental procedure. There was a delay in the normal increase of body weight in the first postoperative period, but after that they gained weight. However, the extractions interfered with the chewing of pellets (Text Fig. I) more than did experimental fractures of the mandibular condyle (*Gilhuus-Moe*, 1968).

In all the sections of the extracted teeth remnants of the periodontal membrane were seen on the lingual surface (Fig. 3).

After 6 hours the epithelium at the margins of the wound started to proliferate, and it appeared as if both the pocket epithelium and the oral epithelium were involved. Where bone fragments remained in the orifice of the alveolus, the epithelium surrounded these. Keratinization of the epithelium was observed after 5 days.

The enamel epithelium, which was left in the alveolus after extraction, degenerated. After 3 days thin strands of this epithelium were seen in the clot, later they could not be distinguished.

The enamel organ was completely removed in all but one case, in which a small portion had proliferated and produced enamel and osteodentin orientated in an opposite direction to that of the original tooth (Figs. 4, 5 and 6).

A blood clot regularly filled most of the alveolus, although shrinkage occurred. By 6 hours the clot was seen to be made up by the normal blood constituents, and after 24 hours fibrin strands and a few mesenchymal cells were seen. By 48 hours the clot was invaded by fibroblasts and endothelial cells, revealing H^3TDR labeling in the autoradiographs (Fig. 7). The organi-

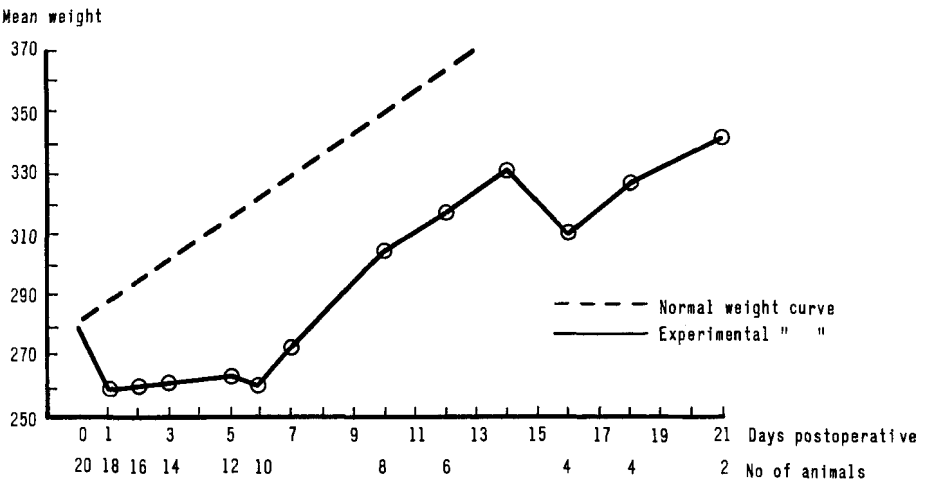

Fig. I. Animal weights

zation of the clot differed in the various parts of the alveolus, the middle portion of it being initially more organized than in the orificial and fundal portions. At longer observation periods, the clot was replaced by granulation tissue undergoing intramembraneous ossification.

Remnants of the periodontal membrane always covered the entire alveolus in the early observation periods and they never showed any sign of degeneration. After 48 hours labelled fibroblasts in these remnants were observed, and the highest number of these labelled cells was found in the vicinity of the vascular canals in the compact bone plate of the alveolus. (Figs. 8, 9 and 10). The transformation of the blood clot into mature granulation tissue took place earlier on the lingual side than on the labial side, the remnants of the enamel epithelium apparently acted as a barrier. Also, the proliferation of the fibroblasts was more pronounced in the middle portion of the socket. After 5 days it was impossible to distinguish between the original periodontal membrane remnants and the granulation tissue. At the end of the experiment the sockets were filled with cancellous bone.

By 6 hours there were only minor changes in the bony surface of the alveolus and in the regional mandibular periosteum. However, by 24 hours the periosteum on the extraction side appeared edematous and infiltrated with inflammatory cells, and the number of labelled preosteoblasts was definitely increased compared to that of the other, non-extracted side. This increase in labelled mesenchymal cells was apparent throughout the observation periods, and about twice as much bone was formed on the extraction side as on the control side (Figs. 11, 12 and 13). Bone formation started later within the alveolus than in the periosteum. The periodontal membrane remnants acted as an integrated part of the membraneous bone formation filling out the alveolus at the end of the experiment (Fig. 14). It appeared as if the formation of bone was more rapid on the lingual side than on the labial side, and that the middle portion of the socket was filled with bone before the opening and the fundus, according to the pattern described above. Dense labelling of both osteoprogenitor cells and fibroblasts was seen in these sections. At the end of the experimental period the outline of the bony walls of the original socket still could be discerned (Fig. 15) although the socket was filled with new bone. This indicates that the remodelling phase of the bone in the alveolus was not finished, despite the finding of osteoclasts in the marrow spaces after 48 hours.

DISCUSSION

The speed of migration of the epithelial covering of the extraction wound did not seem to be related to the degree of inflammation in the marginal

part of the socket. Thus, our finding do not corroborate those made by *Smith* (1958). In our material the epithelium was keratinized after 5 days, which is somewhat earlier than reported in humans by *Carlsson et al.* (1967), but obviously no direct comparison between two so different species can be made. In one case a small part of the dental papilla was left behind after extraction. This part had a remarkable capacity of production of dental hard tissues which may be in accordance with the reported ability of forming the usual dental tissue after transplantation to abnormal locations as e.g. anterior chamber of the eye (*Fleming* 1956).

The organization of the clot revealed regional differences. There was a definite trend for the middle portion to undergo a more rapid organization than the fundal and the orificial portions of the socket. The explanation for this may be sought in the length of the guinea pig incisor alveolus. In the fundus, evidence of secondary hemorrhage could be observed, and a high number of macrophages with engulfed hemosiderin were observed even at late observation intervals. It appears reasonable that these features would delay the repair processes in the fundus of the socket. In the orifice, inflammatory processes occurred and retarded the organization of the original blood clot. In all specimens a blood clot filled out the entire alveolus. Shrinkage was observed in some instances, but in no case was the alveolus empty. Therefore, it could not be decided whether or not a complete blood clot was essential for the described healing pattern.

The early observations in the periodontal membrane remnants were noteworthy. *Euler* (1923) observed hyalinization and calcification in these remnants in dogs. *Hüebsch et al.* (1952) in similar studies in the rat did not make specific comments on the periodontal membrane remnants. In the present study the distribution of H^3TDR labelled cells in these remnants indicated that these cell compartments participate in the formation of the granulation tissue filling out the socket. Further, the observations demonstrating a concentration of labelled cells in the tufts of the periodontal remnants, adjacent to the vascular canals penetrating the compact bone plate of the alveolus, indicate that the vascularization of these areas may be of importance in the pattern of healing.

Our study indicates that bone apposition starts earlier on the outer periosteal surface than in the socket. This confirms the observations made by *Boyne* (1966), and is somewhat in contradiction to similar studies in rats (*Hüebsch et al.*, 1952), monkeys (*Simpson*, 1960) and humans (*Carlsson et al.*, 1967). In addition, the extraction induced an increased proliferation of periosteal cells not only within the post-extraction alveolus, but also in the molar region of the mandible (Figs. 16 and 17). This increased proliferative rate

in the periosteum, remote from the injured areas, is in agreement with the observations made by *Tonna et Cronkite* (1961) in experimental fracture healing in mice.

Osteoclasts were found in the marrow spaces in the alveolus as well as in the compact bone plate of the socket at all observation intervals. However, a complete break down of the compact bone plate of the socket could not be discerned in any specimen before new bone was laid down on the compact bony walls of the socket. Thus, both intense peripheral bone formation as well as bone formation within the socket proper were present before any complete disappearance of the compact bony wall of the alveolus occurred.

SUMMARY

Following extraction of a mandibular incisor in young, male guinea pigs the pattern of healing was investigated. By means of histologic and autoradiographic (with tritiated thymidine) techniques the proliferative capacities of the tissues involved have been examined. The observations indicated:

The epithelium proliferated over the surface of the wound and was keratinized after 5 days. Both the oral epithelium and the cuff epithelium appeared to take part.

The enamel epithelium, left in the alveolus, could not be distinguished after 5 days observation time.

In one animal a small part of the enamel organ, left behind in the alveolus, started to form dental hard tissues.

The blood clot was more rapidly organized in the mid portion of the post extraction alveolus than in the orifical and the fundal portions of the socket.

Periodontal membrane remnants were seen attached to the teeth and to the socket walls after extraction. The autoradiographic study revealed in these cell areas a high number of labelled cells. These cells were concentrated in areas where vascular canals penetrated the compact bone plate of the alveolus. Degenerative changes in the periodontal membrane remnants were not observed. Bone apposition was first observed periosteally. Later, bone formation took place in the alveolus directly on the compact alveolar plate without any previous resorbtion of the same.

RÉSUMÉ

CICATRISATION DE L'ALVÉOLE APRES EXTRACTION CHEZ LE COBAYE ÉTUDE HISTOLOGIQUE ET AUTORADIOGRAPHIQUE (H^3-THYMIDINE)

Après extraction d'une incisive inférieure, l'évolution de la cicatrisation a été étudiée chez de jeunes cobayes mâles. Le pouvoir de prolifération des

tissus en cause a été examiné en utilisant des techniques histologiques et autoradiographiques (avec thymidine tritiée). Les résultats suivants ressortent des observations:

L'épithélium proliférait pour recouvrir la surface de la plaie et était kératinisé au bout de 5 jours. On constatait que l'épithélium buccal ainsi que l'épithélium du repli gingival prenaient part à ce processus.

L'épithélium adamantin restant dans l'alvéole ne pouvait plus être distingué au bout de 5 jours d'observation.

Chez un des animaux, la formation de tissus dentaires durs avait commencé à partir d'une petite partie de l'organe adamantin qui était restée dans l'alvéole.

Le caillot sanguin était plus rapidement organisé dans la portion moyenne de l'alvéole déshabité que dans le fond de l'alvéole et qu'à l'entrée de l'alvéole.

Des restes du desmodonte pouvaient être observés après l'extraction, adhérant à la dent et aux parois de l'alvéole. L'étude autoradiographique décelait parmi les cellules de ces zones un grand nombre de cellules marquées. Ces cellules étaient concentrées aux régions où des canaux vasculaires pénétraient la corticale alvéolaire. On n'observait pas d'altérations dégénératives dans les restes du desmodonte. L'apposition osseuse s'observait d'abord au niveau du périoste. Plus tard, la formation osseuse avait lieu dans l'alvéole directement sur la corticale alvéolaire, sans aucune résorption préalable de celle-ci.

ZUSAMMENFASSUNG

HEILUNG DER ALVEOLE NACH ZAHNEXTRAKTION BEIM MEERSCHWEINCHEN

Nach dem Ziehen der Unterkieferfrontzähne bei jungen männlichen Meerschweinchen, wurde die Heilungsvorgänge untersucht. Mit Hilfe von histologischer und autoradiographischer (mit H^3 Thymidin) Methode wurde die proliferative Kapazität des in Frage stehenden Gewebes untersucht. Die Beobachtungen ergaben:

Das Epithel breitete sich über die Oberfläche der Wunde aus und keratinisierte nach 5 Tagen. Das orale sowie das gingivale Epithel beteiligten sich an dem Vorgang. Das in der Alveole verbliebene Schmelzepithel war nach 5-tägiger Beobachtung nicht mehr nachzuweisen.

Bei einem Versuchstier begann ein kleiner in der Alveole verbliebener Teil des Schmelzorgans hartes Zahngewebe zu bilden.

Das Koagulum organisierte sich schneller im mittleren Teil der Alveole als im äusseren und tieferen Teil derselben. Wurzelhautreste wurden nach der Extraktion an den Zähnen und den Alveolenwänden beobachtet. Die

autoradiographische Untersuchung zeigte in diesem Zellgebiet eine hohe Anzahl H^3 Thymidin-markierte Zellen. Diese Zellen konzentrierten sich in Gebieten, wo Blutgefässe die Kompakta der Alveole durchdrangen.

Degenerative Veränderungen der periodontalen Membranreste wurden nicht beobachtet.

Knochen-Apposition wurde zuerst im Periost beobachtet. Später erfolgte Knochenbildung in der Alveole direkt an der Kompakta, ohne vorhergehende Resorption derselben.

REFERENCES

Amler M. H., P. L. Johnson & I. Salman: Histological and histochemical investigation of human alveolar socket healing in undisturbed extraction wounds. J. Amer. dent. Ass. *61:* 32, 1960.

Amler M. H., I. Salman & Helen Bungener: Reticular and collagen fiber characteristics in human healing. — Oral Surg. *17:* 785, 1964.

Boyne P. J.: Osseous repair of the postextraction alveolus in man.— Oral Surg. *21:* 805, 1966.

Boyne P. J. & G. O. Kruger: Fluorescence microscopy of alveolar bone repair. — Oral Surg. *15:* 265, 1962.

Carlsson G. E., H. Thilander & B. Hedegård: Histologic changes in the upper alveolar process after extractions with or without insertion of an immediate full denture. Acta odont. scand. *25:* 21, 1967.

Christopher E. R.: Hi o gical study of bone healing in relation to the extraction of teeth. — Northw. Univ. Bull. *43:* 5, 1942.

Claflin R. S.: Healing of disturbed and undisturbed extraction wounds. — J. Amer. dent. Ass. *23:* 945, 1936.

Erichsen S.: Standardforblandinger til forsoksdyr. — Nord. Vet.-Med. *18:* 417, 1966.

Euler H.: Die Heilung von Extractionswuden. — Dtsch. Mschr. Zahnheilk. *41:* 685, 1923.

Fleming H. S.: Experimental transplantation of teeth in lower animals. — Oral Surg. *9:* 3, 1956.

Gilhuus-Moe, O.: Subcondylar Fractures in Young Guinea Pigs. A morphologic, histologic and autoradiographic study. To be published.

Hüebsch R. F., R. D. Coleman, A. M. Frandsen & H. Becks: Healing process following molar extraction. I. Normal male rats. (Long Evans strain). — Oral Surg. *5:* 864, 1952.

Joftes D. L.: Radioautography, principle and procedures. — J. nucl. Med. *4:* 143, 1963.

Mangos J. F.: The healing of extraction wounds. An experimental study based on microscopic and radiographic investigations. — N.Z. dent. J. *37:* 4, 1941.

Mowry R. W.: The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical studies of mucins. With revised directions for the colloideal iron stain, the use of Alcian blue G8X and their combinations with the periodic acid-Schiff reaction. — Ann. N.Y. Acad. Sci. *106:* 402, 1963.

Schram E. R.: A histologic study of repair in the maxillary bones, following surgery. (Extraction). — J. Amer. dent. Ass. *16:* 1987, 1929.

Simpson H. E.: Experimental investigation into the healing of extraction wounds in macacus rhesus monkeys. — J. oral Surg. Anesth. *18:* 391, 1960.

Simpson H. E.: Healing of surgical extraction wounds in macacus rhesus monkeys. I. The effect of burs. — J. Oral Surg. Anesth. *19:* 3. 1961.

Simpson H. E.: Healing of surgical extraction wounds in macacus rhesus monkeys. II. The effect of chisels. — J. oral Surg. Anesth. *19:* 126, 1961.

Smith R. L.: The role of epithelium in the healing of experimental extraction wounds. — J. dent. Res. *37:* 187, 1958.

Tonna E. A. & E. P. Cronkite: Cellular response to fracture studied with tritiated thymidine J. Bone Jt. Surg. *43A:* 352, 1961.

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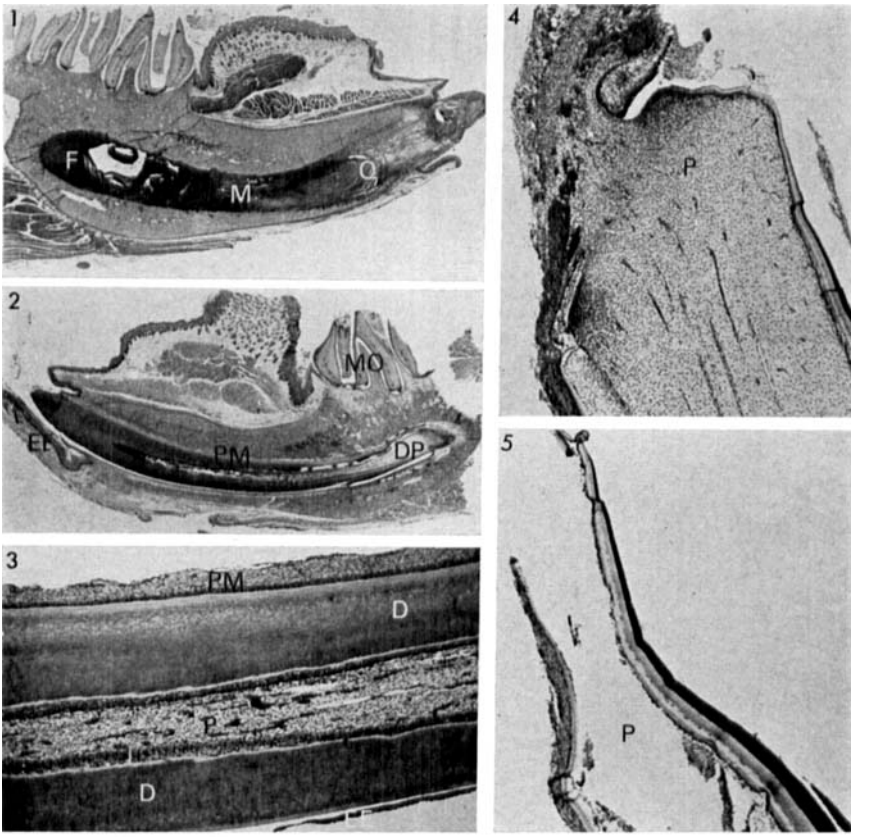


Fig. 1. Sagital section through the anterior part of the guinea pig mandible. ×5. Post-extraction alveolus 48 hours after extraction of incisor.
O: outer (orificial) portion of the socket.
M: middle portion of the socket.
F: deep (fundal) portion of the socket.

Fig. 2. Plane of sectioning as in Fig. 1, revealing the guinea pig incisor in situ. ×5.
Mo: molar teeth.
DP: dental papillae.
PM: periodontal membrane.
EE: enamel epithelium.

Fig. 3. Section of the extracted incisor, middle portion. ×28.
PM: periodontal membrane attached to the lingual surface of the tooth.
D: dentin.
P: pulp.
EE: enamel remnants at the labial surface of the tooth.

Fig. 4. Section of the extracted incisor, apical portion. ×28. The entire dental papillae (P) is attached to the extracted tooth.

Fig. 5. Section of the extracted incisor, apical portion. ×28. A small portion of the dental papillae (P) is disrupted from the extracted tooth and remains in the socket.

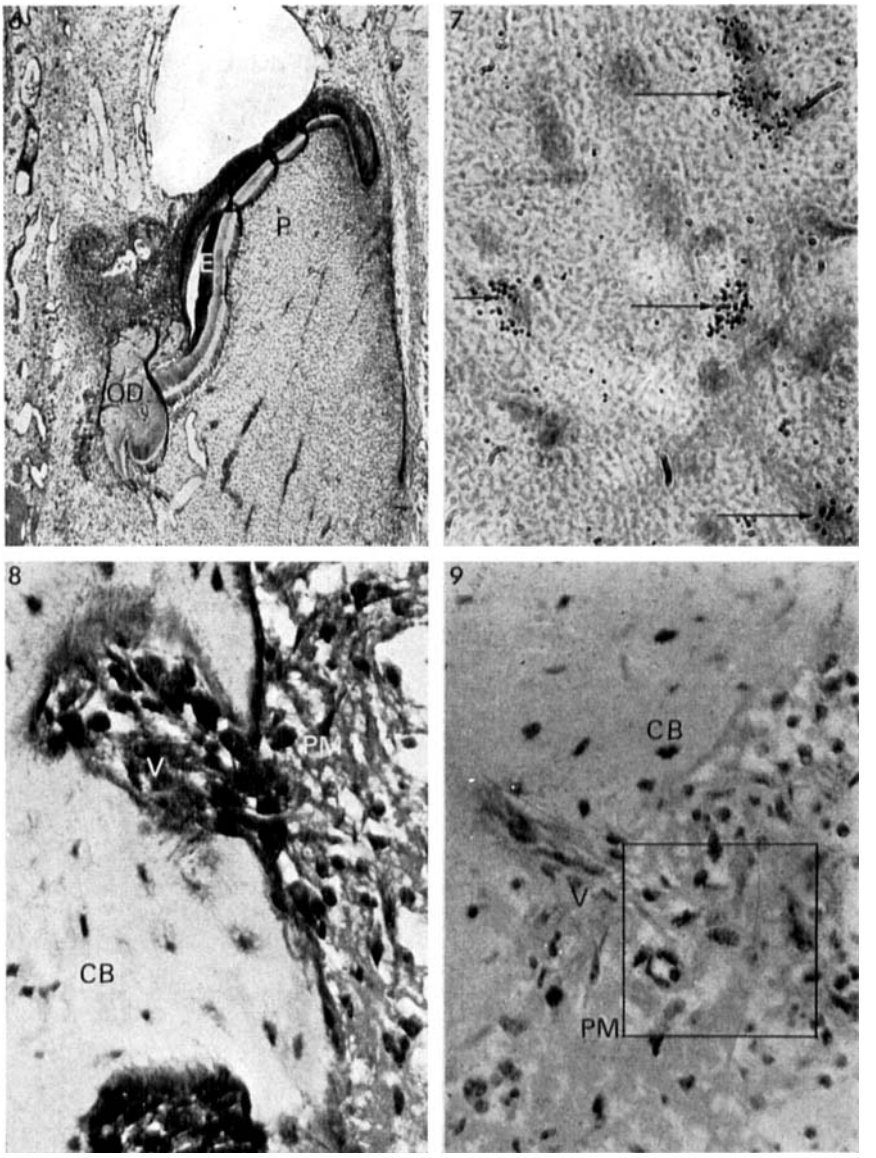
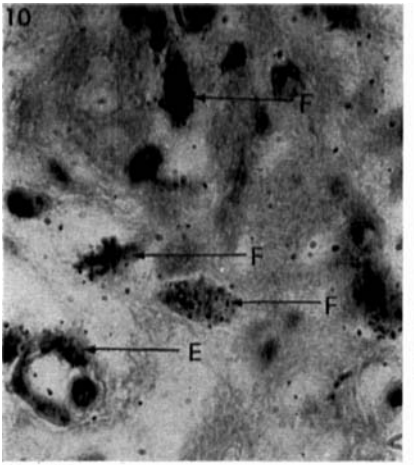


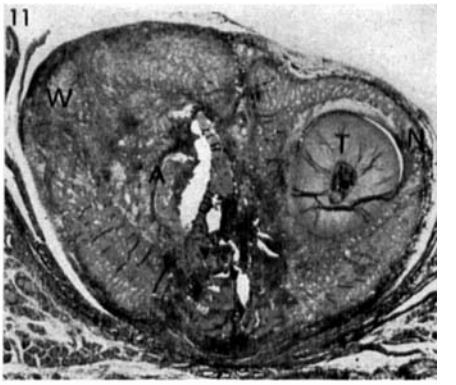
Fig. 6. Fundal portion of the post-extraction alveolus, 10 days after extraction ×28. (Same animal as in Fig. 5). Remnants of the dental papillae and enamel organ left in the socket have produced enamel (E), osteodentin (OD) and pulp tissue (P).

Fig. 7. Post-extraction alveolus (middle portion) 48 hours after extraction. Autoradiograph. ×700. Arrows: H3TDR labelled spindle shaped cells are observed in blood clot filling out the socket.

Fig. 8. Post-extraction alveolus (middle portion) 48 hours after extraction. ×360. Vascular canal (V) is observed penetrating the compact bone plate of the alveolus (CB). Arrows: spindle shaped cells are observed projecting into a tuft of the retained periodontal membrane. (PM).

Fig. 9. Same area as in Fig. 8. Autoradiograph. ×175. H3TDR labelled fibroblasts and endothelial cells are observed in the periodontal membrane remnants (PM) and in the canal (V) penetrating the compact bone plate (CB) of the alveolus.





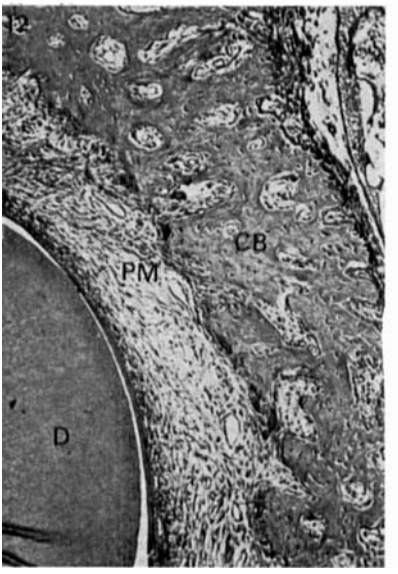


Fig. 10. Same specimen as Fig. 9, encircled part. High power magnification. Autoradiograph. ×700. H3TDR labelled cells in periodontal membrane remnant 48 hours after extraction.
F: labelled fibroblasts.
E: endothelial cells.

Fig. 11. Frontal section through the anterior part of the guinea pig mandible, 7 days after extraction of the right mandibular incisor. ×5.
A: post:extraction alveolus.
T: left mandibular incisor in situ.
Note increased thickness of the left alveolar wall (W), compared to the noninjured right alveolar wall (N).

Fig. 12. Same section as Fig. 11. Higher magnification of the alveolar wall of the nonextracted incisor. ×175.
CB: compact bone plate of socket.
PM: periodontal membrane.
D: dentin.

Fig. 13. Same section as Fig. 11. Higher magnification of the thickened alveolar wall at the side of extractoin. ×175. Newly formed bone (NB) is observed directly at the old compact bone plate of the alveolus (CB).

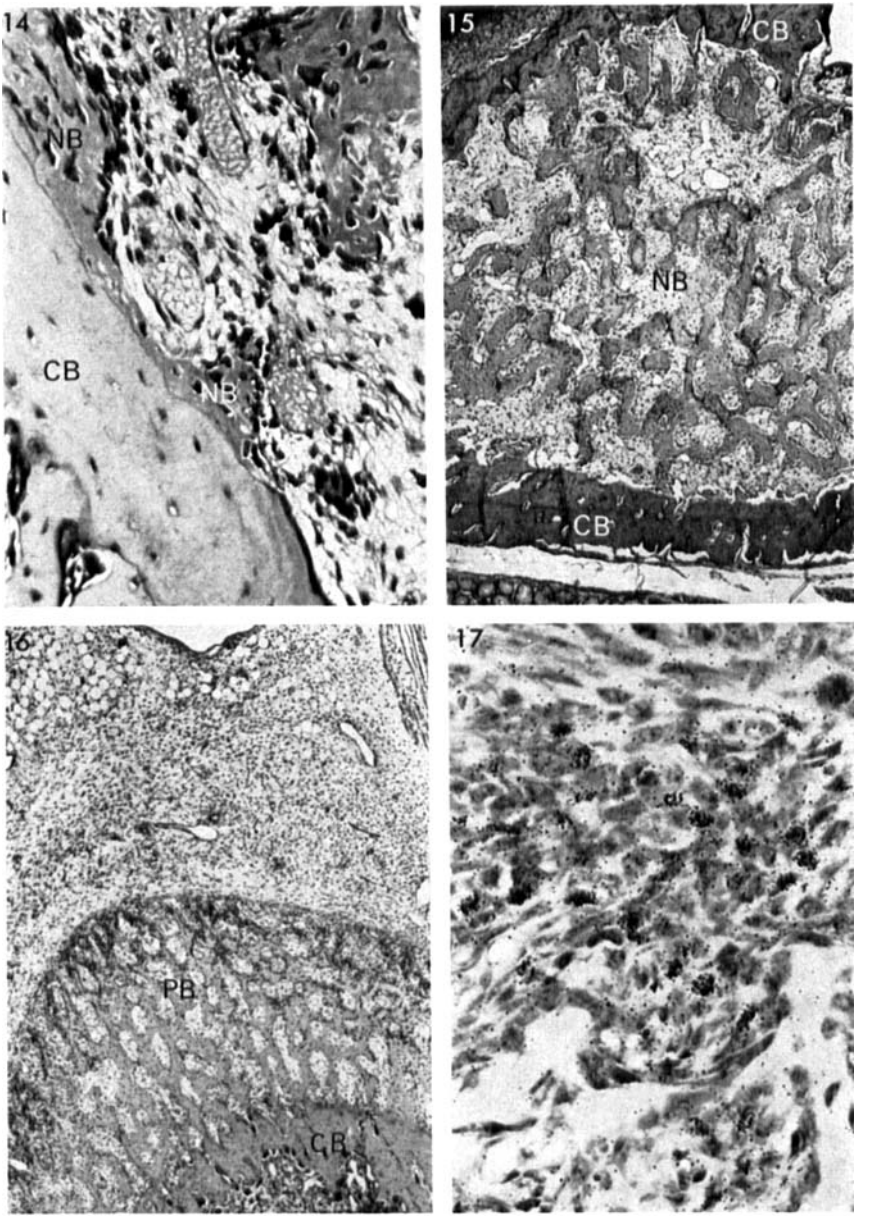


Fig. 14. Post-extraction alveolus 7 days after extraction. ×280. Newly formed bone (NB) is observed directly on the old compact bone plate (CB), separated by a resting line.

Fig. 15. Post-extraction alveolus 21 days after extraction. Middle portion of socket. ×175. The post-extraction alveolus issfilled out with trabeculae of cancellous bone (NB). The compact bone plates of the original socket (CB) are clearly demonstrated.

Fig. 16. Post-extraction alveolus 5 days after extraction. Fundal portion of socket. ×75. Abundant periosteal bone formation (PB) is observed projecting from the compact bone of the mandible (CB). ×280.

Fig. 17. Same specimen as Fig. 16. Autoradiograph. A high number of H3TDR labelled preosteoblasts are observed in the periosteum in the area of the fundal portion of the socket.