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REIMPLANTATION OF MANDIBULAR INCISORS IN THE GUINEA PIG

A HISTOLOGIC AND AUTORADIOGRAPHIC STUDY

by
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INTRODUCTION

Reimplantation and transplantation of dental tissues have attracted the interest of clinicians and researchers for many years. Therefore, numerous case reports and experimental results that deal with these problems are on record. A number of variables have been studied such as the treatment of the periodontal membrane remnants attached to the tooth after extraction, the treatment of the pulp before reimplantation as well as the immunologic response of the host to heterotransplants, homotransplants and autotransplants. For details of these procedures the reader is referred to comprehensive reviews by *Fleming* (1959), *Costich et al* (1963) and *Nordenram* (1963) together with bibliographies by *Coburn & Henriques* (1962) and *Ivanyi* (1962).

Histologic material of the successful reimplantation of teeth with varying observation intervals have been described in man (*Andreasen & Hjörting-Hansen*, 1966) and experimental animals (*Löe & Waerhaug*, 1963). In these studies the most favorable results followed when the remnants of the periodontal fibers were left on the tooth and when the tooth was left out of the socket for only a short period of time. The initial reactions in the periodontal membrane of the reimplanted teeth, so crucial to the outcome of the procedure, have not been described as yet. When a tooth is reimplanted

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in a socket and reattached, the following possibilities exist: the periodontal fibers left in the alveolus and on the tooth reunite without formation of new bone and cementum, the tooth is reattached by formation of new bone, new periodontal fibers and new cementum, the tooth is reattached by formation of cementum and cemental fibers which splice with the periodontal fibers that remain on the socket wall, the tooth is reattached by new fibers formed on the socket wall which splice with the remaining fibers of the cementum. Collagen formation and cellular activity are necessary in any of the hypothetical ways of reattachment listed above.

In order to study the initial reactions in the periodontal tissues after reimplantation, 3H proline was used in the present study. By this experimental approach an estimation of the rate of collagen formation can be assessed by the uptake of 3H proline. Due to the high cost of isotopes, a small experimental animal seemed preferable. The quinea pig was chosen since previous experiments (*Johansen & Gilhuus-Moe*, 1969) showed that periodontal fiber remnants remained attached both to the tooth and to the socket wall after extraction of the incisor. The same situation is found in humans after extraction (*Mangos*, 1941) and in experimental animals as dogs (*Euler*, 1923), rats (*Huebsch et al.*, 1958; *Frandsen*, 1963; *Todo*, 1968), monkeys (*Simpson*, 1960).

MATERIAL AND METHOD

The guinea pig incisor had never been used for reimplantation, wherefore no information on this experimental model was available. Reimplantation and transplantation of incisors have been performed in mice (*Grewe & Felts*, 1968) and the dental anatomy in these two species is comparable. Therefore, in the first group of animals the following procedure was used.

ANIMAL GROUP I

Twelve male albino guinea pigs were kept in separate cages for one week prior to the experimentation. Under Nembutal® anesthesia (0.285 g/kg body weight) the right lower incisor was extracted in each animal by means of a modified hemostat. The extractions were performed with caution in order to reduce trauma to the teeth and jaws to a minimum. Immediately after extraction the tooth was reimplanted into the socket. The animals were weighed regularly in the postoperative periods and fed Purina pellets

Table I.

Distribution of Material According to Observation Time After Reimplantation

Animal Groups	Observation Time in Days	Number of Animals	Mean Weight 0 Day	Standard Deviation
	1	2		
	3	2		
Group I	5	2	232.4 g	23.26 g
Immediate reimplantation	7	2		
	11	2		
	20	2		
	8	2		
Group II	14	2	344.4 g	63.17 g
Immediate reimplantation	18	2		
Liquid diet	25	2		
Group III Immediate reimplantation Fixation, liquid diet and isotopes.	11	12	187.3 g	31.30 g
Total	1—25	32		

and water *ad libitum*. After varying observation periods (Table I) the animals were sacrificed by exanguination in Nembutal® anesthesia. For standardization purposes all experimental procedures, weighing and sacrifice were performed on the same hour of the day.

Two animals were sacrificed at each postoperative interval. The jaws were dissected and immediately placed in Lavdowsky's fixative (*Williams*, 1948) for one week and decalcified in Decal® *. In each group one lower jaw was orientated for longitudinal sectioning and the other for sectioning in the frontal plane and embedded in paraffin. Stepwise serial sections were cut at 5 μ and stained with hematoxylin and eosin.

OBSERVATIONS

Gross Observations

All animals survived. A lag in gain of body weight the first days after the operation was noticed. As visualized in Table II the gross observations

* Scientific Products, Cambridge, Georgia.

Plate I

Fig. 1. One day after reimplantation. Longitudinal section. The enamel epithelium (e) is separated from the enamel matrix (Em) by an inflammatory exudate (ex). Dentine (D). Alveolar bone (B). Hematoxylin and eosin (H+E). Original magnification × 80.

Fig. 2. Three days observation time. Frontal section. It appears as if a splicing of the periodontal fibers from the cementum (C) and the bone (B) has taken place. A few inflammatory cells are seen. Osteoclasts (oc) are seen along the bony alveolus. H+E. Original magnification × 200.

Fig. 3. Three days observation time. An inflammatory exudate (ex) is separating the periodontal fibers from the cementum (C) and the bone (B) in the area where the break in the periodontium occurred when the tooth was extracted. H+E. Original magnification × 80.

Plate II

Fig. 4. A higher magnification of the same section as Fig. 3. The inflammatory exudate (ex) is separating the periodontal fibers from the cementum (C) and the bone (B). The number of cementoblast is reduced in this area. Osteoclasts are lining the bone at the top of the illustration. In this area more cementoblasts are seen along the cementum surface. H+E. Original magnification × 200.

Fig. 5. This illustration shows a survey of a frontal section near the apex of the tooth five days after reimplantation. Abundant periosteal bone proliferation is seen to the left in the picture (Pb). To the right an area of reattachment can be seen (Ra). The pulp is necrotic (P). The thin dentine has been deformed and parts of the enamel matrix have been lost in the histologic procedure. H+E. Original magnification × 20.

Plate III

Fig. 6. This illustration shows some details from the same section as Fig. 5. Osteoclasts are lining the bony alveolus (B) in an area of reattachment. There is no trace of the vascular zone. The number of cementoblasts along the cementum surface (C) is reduced and the direction of the periodontal fibers is changed. The space between the connective tissue of the periodontal membrane and the bone is an artifact (a). H+E. Original magnification × 80.

Fig. 7. From the control side in the same specimen as Figs. 5 and 6. Note the number of cementoblasts along the cementum surface (C), the orientation of the periodontal fibers and the large vessels (v) of the vascular zone. Alveolar bone (B). H+E. Original magnification × 80.

Fig. 8. Shows the pulp in the control tooth. Odontoblasts (ob), dentine (D) and the numerous sinusoid vessels (v) in the pulp are demonstrated. H+E. Original magnification × 80.

Fig. 9. The pulp is necrotic in the reimplanted tooth. The normal morphology seen in Fig. 8 has disappeared. H+E. Original magnification × 200.

Plate IV

Fig. 10. A survey of a frontal section from the incisal part of the reimplanted tooth after eleven days. Reattachment (Ra) is seen at the bottom left part of the tooth. Areas of cementum resorption are present at the lateral part of the tooth. Inflammation is more prominent at the labial side of the tooth (L). The enamel epithelium is absent. (Es) is the space occupied by the enamel which had been removed in the decalcifying process. H+E. Original magnification × 20.

Fig. 11. Higher magnification of an area of reattachment from the same specimen as Fig. 10. The original cementum has been resorbed, but a new layer of cementum with periodontal fibers has been formed later. These fibers do not have the normal orientation. Compare this to the fiber direction in Fig. 7. H+E. Original magnification × 200.

PLATE I

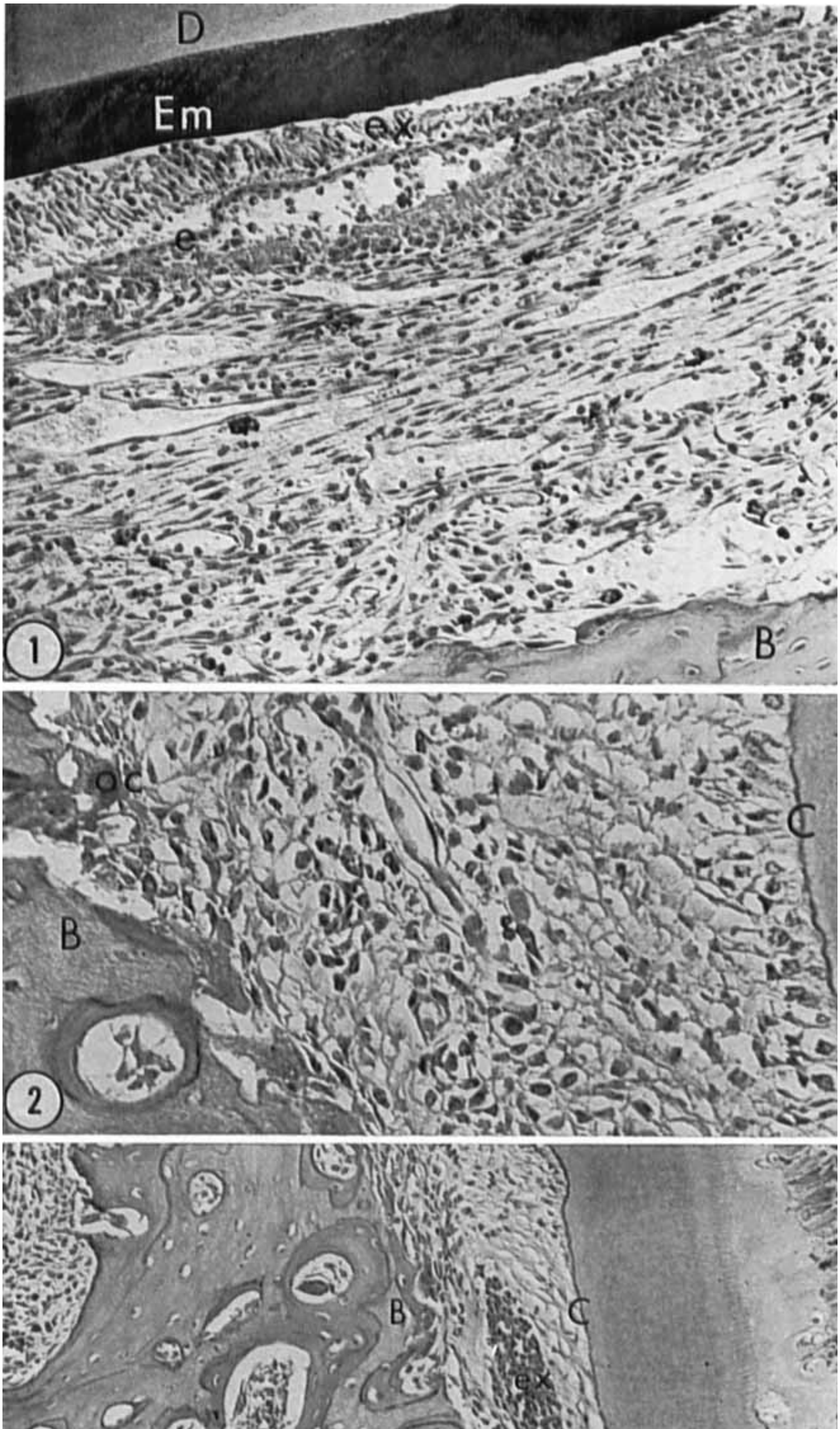
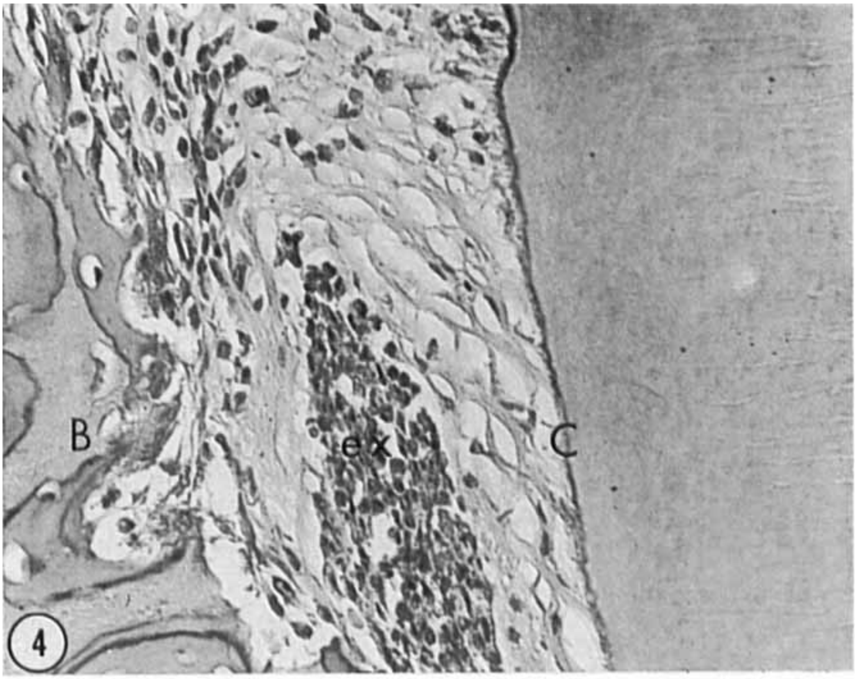
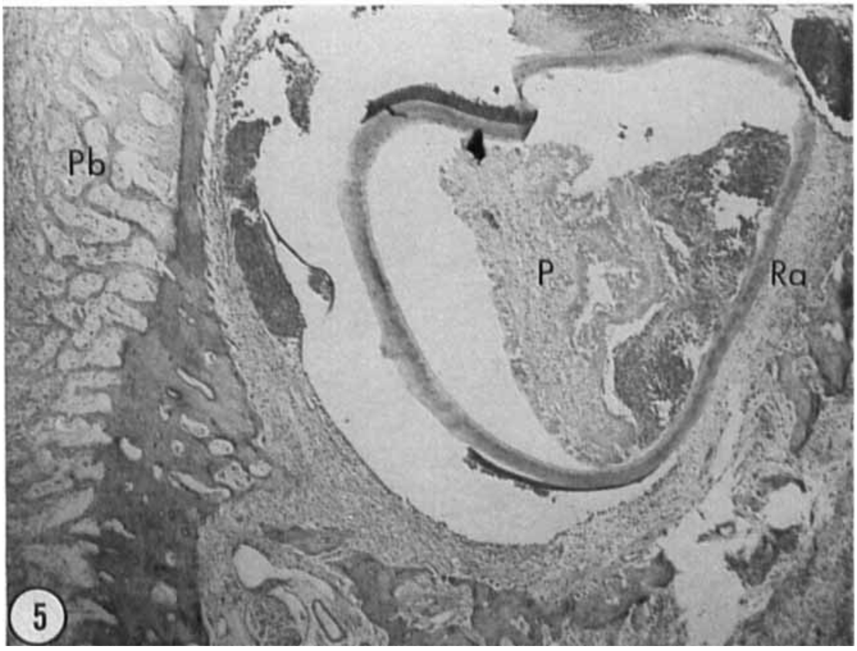


PLATE II





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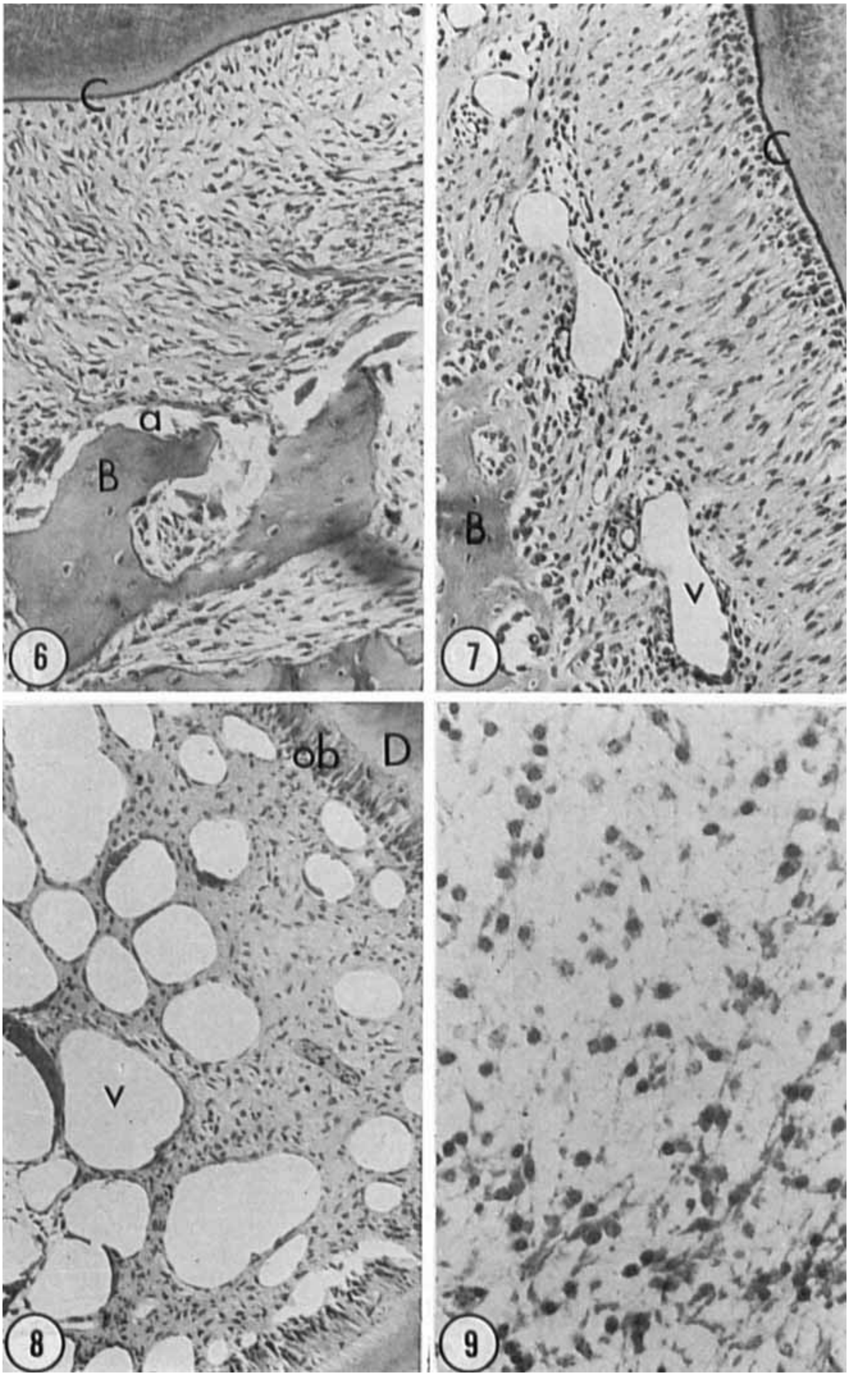

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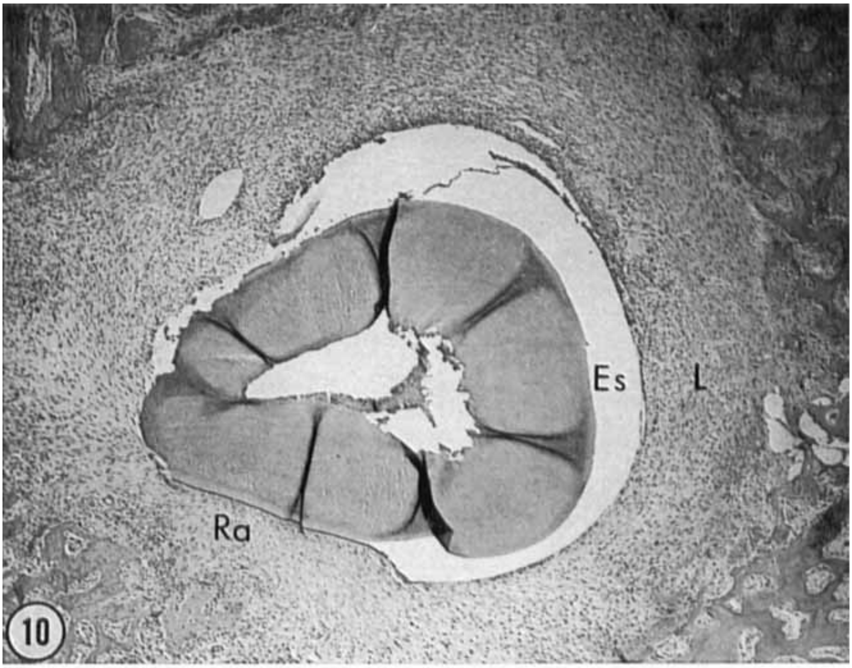


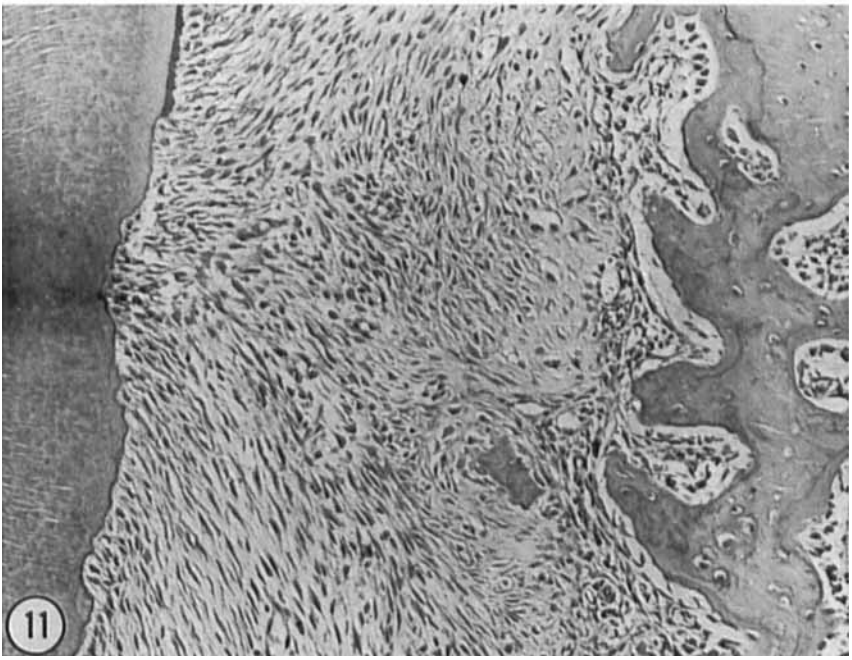
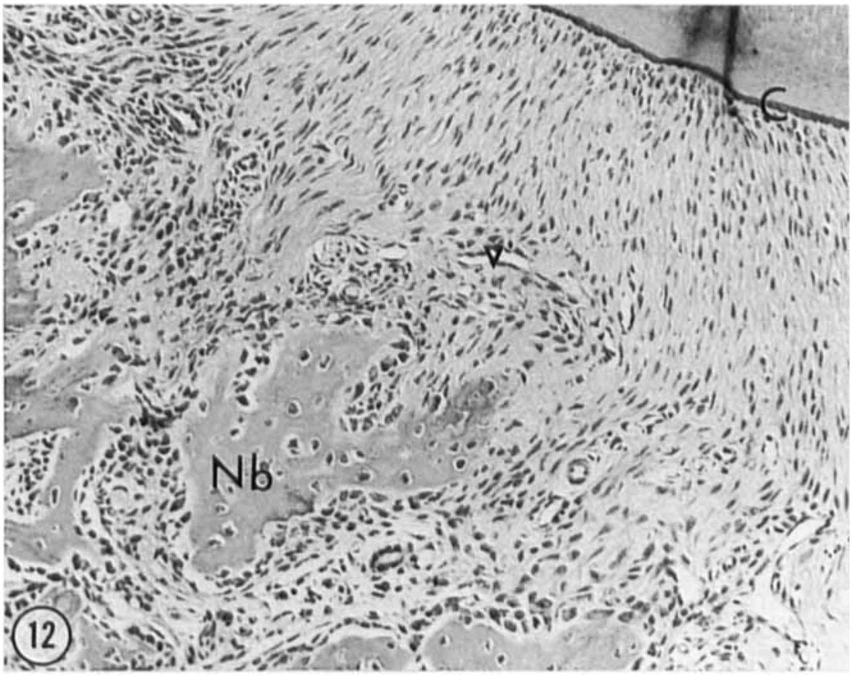


PLATE V



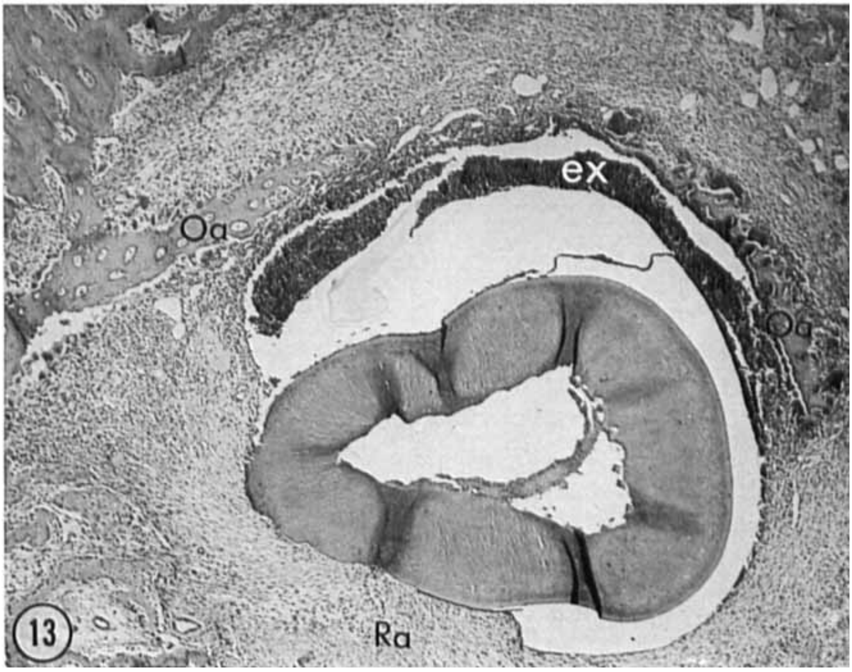
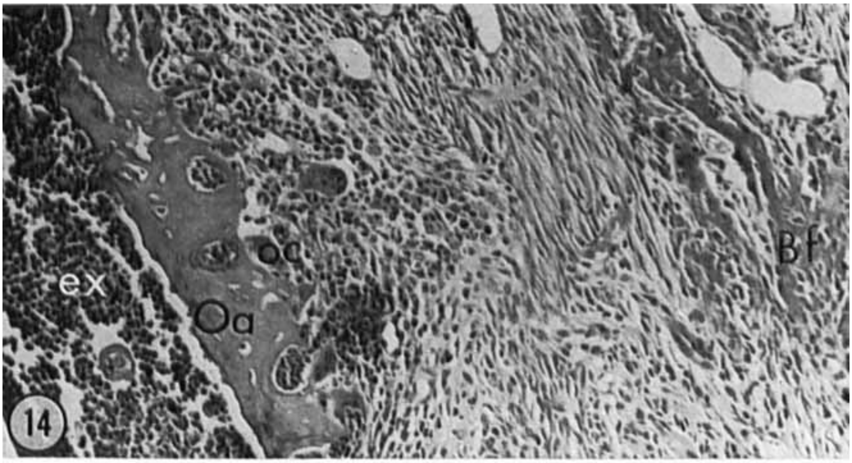


PLATE VI



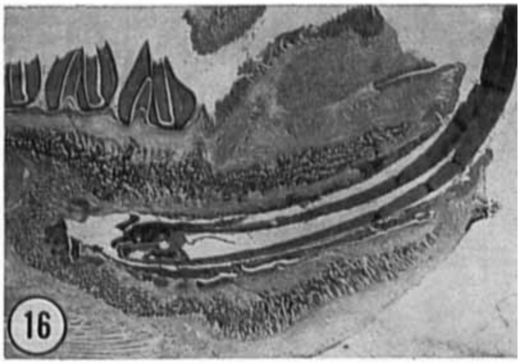
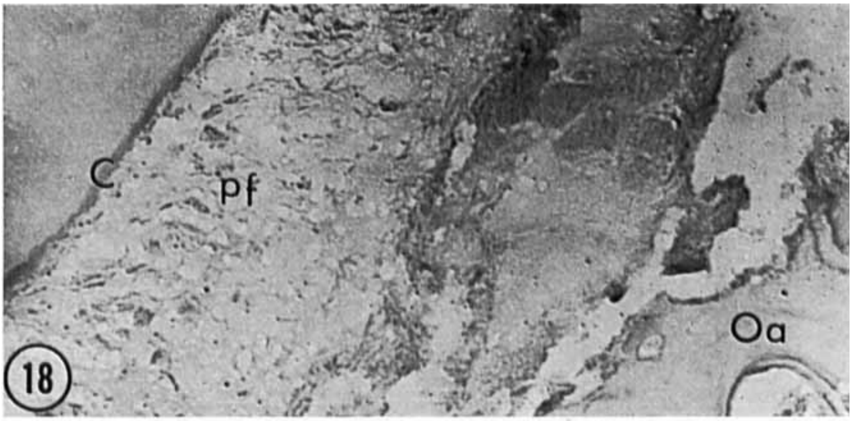


PLATE VII



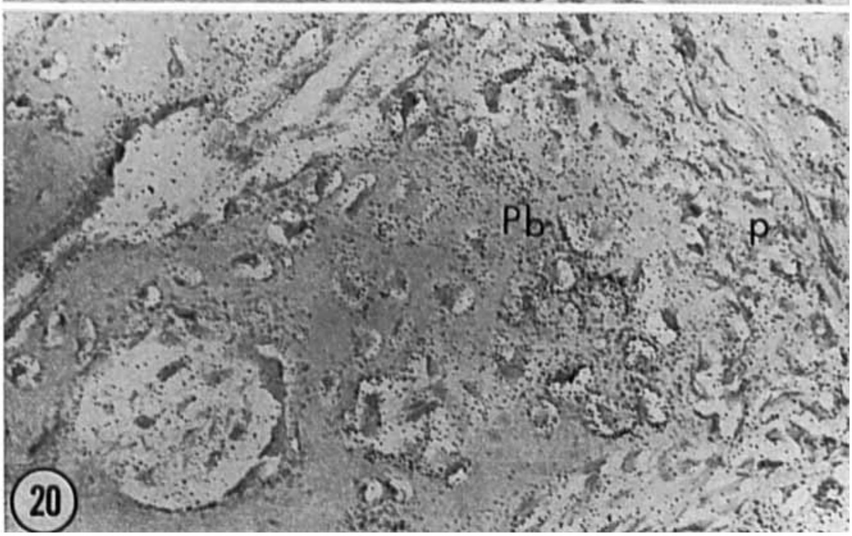


PLATE VIII

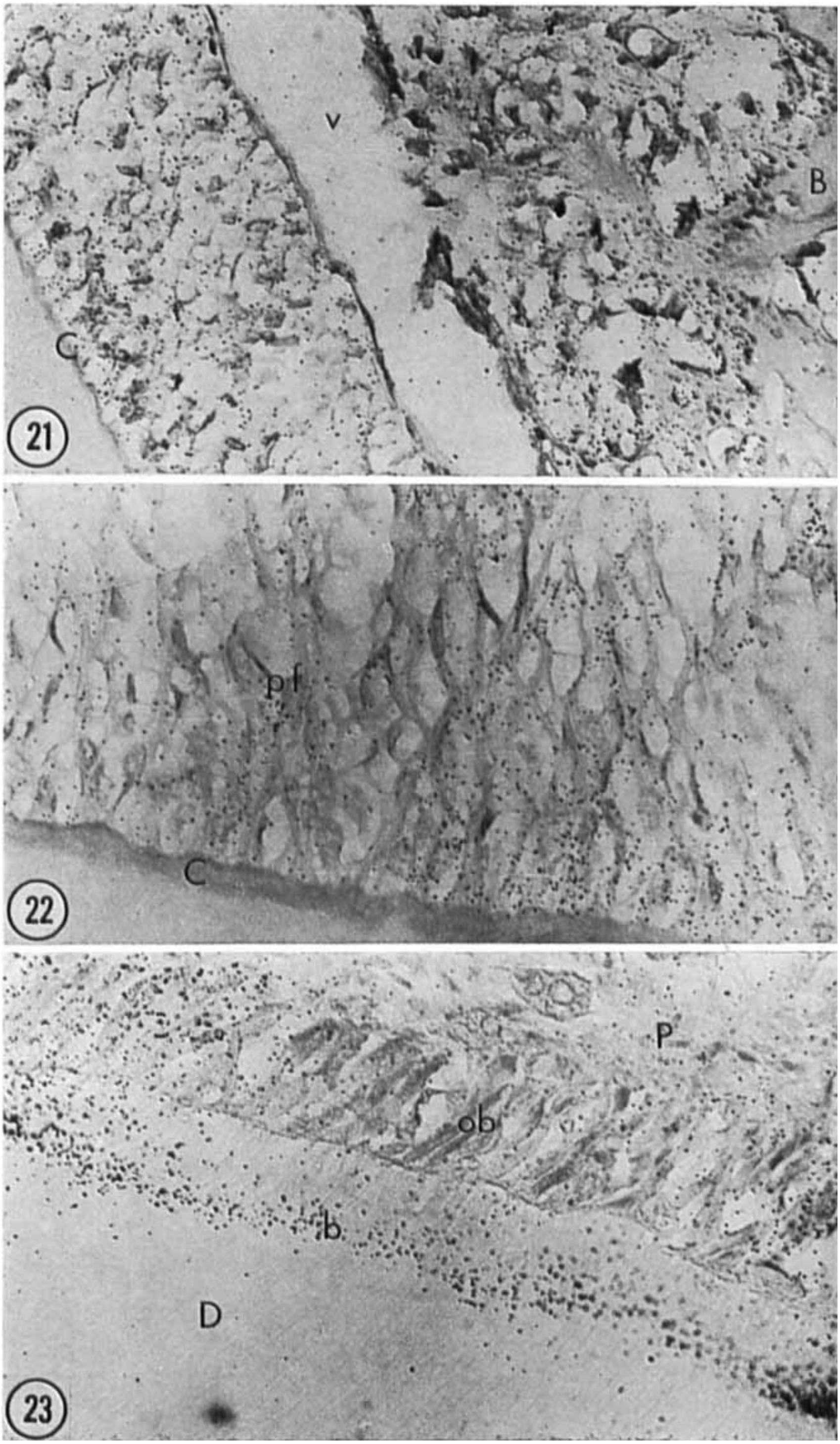


Plate V

Fig. 12. This a higher magnification of the reattachment area in Fig. 10. The perioadontal fibers from the cementum (C) show normal direction. New bone (Nb) is formed at the alveolar side of the periodontal membrane. New vessels (v) are being formed in this area which now resembles the vascular zone seen on the control side (Fig. 7), but the number and the size of the vessels area still smaller than normal. H+E. Original magnification × 200.

Fig. 13. This represents a section more apical in the reimplanted tooth than Fig. 10. The reattachment area (Ra) is still seen. Note the presence of the inflammatory exudate (ex) which extends from the labial side to the lateral side (top of the illustration). Osteoclasts are resorbing the original alveolus (Oa) in these areas.

Plate VI

Fig. 14. A higher magnification of a part of the section shown in Fig. 13. The original alveolus (Oa) is resorbed by osteoclasts (oc) from the marrow spaces. Bone formation (Bf) is seen in the right side of the illustration. Pus is (ex) present in the space between the tooth and the original alveolus. H+E. Original magnification × 200.

Fig. 15. This picture is taken from a specimen fourteen days after reimplantation in animal group II. Frontal section. The alveolar bone around the reimplanted tooth (to the right in the illustration) is much thicker than the alveolar bone on the control side (to the left). The new bone is surrounding the reimplanted tooth and the original alveolar bone. H+E. Original magnification × 4.

Fig. 16. Longitudinal section eleven days after reimplantation of the tooth in animal group III. Abundant new bone formation on the lingual side (top of picture) and on the labial side (bottom of picture) as well as on the dorsal side (left in the picture). The original alveolus is surrounded by dense accumulations of inflammatory cells especially on the labial side. The pulp chamber is empty. H+E. Original magnification × 4.

Fig. 17. A higher magnification of the orificial, lingual part of the periodontium of the section in Fig. 16. The epithelium (e) is proliferating along the necrotic original alveolus (Oa). New bone (Nb) surrounds the original alveolus which gradually is resorbed. There are almost no periodontal fibers attached to the tooth (T) and to the bone (Oa). H+E. Original magnification × 80.

Plate VII

Fig. 18. A frontal section from a specimen in animal group III, eleven days after reimplantation. There is no labelling with 3H proline in the cementum (C) or the periodontal fibers (pf) attached to the reimplanted tooth. The original alveolus (Oa) is non-labelled. Harris hematoxylin Autoradiograph. Original magnification × 200.

Fig. 19. Higher magnification of a part of the original alveolus in Fig. 18. No labelling can be traced in the marrow spaces. Autoradiograph. Harris hematoxylin. Original magnification × 320.

Fig. 20. Intense labelling of the periosteum (p) and newly formed periosteal bone (Pb) at the reimplantation side. Harris hematoxylin. Autoradiograph. Original magnification × 200.

Plate VIII

Fig. 21. This illustration is from the non-reimplanted side in the same specimen as Figs. 18, 19, 20. The entire width of the periodontal membrane from the cementum (C) to the bone (B) shows uptake of 3H proline. A large vessel (v) in the vascular zone of the periodontal membrane is seen in the center of the picture. Harris hematoxylin. Autoradiograph. Original magnification × 200.

Fig. 22. A higher magnification of the cementum (C) and the periodontal fibers (pf) in Figure 21. Dense labelling of the fibers is evident. Harris hematoxylin. Autoradiograph. Original magnification × 320.

Fig. 23. This picture shows the pulp (P), the odontoblasts (ob) and the dentine (D) of the non-reimplanted tooth in the same specimen as Figs. 18—22. There is a lebelling band (b) in the dentine away from the odontoblasts. This distance indicates the amount of dentine formation in the guinea pig incisor in 24 hours. Harris hematoxylin. Autoradiograph. Original magnification × 320.

Table II.

Gross observations in animal group I.
Normal diet (purina pellets)

Observation Period in Days	No. of Animals	Course of Experimental Procedure	Observations at Sacrifice: Gingival Margin	Position of Reimplanted Tooth	Mobility of Reimplanted Tooth
20	1	Dental sac remained in alveolus	Normal	Labial	Slightly increased
20	1	½ Crown fractured, but the tooth reimplanted	Normal	Labial	Slightly increased
11	1	Uncomplicated	Mild inflammation, pus	Normal	Normal
11	1	Uncomplicated	Mild inflammation	Normal	Normal
7	1	Uncomplicated	Pus & inflammation	Overeruption ca. 2 mm	Increased
7	1	Uncomplicated	Pus & inflammation	Normal	Increased
5	1	Fracture of crown tooth replanted	Pus & inflammation	Normal	Increased
5	1	Uncomplicated	Mild inflammation	Labial	Increased
3	1	Uncomplicated	Food pack, inflammation	Crown not visible	Not observed
3	1	Uncomplicated	Inflammation	Fractured labial	Loose
1	1	Fractured	Inflammation	In the socket	Not observed
1	1	Uncomplicated	Inflammation	Normal	Normal

varied. The most constant observation was increased mobility of the reimplanted tooth. The gingiva around the teeth were slightly red to definitely inflamed and in some instances pus was seen.

Histologic observations

One day specimens. One day after reimplantation the location of the rupture of the periodontal membrane after extraction was visible. There was a clear difference between the lingual and the labial part of the periodontium. When the tooth was extracted the rupture of the continuity between the tooth and the bone occurred between the enamel and the enamel epithelium on the labial surface. The enamel epithelium in the outer portion of the socket could be distinguished in various relationships to the tooth, either in close contact with the partly calcified enamel matrix or separated from the matrix by an exudate (Fig. 1). In the apical parts the enamel epithelium was degenerated and reduced to a flat layer of cells. In the middle part of the labial periodontal membrane the fibers appeared disorganized and edematous. Macrophages with engulfed hemosiderin were abundant. Towards the socket wall the blood vessels were dilated and the connective tissue edematous. On the lingual and lateral sides of the tooth where periodontal fibers normally run from cementum to bone, the rupture after extraction took place in the outer highly vascularized zone. A brief description of the anatomy of the incisor and the periodontium in the guinea pig has been presented previously (*Johansen & Gilhuus-Moe*, 1969), and the same terminology will be used in this report. Periodontal fibers were seen attached to the tooth and to the bone separated by an inflammatory exudate. The number of fibroblasts was reduced and the fibers appeared edematous and had lost their normal direction. Inflammatory cells were seen but less macrophages were present here as compared to the labial side.

The pulp in the reimplanted tooth showed intensive edema with varying number of inflammatory cells. The inflammation was pronounced in the apical region of the pulp.

The periosteum on the labial side of the reimplanted tooth had increased in thickness and the layer of osteoid was thicker than on the control side. No reattachment of the tooth had taken place.

Three days specimens. After three days the enamel epithelium was seen in different phases of degeneration, and in the fundal part it was in some places completely necrotic. The labial part of the periodontium appeared edematous with macrophages and other inflammatory cells. The lingual part of the periodontium also showed varying degrees of inflammation. A reattachment took place in an area about one-fourth of the circumference of the lingual part of the periodontal membrane. It seemed as if a direct splicing of the fibers from the bone and from the cementum took place (Fig. 2). The splicing occured in the »vascular zone» which could not be distinguished. Some osteoclasts were seen along the alveolar wall in this location. In other parts of the lingual periodontal membrane proper an inflammatory exudate separated the tooth from the alveolar wall. In these areas osteoclasts were numerous in the adjacent marrow spaces. The inflammatory exudate separated the fibers remaining on the cemental surface and the fibers on the alveolus after extraction (Fig. 3) and no resorption was seen on the tooth surface. The number of cementoblasts was decreased (Fig. 4). The pulp exhibited engorged vessels, extravasation of blood, and a communication between the pulp and the periodontal membrane proper was also seen in one specimen. The periosteum around the mandible at the labial and lateral surfaces showed proliferation of the cells of the cambium layer.

Five days specimens. After five days (Fig. 5) the enamel epithelium was completely atrophied and the labial part of the periodontium was infiltrated with inflammatory cells. In some parts of the periodontal membrane proper where inflammation was less prominent, osteoclasts were lining the alveolar wall (Fig. 6). In these areas the number of cementoblasts was reduced on the cementum surface, but disorganized fibers could easily be distinguished. No trace of the »vascular area» present on the control side (Fig. 7) was found in this region. Some reattachment had taken place but it seemed that this was gradually being destroyed by progressing inflammation that spread along the bony alveolar wall. In the areas of dense inflammation no reattachment was seen. The fibers from the cementum could still be discerned, but the cementoblasts and fibroblasts were atrophied and in places completely absent.

The pulp was undergoing necrosis. The vessels present in the normal pulp (Fig. 8) had disappeared (Fig. 9). The necrosis was complete in the apical part of the tooth.

Periosteal bone proliferation had taken place and the mandible on the reimplantation side was much thicker than on the control side. The original alveolus was being resorbed and the new bone was in progress to envelope the parts of the mandible that had been in close relation to the tooth (Fig. 5).

Seven days specimens. At seven days a small area of reattachment was seen in the lingual part of the periodontal membrane. The earlier observations of necrosis of the pulp, the enamel epithelium and periosteal bone proliferation had progressed even further than in the five day specimens. Separation of the original alveolar bone by osteoclasts in the marrow spaces was a dominant feature.

Eleven days specimens. Eleven days after reimplantation almost complete reattachment was seen in the incisal part of one specimen (Fig. 10). Some parts of the cementum (Fig. 11) were resorbed, particularly in the vicinity of the inflammation present along the tooth surface on the labial part. Whether there had been complete reattachment in the areas of resorption prior to the present resorption was impossible to decide but only minor inflammation was seen in the region and a thin layer of newly formed cementum was evident (Fig. 11). The fibers here were not oriented in a normal fashion but ran in a circumferential direction (Fig. 10). On the medial surface of the tooth (towards the symphysis) an almost normal periodontal membrane was seen. The fibers from the cementum were distinctly oriented and the number of cementoblasts was normal. New bone formation was evident, and from this new bone fibers extended and met the cemental fibers in the vascularized zone, which was seen to reappear (Fig. 12). The number and size of vessels were still less than normal (Fig. 7).

Sections more apical to those described above showed increased spread of the inflammation on the labial surface of the tooth (Fig. 13). It was noticeable that the resorption of the alveolar bone started from the marrow spaces mainly (Fig. 14), while bone apposition started around the periphery of the mandible on the reimplantation side (Fig. 5). The true reattachment seen on the medial side against the symphysis was present although the labial and lateral portions of the periodontal membrane showed an intense inflammatory reaction. The inflammation was in accordance with the finding of pus in the gross examination of the animal prior to sacrifice. The pulp was necrotic and the enamel epithelium could not be seen.

Twenty days specimens. After twenty days there was no reattachment. The alveolus around the tooth was completely separated from the rest of the mandible by the inflammatory exudate. No osteocytes were present in the necrotic bone. In other areas the original alveolar wall had been completely resorbed and bone formation from the periphery was narrowing the space between the tooth and the newly formed bone. It appeared as if the tooth and the original bone of the periodontal membrane adjacent to it was enveloped like a sequestrum. Degenerated periodontal fibers attached to the cementum and to the original alveolus were seen, separated by the inflammatory cells. The pulp was necrotic and the pulp chamber was wider in the reimplanted tooth than in the control tooth, which showed that the continuous formation of dentine had ceased in the reimplanted tooth. The labial and lateral surface of the mandible at the reimplantation side was two to three times as thick as on the control side.

ANIMAL GROUP II

MATERIAL AND METHOD

Reattachment of the reimplanted tooth in animal group I was an unpredictable result. Failures might be due to several factors. One of these might be the effect of chewing of the Purina pellets with the reimplanted tooth. In order to study this further, a new group of animals was used. The experimental procedure was identical to the procedure in group I, but in order to

Table III.

Gross observations in animal group II.
Liquid diet

			Observations at Sacrifice		
Observation Period in Days	No. of Animals	Course of Experimental Procedure	Gingival Margin	Position of Reimplanted Tooth	Mobility of Reimplanted Tooth
8	1	Uncomplicated	Mild inflammation	Overerupted 2 mm	Increased
8	1	Tooth fractured in the forceps outside socket Both pieces reimplanted	Mild inflammation	Normal	Normal
14	1	Tooth fracture, incisal part reimplanted	Normal	1/2 the crown missing	Increased
14	1	Uncomplicated	Normal	1 1/2 mm shorter than control	Normal
18	1	Crown fracture Tooth reimplanted	Inflammation	3 mm shorter than control	Normal
18	1	Uncomplicated	Inflammation	Labial position	Increased
25	1	Crown fracture but reimplanted	Inflammation	At the level of gingival margin	Difficult to assess
25	1	Complicated reimplanted after fracture of crown 2 mm above gingival margin	Mild inflammation almost normal	Labial	Loose

reduce the trauma to the reimplanted tooth, a liquid diet (Sustagen ®)* was used. The animals were placed on this diet one week prior to the reimplantation procedure and kept on the liquid diet during the postoperative periods. In order to obtain information from a wider range of postoperative observation periods than in group I, these animals were sacrificed in pairs after eight, fourteen, eighteen and twenty-five days (Table I). The mode of sacrifice and the histological techniques were the same as in group I.

* Collier Drug Company, 2 North 4th Place, Birmingham, Alabama.

OBSERVATIONS

Gross Observations

All animals survived the experiment. The body weights were presented in Table I. It was evident that the animals gained weight normally on the liquid diet. Gross observation at the time of sacrifice are presented in Table III.

Histological Observations

The main features in these animals corresponded to the findings in animal group I such as inflammation around the tooth and progressive sequestration of the alveolus with the tooth in it (Fig. 15) and increased thickness of the mandible at the reimplantation side. Reattachment was not seen later than 8 days. The pulp was necrotic in all specimens.

ANIMAL GROUP III

MATERIAL AND METHOD

From the experience gained in the two proceeding animal groups it was clear that the change from hard to liquid diet had no effect on the chances for reattachment. Therefore, a third group of twelve animals were used. The experimental procedure was the same in this group as in the two previous ones. In the present group, however, the reimplanted tooth was ligated to the neighboring incisor by means of a soft stainless steel wire. The wire was applied as a digit eight around the two incisors. After tightening, the wire was painted with self-curing acrylic to inforce the stability of the splint. The animals were fed the same liquid diet as in group II. The purpose of this procedure was to evaluate the effect of splinting on the success of reattachment after reimplantation. Based on the experience gained in animal group I all animals in group III were killed after 11 days, since this postoperative interval had proved to be the most suitable for studying reattachment in group I. Twenty-four hours prior to sacrifice all animals were injected intraperitoneally with 3H proline* (3 μ c/g body weight). The animals were killed by exanguination after an overdose of Nembutal.® The lower jaws were dissected, fixed in Lavdowsky's solution, decalcified in Decal ®. Six mandibles were cut in a longitudinal plane at 5 μ, and the other six mandibles were

* Specific activity 0.5 c/m mole., Schwarz Bioresearch, Orangeburg, New York.

cut in a frontal plane at 5 μ. The selection for the choice of sectioning plane was made at random. Serial sections were prepared. The sections were stained with hematoxylin-eosin, Alcian blue-PAS ph 2.7 (*Mowry*, 1963). Other sections were prepared for autoradiography by the dipping procedure (*Joftes*, 1963) using Kodak NTB2 emulsion *. After drying the slides were kept in light- tight boxes in a dry atmosphere at 4°C for three weeks, developed and stained through the emulsion with Harris hematoxylin. The autoradiographic procedures were made in complete darkness.

OBSERVATIONS

Gross Observations

No animals died. Their mean weight is shown in Table I and a summary of the clinical findings at the time of sacrifice is given in Table IV.

Histologic Observations

No reattachment was seen in any of the specimens. In all animals with uncomplicated extraction and reimplantation varying degrees of sequestration of the tooth and parts of the surrounding alveolus were noted (Fig. 16). The oral epithelium proliferated apically. The epithelial proliferation did not take place between the tooth and the alveolus but along the dental part of the original alveolar bone which was necrotic and in different stages of resorption (Fig. 17).

Increased thickness of the bone of the mandible was evident in all specimens at the reimplantation side, and the pulp was necrotic. In short, the findings in this series were the same as in the two proceeding series. The difference was that in animal group I a partial reattachment was found, which was not seen in any of the animals in group III.

Autoradiographic Observations

The injected 3H proline was taken up by all animals in group III. The distribution of the label confirmed the histologic observations. There was no labelling of the sequestrating alveolus or the cementum and the periodontal fibers at the reimplanted side (Figs. 18 and 19). On the non-reimplanted side

* New England Nuclear Corporation, Boston, Massachusetts, U.S.A.

Table IV.

Gross observations in animal group III
Liquid diet with fixation. 3H proline injected

			Observations at Sacrifice		
Observation Period in Days	No. of Animals	Course of Experimental Procedure	Gingival Margin	Position of Reimplanted Tooth	Mobility of Reimplanted Tooth
11	1	Uncomplicated, reimplanted 2 mm below the other incisor	Mild inflammation Periosteal bone proliferation palpable	2 mm lower than neighbor, but splint came out 2 days before sacrifice	Increased
11	1	Uncomplicated	Mild inflammation Periosteal bone increase can be palpated	Normal	Not assessed
11	1	Uncomplicated	Food pack, Periosteal bone increase can be palpated Mild inflammation. No pus	Normal	Not assessed
11	1	Uncomplicated	Mild inflammation Pus upon palation Periosteal bone increase can be palpated	Splint is loose	Increased
11	1	Uncomplicated	Normal. Periosteal bone increase can be palpated	Normal	Not assessed
11	1	Uncomplicated	Some pus upon palpation. Periosteal bone increase can be palpated	Normal	Not assessed
11	1	Uncomplicated	Periosteal bone proliferation palpable. Mild inflammation	Normal	Not assessed
11	1	Uncomplicated	No pus. Mild inflammation. Bone proliferation palpable	Normal	Not assessed
11	1	Crown fracture but reimplanted with fixation.	Food pack. Mild inflammation. No pus. Palpable bone prolif.	Ca 4 mm below control tooth	Not assessed
11	1	Crown fracture Reimplanted in the same occlusal plane as control tooth.	Pus, palpable bone proliferation. Inflamed.	Normal	Not assessed
11	1	Crown fracture Reimplanted with fixation	Food pack. Mild inflammation. Bone proliferation palpable.	3 mm lower than control tooth	Not assessed

the periodontal membrane showed uptake of 3H proline in its entire width from cementum to bone (Figs. 21 and 22). A labelling band was found in the dentine of the tooth on this side and the pulp also was labelled (Fig. 23).

DISCUSSION

The enamel epithelium that normally covers the labial part of the guinea pig incisor was always seen at different degrees of atrophy after the reimplantation procedures. It appeared that this tissue had no capacity of repair after the extraction and reimplantation trauma. It has been shown earlier (*Johansen & Gilhuus-Moe*, 1969) that the enamel epithelium will remain in the socket after extraction. Thus, the connection between the enamel in various phases of mineralization, and the enamel epithelium is weaker than the connection between the enamel epithelium and the surrounding connective tissues of the labial part of the periodontium. By increasing observation periods inflammatory exudate was seen in the space between the enamel and the degenerating enamel epithelium, in accordance with the postulate that pus follows the path of least resistance.

The sensitivity of the enamel epithelium to trauma seen in this experiment and also reported previously (*Johansen & Gilhuus-Moe*, 1969) contrasted with the findings reported by *Grewe & Felts* (1968) in mice incisors. They reported that »75 % of the replanted incisors exhibited further growth and maturation». In the present study no further growth of the reimplanted incisor was seen in any animal after complete extraction and subsequent reimplantation. In one animal (TP9 in animal group III) the tooth fractured deep in the socket and the incisal fragment was reimplanted. In this animal the tooth fragment left in the socket continued to form normal dental hard tissues. Osteodentin was seen corresponding to the formative region at the time of experimentation. This finding corresponds closely to the reported dental hard tissue formation that was found when the dental papilla was left behind in the alveolus in a previous report on extraction of guinea pig incisors (*Johansen & Gilhuus-Moe*, 1969). It is difficult to explain the differences between the observations of *Grewe & Felts* (1968) and the present. It seems unlikely that the species difference alone would account for the discrepancy. It may be that the use of unerupted and newly erupted teeth in the study by *Grewe & Felts* (1968) could play a role in the discrepancies observed.

The pulp underwent necrosis in all reimplanted incisors. This was surprising since the part of the pulp and the enamel organ left in the alveolus after extraction in a previous study (*Johansen & Gilhuus-Moe*, 1969) showed the capacity to continue to form dental hard tissues. There again, a comparison with the report of *Grewe & Felts* (1968) seems logical. Further growth and maturation of the incisors necessitates a vital pulp. This was not found in this experiment in contrast to the above mentioned report. The further growth of the incisors was anticipated to take place. Especially so since *Myers et al* (1954) reported success after reimplantation of hamster molars with both closed and open apices. They speculated about the possibility of pulp regeneration or repair after initial necrotic changes in the pulp. The possibility of pulp repair in the guinea pig incisor can be excluded. With increasing observation intervals the reimplanted tooth together with part of the surrounding alveolus were sequestrating, rendering any repair impossible. The role of root canal filling in dogs prior to reimplantation of teeth with closed apices has been discussed by *Knight et al* (1964), who stated that better results were obtained in treated than the non-treated teeth after reimplantation, even though reattachment of the teeth in the sockets as well as some evidence of pulp repair could be found in the untreated teeth. The guinea pig has an open apex whereby the possibility of repair after reimplantation should be optimal if the speculations of *Knight et al* (1964) were correct and a comparison of the guinea pig incisor with hamster molar (*Myers et al.*, 1954) and the incisors of dogs (*Knight et al.*, 1964) were possible. It was therefore surprising that in the present study survival of the pulp following reimplantation could not be verified.

No formation of cementum was found after the reimplantation. The cementoblasts decreased in number and eventually disappeared with increasing observation periods. Some areas of resorption of the cemental surface was seen. This was clearly evident in one of the seven day specimens in animal group I. The fibers of the cemental surface were also degenerating by prolonged postoperative intervals, but the configuration of collagen fibers from the cementum out in the periodontal membrane space could be seen more clearly here than in the fibers originating from the bony wall of the periodontium. New bone formation inside the periodontal membrane was found in the seventh day specimen in the region of reattachment. (Reattachment in this study is defined as a restored periodontal fiber apparatus running from the cemental surface to the alveolar wall). This was the only specimen where this phenomenon was observed. In other areas in the same specimen this bone formation could not be seen, but in these locations no reattachment was present.

Partial reattachment was seen in the three, five, seven and eleven day specimens in animal group I. No reattachment was seen in animal group II and III. It was speculated during the progress of this experiment that the chewing of pellets in group I and the lack of fixation of the reimplanted tooth in group II could be responsible for the unpredictable success of the reimplantation procedures. Obviously this was not so. Animal group III in which both splinting and soft diet was used did not show any reattachment. Thereby the observed reattachment in the eleventh day specimen in group I is difficult to explain. It might be, that eleven days is too long observation time, and that reattachment takes place prior to eleven days. This can be substantiated by the findings of increasing pulp necrosis and subsequent exudation which spreads in the area of the split between the periodontal fibers from cementum and bone. By this exudation and new splicing of the fibers from the cementum and the bone is broken and reattachment therefore will not be seen in later postoperative intervals, since the pulp remains necrotic and the tooth with the surrounding alveolar bone is eventually discharged as a sequestrum. It is important to note that in the longer postoperative intervals the proliferating oral epithelium was not seen between the tooth and the bone, but surrounding the sequestrating alveolar bone (Fig. 17), and that the separation of this part of the alveolar bone by osteoclasts started in the marrow spaces and not from the necrotic periodontal membrane side. This observation resembles the observations of *Reitan* (1951), who reported that resorption of the bone of the periodontal membrane took place from the marrow spaces when excessive force was used in experimental orthodontic movement of teeth. In the present study necrosis of the cells of the periodontal membrane might be caused by the extension of the inflammation of the pulp that spreads within the periodontal membrane. This inflammatory exudate follows the weak connection between the enamel and the enamel epithelium in the short observation periods. At this stage reattachment may already have taken place at the lingual side. After longer observation periods, the exudation increases, pus spreads in the entire circumference of the tooth and the previous attachment is broken. Necrosis of all cells between the tooth and the bone follows. Therefore, no resorption of the tooth or the bone can take place in the space previously occupied by the periodontal membrane. The separation of the tooth and the bone takes place and starts from the marrow spaces by osteoclastic activity. This description resembles the demarcation process found in osteomyelitis.

It appeared as if the reattachment that took place in the third, fifth, seventh and eleventh day specimens in group I took place in the »vascularized area« (*Johansen & Gilhuus-Moe*, 1969). No bone formation or cementum formation seemed necessary for the first splicing of the fibers. It may be speculated that the reunion of fibers takes place without new formation of the entire fiber apparatus across the periodontal membrane. The most striking difference between the control tooth (Fig. 13) and the reimplanted tooth was the disappearance of the »vascular zone» on the experimental side. Also new bone formation in this region was seen. It seems therefore that the first reattachment took place by healing by the first intention (after three and five days). By the eleventh day the fibers and the bone along the original alveolar wall was replaced by new fibers embedded in a layer of new bone. Unfortunately no reattachment was found in the 3H proline injected animals, whereby no confirmation of this hypothesis was obtained.

Periosteal bone proliferation at the reimplantation side was noticed in the sections to such an extent that it could be palpated clinically. The same observations were made after extraction of incisors in the guinea pig (*Johansen & Gilhuus-Moe*, 1969) and after extraction of molars in rats. This proliferation of bone was found in all groups of animals in this study. Therefore is seems reasonable to explain this observation as a response to inflammatory stimuli rather than to mechanical instability of the mandible after extraction, since the ligated tooth in group III can be expected to be more resistant to stress than the unligated tooth in group I. Mechanical stress may play a role as an inductor of periosteal proliferation, but other stimuli undoubtedly are equally important.

The 3H proline observations confirmed the light microscopic results. It seemed that the injection of 3H proline 24 hours prior to sacrifice was an acceptable time span. Labelled periosteal bands were seen clearly at the reimplantation side, and as expected much less labelling was seen on the control side. Also the absence of labelling bands in the dentine of the reimplanted tooth and the sequestrating alveolus together with the periodontal fibers and the cementum supported the histologic observations. The 24 hour exposure to proline has been used previously by *Stallard* (1963) and by *Crumley* (1964) in experimental reactions of the periodontium in mice. The observations made in this study confirm that the injected 3H proline has passed through the cells and is found extracellularly after 24 hours in guinea pigs also. The early reattachment changes after reimplantation should therefore be tracable by the technique used in this study, if the reproducibility of these changes could be increased. An approach applying root filling of the tooth prior to reimplantation will be reported in a subsequent paper.

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SUMMARY

Immediate reimplantation after extraction of one of the mandibular incisors was studied by histologic and autoradiographic techniques in 32 young male guinea pigs. The effects of soft versus liquid diet and splinting versus non-splinting on the reattachment of the extracted teeth were evaluated. None of these factors seemed to influence the chances for reattachment. True reattachment was an unpredictable occurrence. It is postulated that reattachment takes place in the »vascular area» of the periodontal membrane without new formation of bone and cementum as a prerequisite. This »healing by first intention» is the initial phase of reattachment of the extracted tooth.

The experimental model seems suitable for reimplantation studies provided the predictability of the experimental results can be increased. An evaluation of the influence of root filling on the success of reattachments is in progress.

RÉSUMÉ

RÉIMPLANTATION D'INCISIVES INFÉRIEURES CHEZ LE COBAYE. ÉTUDE HISTOLOGIQUE ET AUTORADIOGRAPHIQUE.

La réimplantation immédiate de l'une des incisives inférieures après extraction a fait l'objet d'une étude histologique et autoradiographique chez 32 jeunes cobayes mâles. L'action d'un régime mou par rapport à un régime liquide et celle de la présence d'une fixation par attelle par rapport à l'absence de fixation par attelle ont été évaluées. Aucun de ces facteurs n'a semblé avoir d'influence sur les possibilités de réattachement. Un réattachement véritable se présentait comme un fait imprévisible. On considère qu'un réattachement se produit dans la »zone vasculaire» du périodonte, sans qu'il y ait nécessité préalable de néoformation d'os et de cément. Cette cicatrisation par première intention constitue la phase initiale du réattachement de la dent extraite.

Ce modèle expérimental semble convenir aux études de réimplantation, à la condition qu'il soit possible d'augmenter la prévisibilité des résultats expérimentaux. Une évaluation de l'influence d'une obturation radiculaire sur le succès du réattachement est en cours.

ZUSAMMENFASSUNG

REIMPLANTATION VON MANDIBULÄREN SCHNEIDEZÄHNEN DER MEERSCHWEINCHEN

EINE HISTOLOGISCHE UND AUTORADIOGRAPHISCHE STUDIE

Unmittelbare Reimplantation nach Extrahierung eines mandibularen Schneidezahnes wurde beobachtet mit histologischen und autoradiographischen Methoden bei 32 jungen männlichen Meerschweinchen. Die Wirkungen von weicher im Gegensatz zu flüssiger Diät und Fixierung im Gegensatz zu non-Fixierung auf das Reattachment der extrahierten Zähne wurden geschätzt. Keine von diesen Faktoren scheinen einen Einfluss auf das Reattachment zu haben. Echtes Reattachment konnte nicht vorausgesehen werden. Es ist aber anzunehmen, dass Reattachment in der »vascularen Zone» statt findet, ohne dass neue Bildung von Knochen und Zementum erforderlich ist. Diese »Heilung bei erster Intention» ist die intiale Phase des Reattachments des extrahierten Zahnes.

Diese Modelluntersuchungen scheinen geeignet zu sein, wenn es sich um das Reattachment handelt, vorausgesetzt, dass die Vorhersagung der experimentellen Resultate genauer bestimmt werden kann. Untersuchungen des Einflusses einer Wurzelfüllung auf den Erfolg des Reattachments sind in Arbeit.

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