The effect of splinting upon periodontal healing after replantation of permanent incisors in monkeys

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The effect of splinting upon periodontal healing after replantation was studied in 21 green Vervet monkeys (*Cercopithecus aethiops*). Two maxillary incisors were extracted in each monkey and the teeth were endodontically treated extraorally. One incisor was replanted after 18 min. whereas the other was replanted after 120 min. In 8 monkeys an orthodontic band-acrylic splint was applied for 6 weeks. In 6 monkeys the replanted teeth were splinted for 2 weeks. In 7 monkeys no splinting was carried out. The animals were sacrificed 8 weeks after replantation and the replanted teeth were examined histologically. The following histologic parameters were registered for each tooth: surface resorption, inflammatory resorption, replacement resorption (ankylosis), downgrowth of pocket epithelium and periapical inflammatory changes. All teeth replanted after 120 min. showed extensive ankylosis irrespective of the splinting method. In the 18-minute replantation group, the frequency and extent of replacement resorption was significantly lower in the non-splinted teeth compared to the splinted teeth. No significant changes were found among the other examined histologic parameters. It is concluded that splinting did not improve periodontal healing after replantation of mature teeth in monkeys and apparently exerted a harmful effect upon periodontal healing in teeth replanted after a short extraoral period.

Key-words: Periodontal prosthesis; splints; monkeys; tooth reimplantation

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It is generally accepted that splinting is an essential and necessary treatment procedure on traumatically luxated and replanted teeth. However, no clinical or experimental study has confirmed this assumption. The purpose of the present study was, therefore, to examine the effect of splinting upon the healing of the periodontium after replantation of extracted incisors in monkeys.

MATERIAL AND METHODS

The material consisted of 21 male green Vervet monkeys (*Cercopithecus aethiops*) weighing from 1.9 to 2.5 kg. The animals were used in the production of inactivated poliomyelitis vaccine and along with sacrifice the kidneys were removed for this purpose.

All potential animals for this study were examined by intra-oral radiographs and

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only those monkeys were selected which had completed root formation of maxillary incisors and the primary canine in late stages of shedding. This corresponds according to the classification suggested by *Morrees, Fanning & Hunt* (1963)to Stage 6 (root length complete with apical foramen half closed) and Stage 7 (apical foramen closed).

The monkeys were anaesthetised with phencyclidine (Sernylan ®, Park Davis & Co) 2.5 mg/kg of body weight supplemented with penthobarbitone sodium (Mebumal ®) using doses of 30 mg/kg body weight. Prior to surgery, pocket depth and gingival condition were recorded around the maxillary incisors. Sulcus depth was determined by means of a periodontal probe.

The experimental teeth were the maxillary right lateral incisor and the left central incisor. These teeth were extracted with forceps by gentle luxation movements. The time in seconds necessary to extract the tooth (luxation period) was recorded. The tooth was endodontically treated extraorally. During this period the tooth was held by the crown with forceps and the root surface was kept moist with a flow of saline. The crown was opened on the labial side with a bur and the pulp extirpated with a barbed broach. Thereafter the canal was enlarged with a file no 5 and 6 (Giro file ® Micro-Mega) operated with a special contra-angle handpiece (Giromatic ® Micro-Mega). The canal was cleansed with a 5 % sodium hypochlorite solution (Chlorox ® Chlorox Company) and immediately after rinsed with saline. Care was taken to prevent that sodium hypochlorite contacted the root surface. After drying the root canal with paper points the root canal was filled with gutta percha points and a root canal sealer (Kerr Pulp Canal Sealer ® Kerr Co.) using a lateral condensation technique. The endodontic procedure was carried out within 18 minutes after extraction.

In each monkey one maxillary incisor was replanted after 18 minutes (Group 1—3). The teeth were replanted in the alveolus using forceps or finger pressure. No efforts were made to remove clotted blood within the alveolus before replantation. Following these procedures the animals were permitted to assume normal activity and were maintained on the powdered diet (Standard diet for monkeys, Statens Seruminstitut, Copenhagen) supplemented with white bread and bananas. Another maxillary incisor was replanted after 120 minutes (group 4—6). In the period after endodontic treatment (i.e. from 18 to 120 minutes after extraction) and before replantation this tooth was allowed to dry up upon a gauze sponge at room temperature (22,3°, S.D. 1.86°). The relative humidity in the room was (47.2 %, S.D. 6.13 %). In 14 monkeys (group 1 to 4) the replanted teeth were splinted for 2 or 6 weeks with an orthodontic band-acrylic splint (*Andreasen*, 1971, 1972). This splint included all maxillary incisors, Fig. 1. In 7 monkeys no splinting was carried out (Group 3 and 6). Proper repositioning of the replanted teeth was checked radiographically (Fig. 2 A).

The monkeys were sacrificed after 8 weeks. The gingival condition and pocket depth was recorded among operated teeth as well as contralateral control teeth.

The anterior portion of the maxilla was removed including lateral and central incisors. This jaw segment was placed in a special clamp which allowed tooth mobility testing of all maxillary incisors (Fig. 2 B). Horizontal tooth mobility in labio-lingual direction was measured with the macro-periodontometer using 100 p instantaneous

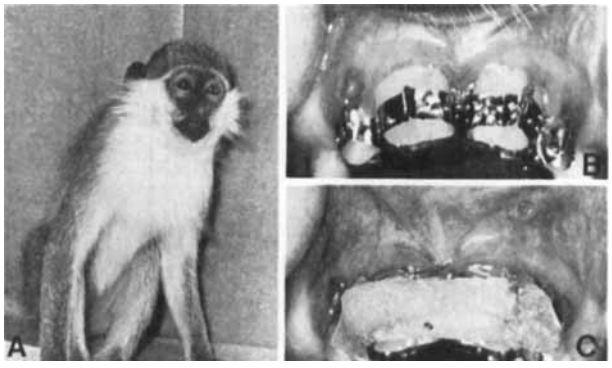

Fig. 1. A. Green vervet monkey. B. Orthodontic bands cemented with carboxylate cementum on replanted incisors and neighbouring teeth. C. Cold-curing acrylic unites the orthodontic bands.

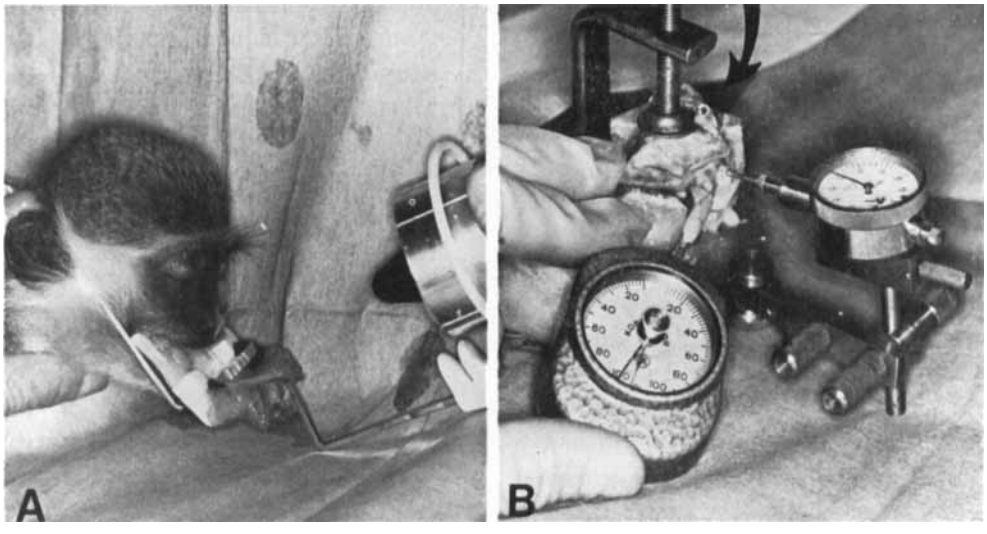

Fig. 2. A. Filmholder used for radiographic examination of replanted incisors. B. Clamp system used for fixation of anterior jaw segment during mobility testing of replanted incisors (arrow).

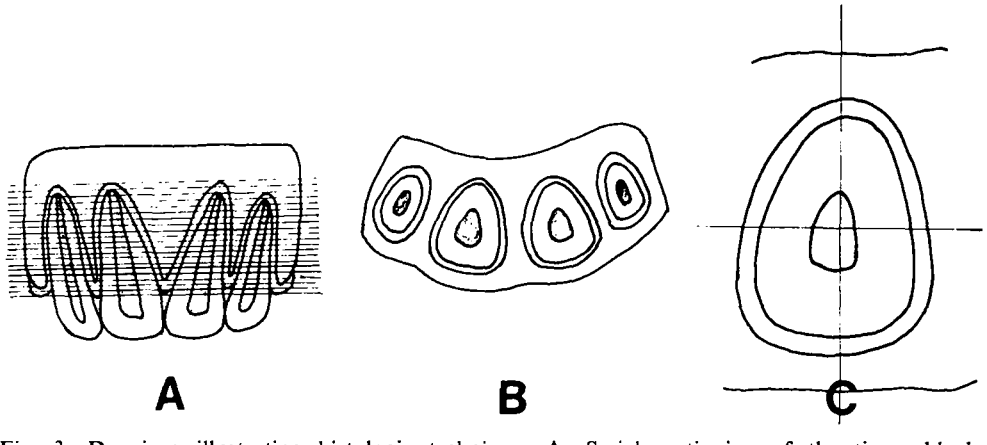

Fig. 3. Drawings illustrating histologic technique. A. Serial sectioning of the tissue block. B. Histologic section including transverse sections of all maxillary incisors. C. Orientation of the grid lines in labio-lingual and mesio-distal direction on the tooth to be examined.

forces (*Mühlemann*, 1960, 1967). The average of 3 recordings was taken as the mean mobility for the tooth. All measurements were performed between 8 a.m. and and 9 a.m. and standardized in relation to feeding of the animals.

After mobility testing the tissue block was placed in 10 % neutral buffered formalin and demineralized in 10 % solution of EDTA at pH 6.9. After double infiltration in celloidin-paraffin the tissue blocks were embedded in paraffin. The specimens were sectioned perpendicular to the long axis of the incisors at a thickness of 5 μm. The entire tooth was sectioned (*Stanley*, 1957) in step-serial sections at 500 μm untervals (Fig. 3 A). At each sectioning level one section was stained with hematoxylin-eosin and used for histometric evaluation (Fig. 3 A).

A projection microscope (Reichert Visopan®) was used for the histometric study. Sections were projected on a 200 mm disc type screen using a magnification of 130 × (Fig. 3 B). All sections were evaluated blindly in relation to extraoral period and splinting. A crossruled grid was placed over the projected cross section of the root and oriented according to the labio-lingual axis of the tooth. In the intersections between the two grid lines which corresponded to the mesio-distal and the labio-lingual axis of the tooth the changes in the periodontium were noted, i.e. four measuring points were used in each tooth (Fig. 3 C). The character of the periodontal changes was classified as follows, (Fig. 4):

1. *Normal periodontium.* Normal structured periodontal ligaments and an intact cementum layer.
2. *Active surface resorption.* Resorption cavities are present on the rooth surface bordered by a normal structured periodontium. The resorption process takes place by uni- or multinucleated cells but without inflammatory changes in the soft tissues.
3. *Arrested surface resorption.* The changes are similar to 2, apart from an arrest of the resorption process.
4. *Repaired surface resorption.* The changes differ from 2 by the repair of the resorption cavities with cellular or acellular cementum.
5. *Active inflammatory resorption.* Active resorption is present on the root surface. The resorption process takes place by uni- or multinucleated cells present in granulation tissue.
6. *Arrested inflammatory resorption.* The changes are similar to 5, apart from an arrest of the resorption process.
7. *Repaired inflammatory resorption.* The changes are similar to 5 apart from a repair of the resorption cavities with cellular or acellular cementum.
8. *Active replacement resorption.* Resorption cavities are present on the root surface adjacent to bone deposition upon the root surface. The resorption process takes place by uni- or multinucleated cells in a loose structured connective tissue.
9. *Arrested replacement resorption.* The changes are similar to 8 apart from an arrest of the resorption process.
10. *»Repaired» replacement resorption.* Alveolar bone is deposited upon the root surface. The root surface may or may not have been resorbed before bone deposition.
11. *Inflammation in the periodontal ligament without root resorption.*
12. *Downgrowth of pocket epithelium below the dentino-enamel junction.*

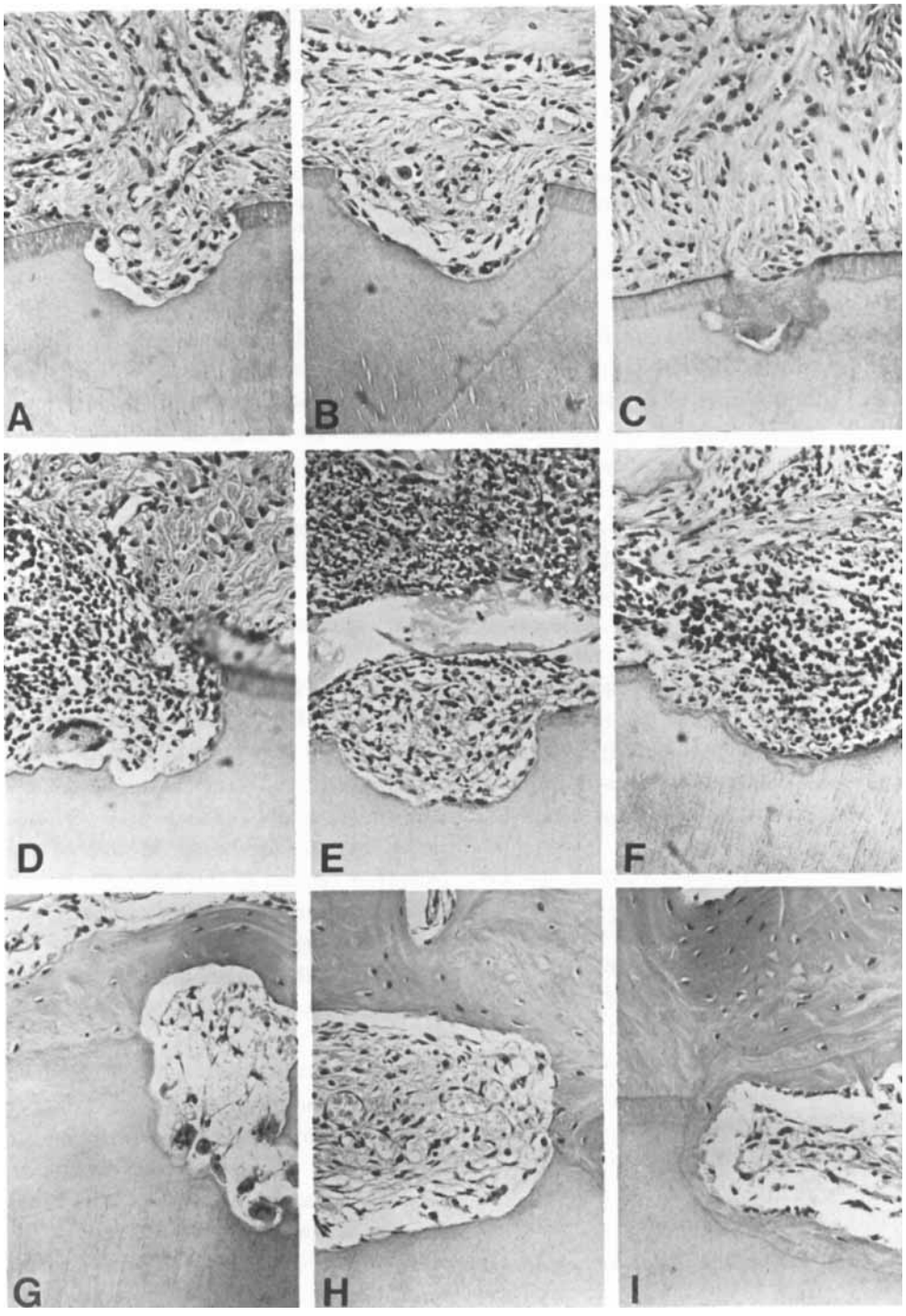

Fig. 4. A. Active surface resorption. B. Arrested surface resorption × 156. C. Repaired surface resorption × 156. D. Active inflammatory resorption × 156. E. Arrested inflammatory resorption × 156. F. Repaired inflammatory resorption × 156. G. Active replacement resorption × 156. H. Arrested replacement resorption × 156. I. »Repaired» replacement resorption × 156.

An average of 15 sections were examined for each tooth. A *pathology index* was calculated for each tooth as follows: The number of measuring points showing a certain type of periodontal change was divided by the total number of measuring points for each tooth × 100. Thus a pathology index value of 0.32 for replacement resorption indicated that 32 % of the measuring points showed replacement resorption. A *resorption activity index* was calculated for each tooth as follows. Out of the total numbers of measuring points with a certain type of resorption, e.g. replacement resorption, the number of points demonstrating active replacement resorption was determined. An arrested replacement resorption activity index of 0.1 thus indicated that among all registered measuring points exhibiting replacement resorption 10 % showed replacement resorption in an arrested phase.

The precision of the pathology index was calculated from 10 double determinations performed at an one-month interval. The precision was calculated from the formula $s(i) = \sqrt{\frac{\Sigma(d)^2}{2n}}$ where d is the difference between duplicated registrations and n is the number of registrations.

In the statistical analysis the following tests were used. Fisher exact probability test and a randomisation test for two independent samples and 5 % was used considered as the critical probability value (*Siegel*, 1956; *Finney*, 1948).

RESULTS

Precision of the pathology index was found to range from 1.2 to 1.5 for the different resorption types (Table I). In Table II the presence of root resorption is related to the clinical variables: splinting and extra-oral period.

Table I. *Precision of different pathology index registrations*

	No. of double registrations	s(i)
Surface resorption	10	1.5
Inflammatory resorption	10	1.4
Replacement resorption	10	1.2
Normal periodontium	10	1.5

In Table III the extent of the histologic changes (pathology index) is related to the same clinical variables. The histologic variables were compared between the 2- and 6-week splinting groups in Table II and III. No significant difference was found between the two splinting periods. Thus in the further statistical analysis the two splinting groups were combined and tested against the non-splinting group.

Extra oral period 18 min (Groups 1, 2, 3)

It appears that *surface resorption* was present in all experimental groups irrespective of splinting and to approximately the same extent (Table III). Surface resorption was seen only in one of the examined control teeth (Table II).

Inflammatory resorption was seen in one case (experiment 2, Table II) and was not registered in the control groups (Table II).

Replacement resorption was found in all splinted teeth except one tooth in group 2. In contrast, 5 out of 7 teeth in the nonsplinting group showed no evidence of replacement resorption. This difference in frequency of replacement resorption between splinted and non-splinted teeth was found to be significant ($p = 0.01$). Also the extent of the replacement resorption was found to be significantly related to splinting time ($p = 0.05$) (Table III).

Downgrowth of epithelium was a rare finding (Table III).

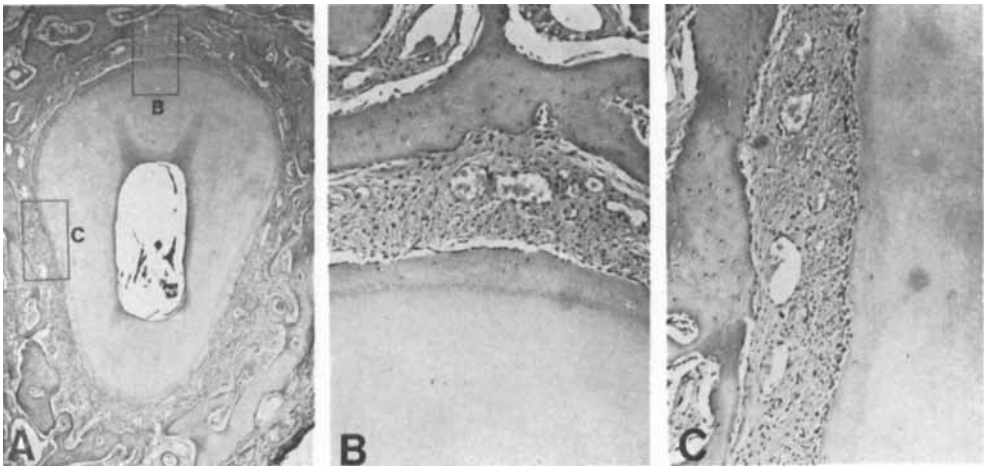

Fig. 5. Histologic changes after replantation without splinting. Extra-oral period 18 minutes. A. Low power view × 13. B. and C. Normal periodontium × 62.

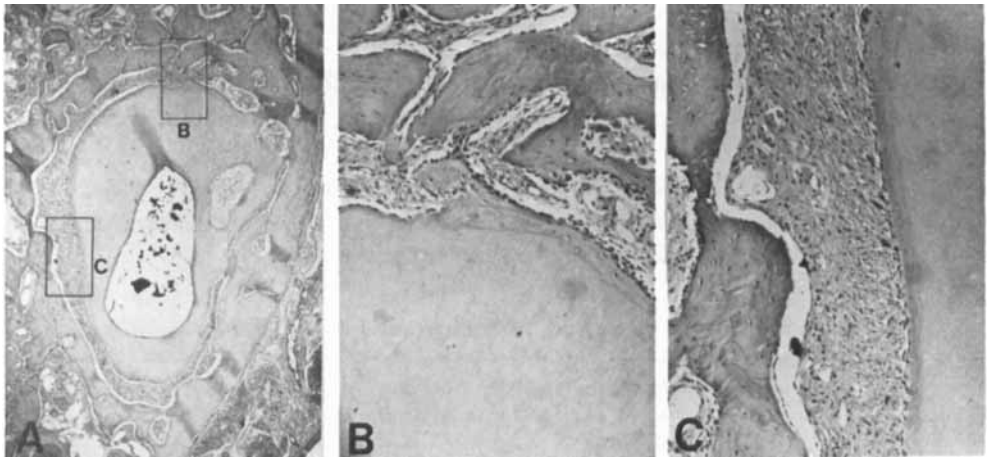

Fig. 6. Histologic changes after replantation with splinting for 6 weeks. Extra-oral period 18 minutes. A. Low power view × 13. B. Replacement resorption × 62. C. Normal periodontium × 62.

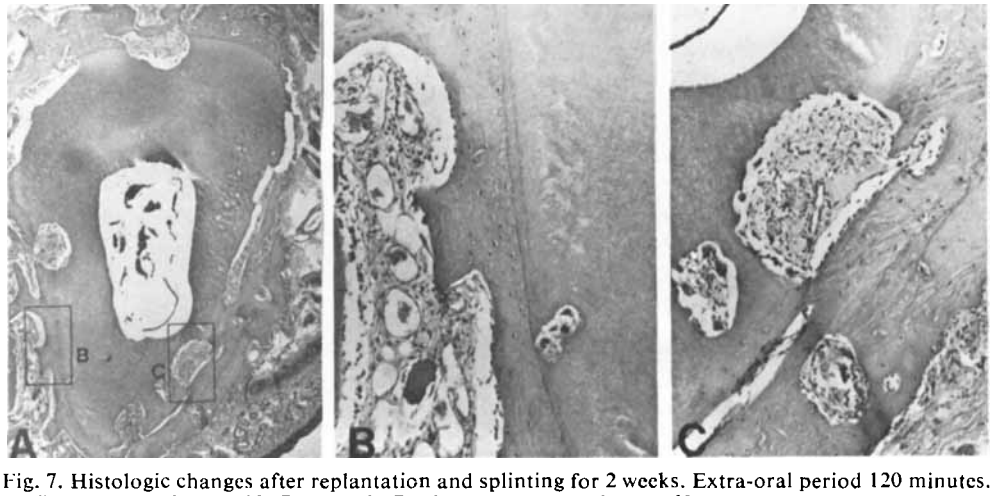

Fig. 7. Histologic changes after replantation and splinting for 2 weeks. Extra-oral period 120 minutes. A. Low power view × 13. B. and C. Replacement resorption × 62.

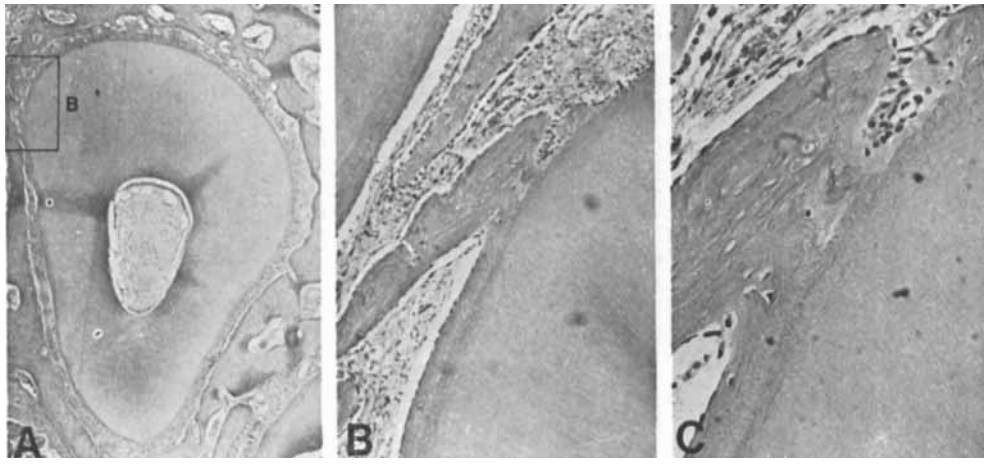

Fig. 8. Histologic changes in control tooth. A. Low power view × 13. B. and C. Small area with replacement resorption × 62 and × 156.

Inflammation in the periodontal ligament was seen with almost the same frequency in all experimental groups.

Extra oral period 120 min (Groups 4, 5, 6) One monkey in group 6 lost a central incisor 1 day after replantation.

Surface resorption was found in most teeth (Table II) and to approximately the same extent in all experimental groups (Table III).

Inflammatory resorption was a rare finding and not significantly related to splinting.

Table II. *Relation between presence of root resorption, splinting, and extra-oral period*

Group	Extra-oral period (min.)	Splinting period (weeks)	Number of teeth	Surface resorption +	Surface resorption —	Inflammatory resorption +	Inflammatory resorption —	Replacement resorption +	Replacement resorption —
Exper. 1	18	6	6	6	0	1	5	6	0
Exper. 2	18	2	8	8	0	0	8	7	1
Exper. 1 + 2	18	6 or 2	14	14	0	1	13	13**	1**
Exper. 3	18	0	7	7	0	0	7	2**	5**
Control 1	—	—	6	1	5	0	6	0	6
Control 2	—	—	8	0	8	0	8	0	8
Control 3	—	—	7	0	7	0	7	0	7
Exper. 4	120	6	6	5	1	1	5	6	0
Exper. 5	120	2	8	4	4	1	7	8	0
Exper. 4 + 5	120	6 or 2	14	10	4	2	12	14	0
Exper. 6	120	0	7	4	3	0	7	7	0
Control 4	—	—	6	0	6	0	6	1	5
Control 5	—	—	8	0	8	0	8	0	8
Control 6	—	—	7	0	7	0	7	0	7

The significant differences between the splinting and non-splinting groups with identical extra-oral periods (Fisher's exact test) are indicated with asterisks: * $0.05 \geq p > 0.01$, ** $0.01 \geq p > 0.001$, *** $p \leq 0.001$.

Table III. *Pathology index related to splinting, extra-oral period, luxation period, and mobility testing*

Group	Extra-oral period (min.)	Splinting period (weeks)	Number of teeth	Surface resorption (%) $\bar{x}$ S.D.	Inflammatory resorption (%) $\bar{x}$ S.D.	Replacement resorption (%) $\bar{x}$ S.D.	Downgrowth of pocket epithelium (%) $\bar{x}$ S.D.	Periapical inflammatory changes (%) $\bar{x}$ S.D.	Normal periodontium (%) $\bar{x}$ S.D.	Luxation period (seconds) $\bar{x}$ S.D.	Mobility testing ($mm \times 10^{-2}$) $\bar{x}$ S.D.
Exper. 1	18	6	6	7.0 ± 4.56	0.3 ± 0.81	9.4 ± 6.65	0	1.0 ± 1.09	82.4 ± 11.13	139.0 ± 33.46	5.5 ± 2.90
Exper. 2	18	2	8	10.1 ± 8.77	0	10.7 ± 14.12	0.2 ± 0.70	3.1 ± 2.47	75.3 ± 12.40	128.1 ± 85.89	7.0 ± 3.90
Exper. 1+2	18	6 or 2	14	9.3 ± 6.76	0.1 ± 0.53	10.2* ± 11.17	0.1 ± 0.53	2.2 ± 2.22	78.4 ± 11.99	132.7 ± 66.58	6.4*** ± 3.47
Exper. 3	18	0	7	13.1 ± 7.75	0	4.7* ± 9.74	0	3.7 ± 3.03	78.7 ± 16.32	151.1 ± 69.05	13.6*** ± 4.42
Control 1	—	—	6	0.3 ± 0.81	0	0	0	0	100	—	9.0 ± 2.10
Control 2	—	—	8	0	0	0	0	0	100	—	10.0 ± 3.84
Control 3	—	—	7	0	0	0	0	0	100	—	11.6 ± 2.43
Exper. 4	120	6	6	2.4 ± 2.16	0.6 ± 1.63	88.1 ± 3.13	0.6 ± 1.63	3.3 ± 3.98	5.3 ± 2.16	155.4 ± 105.37	3.2 ± 1.22
Exper. 5	120	2	8	2.3 ± 2.61	0.2 ± 0.70	87.4 ± 10.79	0	6.2 ± 6.58	3.4 ± 3.38	86.8 ± 40.8	2.3 ± 1.78
Exper. 4+5	120	6 or 2	14	2.4 ± 2.34	0.4 ± 1.15	87.7 ± 8.16	0.2 ± 1.06	5.0 ± 5.62	4.2 ± 2.97	116.2 ± 80.06	2.7 ± 1.58
Exper. 6	120	0	7	2.5 ± 2.76	0	84.0 ± 8.04	0	5.0 ± 2.64	8.1 ± 8.85	135.1 ± 67.74	3.5 ± 1.19
Control 4	—	—	6	0	0	0.1 ± 0.40	0	0	100	—	9.9 ± 2.83
Control 5	—	—	8	0	0	0	0	0	100	—	10.3 ± 2.83
Control 6	—	—	7	0	0	0	0	0	100	—	11.8 ± 1.21

The significant differences between the splinting and non-splinting groups with identical extra-oral period (Randomization test for independent samples) are indicated with asterics: * $0.05 \geq p > 0.01$, ** $0.01 \geq p > 0.001$, *** $p \leq 0.001$.

Table IV. *Replacement resorption activity index related to splinting*

Group	Extra-oral period (min.)	Splinting period (weeks)	Number of teeth	Active replacement resorption (%)		Arrested replacement resorpsion (%)		»Repaired» replacement resorption (%)	
				$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Exper. 4	120	6	7	27.6*	9.95	7.0	8.19	65.1*	8.40
Exper. 5	120	2	6	14.4*	8.86	7.6*	6.54	78.7*	12.76
Exper. 6	120	0	8	12.2	4.49	16.1*	7.55	71.7	7.78

The significant differences between the splinting and non-splinting group (Randomization test for independent samples) are indicated with asterics: * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $p < 0.001$.

Replacement resorption was found in all replanted teeth irrespective of splinting time (Table II). The extent of replacement was also nearly the same (Table III). In one control tooth a small area with replacement resorption was found. This area was apparently in a stage of elimination as revealed by the intense resorption of the ankylosis area.

Downgrowth of pocket epithelium and *periapical inflammatory changes* was found with nearly the same frequency in all groups.

The luxation period showed a great range from 40 to 293 seconds. No significant relationship was found between the luxation period and the degree of resorption.

When the *pathology index* was compared between the 18- and 120-minute group it appeared that the extended extra-oral period, irrespective, led to a significant increase in replacement resorption, irrespective of splinting (Table III).

In Table IV the replacement *resorption activity index* is listed for the 2- and 6-week experimental groups with extra-oral periods of 120 minutes. It appears that active replacement resorption was significantly more frequent in the 6-week group; whereas, »repaired» replacement resorption was less frequent. Arrested replacement resorption was significantly more frequent in the non-splinting group compared to the two splinting groups. A similar resorption activity analysis was not possible for the 18-minute group and for the other resorption types due to the low frequency of resorption in these groups which did not allow a statistical analysis of differences in resorption activity.

Mobility testing values (Table III) reflect the extent of replacement resorption. This is especially evident when experimental groups 1 and 2 are compared with experimental groups 3 through 6.

DISCUSSION

The results from the histologic evaluation indicate that splinting did not improve periodontal healing after replantation, but actually resulted in an increase in replacement resorption in teeth replanted with a short extra-oral period. This unexpected finding indicates that functional stimuli may prevent or eliminate replacement resorption.

The influence of function upon periodontal healing after surgical or traumatic injuries has not been examined previously, thus an exact explanation for

the above-mentioned finding is not available at the present time. Hypothetically one may assume that functional stimuli may depress osteogenesis and enhance fibrous healing, a phenomenon known to occur in insufficiently splinted bone fractures (*Clark & Hayes*, 1963). Another explanation could be that the extent of replacement resorption is proportional to the extra-oral period. Thus in the 18 minute group a resorption process may be able to eliminate the ankylosis area, a phenomenon already demonstrated in rats where the periodontium were surgically injured (*Andreasen & Skovgaard*, 1972). The general lack of replacement resorption in the non-splinting group is hypothetically due to resorption of an initially created ankylosis; whereas the similar ankylosis in splinted teeth possibly becomes permanent or eliminated at a later date. Clinical data from mobility testing of replanted human teeth seems to give some support to this theory (*Andreasen*, 1975).

One may speculate why function did not prevent ankylosis in the 120 minute group. It is possible that the damage inflicted upon the root surface was so extensive that it lead to massive ankylosis which could not be removed by a functionally stimulated resorption process.

The resorption activity index indicated that this parameter was also related to splinting. Whether this relation reflects transient or permanent changes in resorption activity merits further investigations.

The present study raises a number of questions. What is the exact effect of occlusal function upon healing of the periodontium on a cellular level after surgical and traumatic injuries? Can traumatic occlusion prevent the development of ankylosis in teeth with extended extra-oral periods? What is the effect of splinting upon pulpal repair after traumatic injuries to the periodontium? Experiments are now in progress dealing with these problems.

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