The effect of citric acid on repair after delayed tooth replantation in dogs

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Klinge B, Nilvéus R, Selvig KA. The effect of citric acid on repair after delayed tooth replantation in dogs. Acta Odontol Scand 1984;42:351–359. Oslo. ISSN 0001–6357.

An experimental study of maxillary incisors in dogs was carried out to test the hypothesis that demineralization of denuded root surfaces by citric acid to expose collagen matrix fibrils might improve the rate of periodontal regeneration after replantation of traumatically avulsed and dried teeth, provided that the root surface is not traumatized during replantation or postoperatively. Experimental teeth (group 4) were allowed to dry in air for 45 min. A mid-portion of the root was then root-planed extensively, treated with citric acid for 3 min, rinsed with saline, and replanted. The apical region and a cervical collar were not instrumented, ensuring precise reseating and stability of the tooth during the postoperative period. Control teeth were either replanted immediately (group 1), bench-dried for 45 min and replanted (group 2), or bench-dried and the entire root surface root-planed and acid-treated before replantation (group 3). Histological examination of block specimens after 21 days of healing showed complete periodontal regeneration in group 1 and, by contrast, a high rate of ankylosis, inflammatory resorption, and surface resorption in group 2. Root planing and acid treatment of bench-dried teeth (group 3) did not improve the healing response, whereas the mid-root region of teeth in group 4 showed an absence of inflammatory resorption and less ankylosis than groups 2 and 3. These preliminary observations indicate that the frequency of adverse healing results after delayed replantation can be reduced by removing nonvital soft tissue remnants, demineralizing the root surface, and preventing mechanical trauma to the root surface in the postoperative period. □ *Periodontal ligament; tooth reimplantation*

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Successful regeneration of periodontal tissues after tooth replantation after shorter or longer extraoral periods is a subject of great clinical significance.

Clinical observations and experimental studies in dogs and primates have indicated that teeth that are replanted shortly after dislocation may heal, with complete restoration of normal periodontal relationships. On the other hand, teeth that have been dislocated over a longer period of time show an increased tendency towards ankylosis and resorption (1, 2).

Successful regeneration apparently depends on the presence of viable periodontal ligament attached to the reimplanted tooth. The use of saline, saliva, milk, or other storage media may prolong the extraoral period possible (3–5). Andreasen (6) has concluded that 'the only decisive factor for the development of ankylosis after replantation of teeth appears to be the condition of the periodontal ligament upon the tooth, and not the condition of the socket'.

The preservation of the tooth during the extraoral period is therefore critical. Löe & Waerhaug (7) did not find a normal periodontal ligament on any teeth dried for 45 min or longer. Other studies have indicated that a 20–30-min interval between the time of extraction and subsequent replantation is compatible with preservation of viability of the cells of the periodontal ligament attached to the root surface (8, 9). After 2 h, no vital cells remain (3, 10).

Proye & Polson (11), however, were surprised to observe a high degree of acellularity within the periodontal ligament 1 day after replantation of teeth that had been kept extraorally for only 3 min. They ascribed this effect to trauma during luxation. In addition, trauma during luxation seems to stimulate resorption (3), which may be more pronounced on the rounded labial and lingual root surfaces than on the flat or concave approximal surfaces (12–14).

Experimental periodontal surgery has indicated that regeneration of connective tissue attachment to an exposed and planed root surface may be greatly improved if the root surface is demineralized by topical application of citric acid. The demineralization results in exposure of collagen matrix fibrils with which new fibrils may interdigitate (15). In experimental tooth replantation, other acidic solutions have been tried; local application of 5% SnF_2 before replantation reduced the tendency to resorption in replanted molars in rats (16). Since the fluoride solution had a low pH, it is possible that the favorable result in that study was due to the effect of the acid rather than to an effect of the fluoride ion. Treatment with 2% acidulated NaF at pH 5.5 for 30 min, on the other hand, did not reduce root resorption in monkeys (17). Root demineralization with 0.6N HCl for 2 h did not reduce root resorption (18), whereas a brief treatment with citric acid had a remarkable effect on healing in the gingival region after replantation and resulted in connective tissue rather than epithelial reattachment (19, 20). Although histometric data were not presented, Polson & Proye (19) reported that extensive root resorption was present in the intra-alveolar region 21 days after tooth replantation. Thus, whereas the stimulating effect of citric acid treatment in the regeneration of connective tissue attachment to a denuded root surface in periodontal surgery is well established, the effect on replanted teeth has not been explored completely.

On this background, additional experiments should be carried out to test the possibility that demineralization of the root surface might improve the periodontal healing of teeth that have been dislocated for an extended period of time. Preliminary observations in our laboratory and other laboratories have been inconclusive in this respect. A possible explanation is that the demineralized surface layer of the root has been damaged during reinsertion of the tooth or that the tooth has been incorrectly repositioned, resulting in direct contact with the alveolar wall. In the present study, therefore, an attempt was made to avoid this problem by protecting a portion of the periodontal ligament against such damage.

Materials and methods

Maxillary anterior teeth in six young, adult beagles were available for experimentation. The dogs were littermates. All teeth in these animals had completed root formation. Experimental procedures were carried out on the first and second maxillary incisors. The third incisors were included in the specimen blocks as untreated controls.

With the dogs under intravenous pentobarbital sodium anesthesia, the experimental teeth were extracted with forceps, using rotating movements. Immediately after extraction, the teeth were cleansed with a flow of saline and assigned to one of the following experimental groups (Fig. 1):

Group 1. Replanted immediately.

Group 2. Stored dry for 45 min before replantation.

Group 3. Stored dry for 45 min, root-planed with curettes and finishing burs, treated with citric acid (pH 1.0) for 3 min, rinsed with saline, and replanted.

Group 4. Stored dry for 45 min, mid-portion of the root root-planed extensively with diamonds and finishing burs, while apical

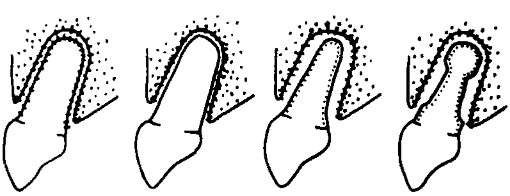

Fig. 1. Experimental design. Group 1: immediate replantation. Group 2: dry storage 45 min, replantation. Group 3: dry storage 45 min, root planing, citric acid treatment, replantation. Group 4: dry storage 45 min, extensive root-planing of mid-root area only, citric acid treatment, replantation.

Table 1. Distribution of experimental teeth by treatment procedure

Dog no.	Tooth			
	URI_2	URI_1	ULI_1	ULI_2
1	2	3	4	1
2	1	2	3	—*
3	—	4	1	2
4	4	1	2	—
5	1	2	3	4
6	2	—	—	1

For description of experimental procedures in groups 1–4, see text.
* Teeth exfoliated after replantation.

and cervical areas were uninstrumented, treated with citric acid, rinsed with saline, and replanted.

The teeth were carefully reseated in the alveolus, using finger pressure. No fixation was used. Distribution of the experimental teeth in accordance with treatment is shown in Table 1.

The dogs were killed after 21 days. The main consideration in the choice of observation period was to enable complete connective tissue healing and possible establishment of a new fiber attachment, while, on the other hand, possible differences in healing result between various levels in group 4 should not be obscured by healing patterns characteristic of one particular level extending into another.

Anterior portions of the right and left maxillae were removed and placed in 10% neutral, buffered formalin for 14 days. After demineralization in 5.2% nitric acid, the tissue blocks were embedded in paraffin, and horizontal sections were made at 7 μm through the first, second, and third incisor. Owing to the nonparallelism of the long axes of the incisors, the resulting sections did not strictly represent transverse sections of all teeth in each block. The difference in angulations of the sections, however, was not considered to influence significantly the evaluation of the experimental results. The sections were stained with hematoxylin and eosin.

Histological examination of the healing events was carried out for each tooth separately. One section per 0.5 mm of root length was selected for histometric analysis. In each section the type of healing was evaluated at 12 points along the root surface, separated by an angle of 30° through the center of the tooth (14, 21). At each measuring point, the following variables were recorded:

Root: presence of surface resorption, inflammatory resorption, ankylosis (replacement resorption), presence of old cementum and new hard tissue.

Gingiva and periodontal ligament: presence of junctional epithelium, supra-alveolar versus intra-alveolar connective tissue, width of periodontal ligament.

Alveolar bone: presence of resorption on the periodontal aspect, presence of new bone.

In addition, the normal width of the periodontal ligament was estimated on the mesial aspect of the uninvolved third incisor, which was included in the sections. Excluding the cervical sections showing junctional epithelium all around the root surface, histometric analysis of connective tissue healing was based on 97–183 measuring points per tooth.

The proportion of the root surface apical to the junctional epithelium which showed either surface resorption, inflammatory resorption, or ankylosis was calculated and expressed as a percentage. For the teeth in group 4, these data were calculated separately for the uninstrumented cervical region, the instrumented mid-root region, and the uninstrumented apical region. To enable a more meaningful comparison with the other experimental groups of healing at various levels of the root, the data from groups 1, 2, and 3 were tabulated separately for the supracrestal region, periodontal region, and apical region. The apical region was arbitrarily designated as the apical fourth of the total root length. The uninstrumented cervical root surface region in group 4 corresponded approximately with the supracrestal region.

Statistical analysis included calculation of means and standard deviations. The significance level of observed differences was tested by Student's *t* test.

Table 2. Resorptions: frequency by treatment group and type of lesion in percentage of available root surface area

Treatment group	No. of teeth	Root surface region: Supra-alveolar		Periodontal		Apical	
		Surface	Inflammatory	Surface	Inflammatory	Surface	Inflammatory
1	6	5.3 ± 13.1	2.0 ± 4.9	4.2 ± 4.2	3.7 ± 5.5	5.8 ± 6.7	2.2 ± 5.3
2	6	28.2 ± 33.3	9.8 ± 20.8	11.2 ± 11.6	5.0 ± 5.7	11.0 ± 14.3	5.7 ± 7.5
3	3	27.7 ± 15.2	8.3 ± 14.4	21.7 ± 21.4	1.0 ± 1.7	10.3 ± 9.1	0
4	4	26.5 ± 25.2	9.5 ± 19.0	20.8 ± 18.7	0	16.0 ± 23.6	0.5 ± 1.0
Difference between groups		1 vs 2: p < 0.01 1 vs 3: p < 0.01 1 vs 4: p < 0.01	1 vs 2: NS 1 vs 3: NS 1 vs 4: NS	2 vs 3: NS 2 vs 4: NS 3 vs 4: NS	2 vs 3: p < 0.01 2 vs 4: p < 0.01 3 vs 4: NS	2 vs 3: NS 2 vs 4: NS 3 vs 4: NS	2 vs 3: p < 0.01 2 vs 4: p < 0.01 3 vs 4: NS

Results

Five of the teeth were lost within a few days after replantation. Significantly, all of these teeth belonged to those experimental groups in which the root surface had been instrumented before replantation.

Analysis of the observations was somewhat complicated by the large variations within each group and by the loss of several experimental teeth, which reduced the sample size of groups 3 and 4.

All teeth in group 1 (immediate replantation) had a lower frequency of adverse healing reaction than any of the teeth in groups 2, 3, and 4. In fact, three of six teeth in group 1 did not have any recorded areas of resorption or ankylosis.

The distribution of adverse healing reactions by treatment group and type of root surface lesion is presented in Tables 2 and 3. The total amount of resorption was approximately the same in groups 2, 3, and 4. Closer examination of the data revealed, however, that the figures for group 4 were greatly influenced by one tooth that had an inordinately high frequency of periodontal surface resorptions.

Supra-alveolar (gingival) resorption occurred frequently in groups 2, 3, and 4, and there was no difference between the groups (Table 2). Surface resorption was three times more prevalent than inflammatory resorption in all groups. Inflammatory resorption at the gingival level was seen in only one or two teeth within each group. However, when it did occur, this type of resorption tended to be of great extension and depth, which accounts for the high mean percentages in groups 2, 3, and 4.

Periodontal and apical resorption were less prevalent than supracrestal resorption (Table 2). The figures in Table 2, however, do not give a complete picture of the extent of root resorption, since some resorptions in groups 2, 3, and 4 had subsequently ankylosed.

Interestingly, inflammatory resorption in the periodontal and apical regions was almost absent in groups 3 and 4 (the acid-conditioned roots).

Ankylosis was insignificant in group 1 and

Table 3. Ankylosis: frequency by treatment group in percentage of available root surface area

Treatment group	No. of teeth	Root surface region	
		Periodontal	Apical
1	6	0.8 ± 2.0	1.0 ± 2.4
2	6	50.2 ± 15.5	48.7 ± 26.5
3	3	35.0 ± 31.2	58.3 ± 52.0
4	4	16.8 ± 12.8	49.5 ± 31.6
Difference between groups		2 vs 3: NS 2 vs 4: $p < 0.01$ 3 vs 4: NS	2 vs 3: NS 2 vs 4: NS 3 vs 4: NS

involved approximately half of the periodontal and apical root surface area in group 2 (Table 3). Citric acid conditioning did not prevent a similar frequency of ankylosis in group 3 and the apical, uninstrumented part of the teeth in group 4. In the instrumented part in group 4, however, ankylosis was significantly lower than in group 2 yet much higher than in group 1.

The extent of ankylosis varied greatly between teeth in groups 3 and 4, as indicated by the large standard deviations. Thus, one tooth in group 3 showed no evidence of ankylosis, and one of the teeth in group 4 showed only a moderate extent of ankylosis, which was limited to the apical region.

Ankylosis occurred mostly directly to the root surface (Fig. 2) but often to resorption defects as well.

The alveoli left by exfoliated teeth were partly filled by new bone tissue. Radially arranged bony lamellae extended up to 2 mm inward from the alveolar bone (Fig. 3). In

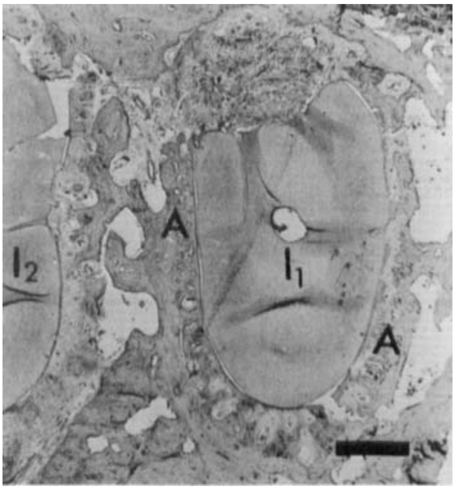

Fig. 2. Complete ankylosis (A) on the mesial, palatinal, and distal root surface of a maxillary first incisor (I_1) replanted after 45 min of bench-drying (group 2). Extensive inflammatory resorption is present labially. The adjacent, immediately replanted second incisor (I_2) shows normal periodontal ligament width and some surface resorptions. Bar = 100 μm. (Magnification, ×10.)

Fig. 3. Extensive bone fill in the alveolus of an exfoliated tooth (I_2). The newly formed bone differs from preexisting bone by the radial arrangement of bony lamellae and a lower ratio of calcified tissue to marrow spaces. Above, the apical, uninstrumented region of a tooth in group 4 shows ankylosis (A) distally (I_1). Bar = 100 μm. (Magnification, ×10.)

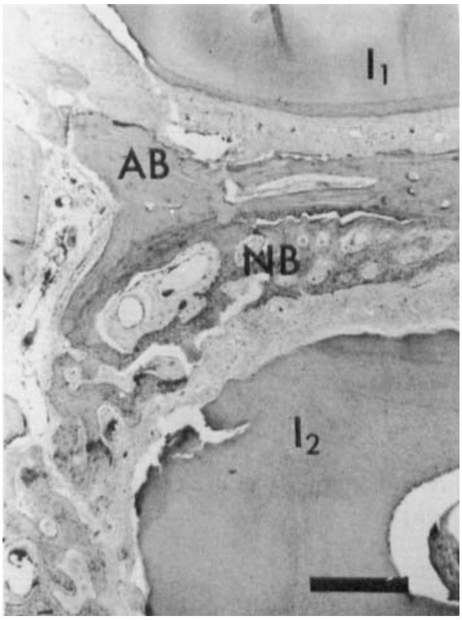

Fig. 4. Periodontal healing around an extensively root-planed, citric acid-treated root (I_2, group 4) and an immediately replanted tooth (I_1, group 1). Bone fill around the instrumented root has resulted in a periodontal ligament space comparable in width to that of the control tooth. AB = preexisting alveolar bone; NB = new bone formed after replantation. Bar = 50 μm. (Magnification, ×25).

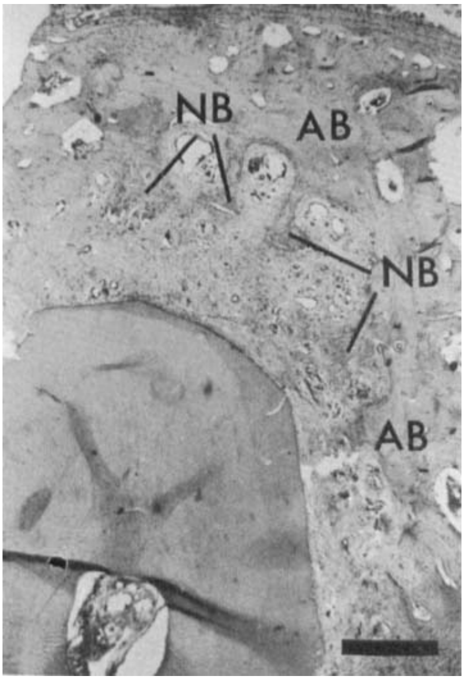

Fig. 5. Bone fill in the periodontal ligament of an extensively root-planed, citric acid-treated tooth (group 4). Lamellae of new bone (NB) have narrowed the width of the periodontal ligament to within the normal range. AB = preexisting alveolar bone. Bar = 50 μm. (Magnification, ×25.)

contrast to old bone tissue, the newly formed bone tissue contained unmineralized spaces that appeared to exceed the mineralized portions in volume.

Around the instrumented part of the teeth in group 4, the relatively wide periodontal ligament space had often been partially filled by new bone (Figs. 4 and 5). Characteristically, but not exclusively, the bony lamellae did not reach the instrumented root surface but were separated from it by a soft tissue space that corresponded in width to the normal periodontal ligament. By contrast, regions of wide periodontal ligament in group 3 had resulted in ankylosis (Fig. 6).

Surface resorptions, at all levels of the root, very often showed signs of reversal, with deposition of a fine layer of new cementum and attachment of new fibers (Fig. 7). Signs of arrested resorption processes occurred in larger defects as well. In these instances, the defects appeared to be filled

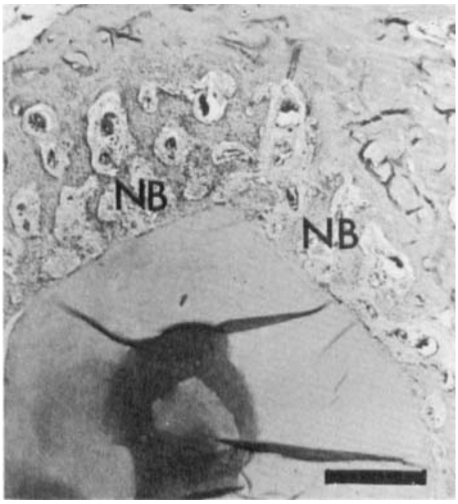

Fig. 6. Complete ankylosis to a root-planed, citric acid-treated root surface (group 3). Bony lamellae (NB) have bridged the relatively wide periodontal ligament space. Bar = 50 μm. (Magnification, ×25.)

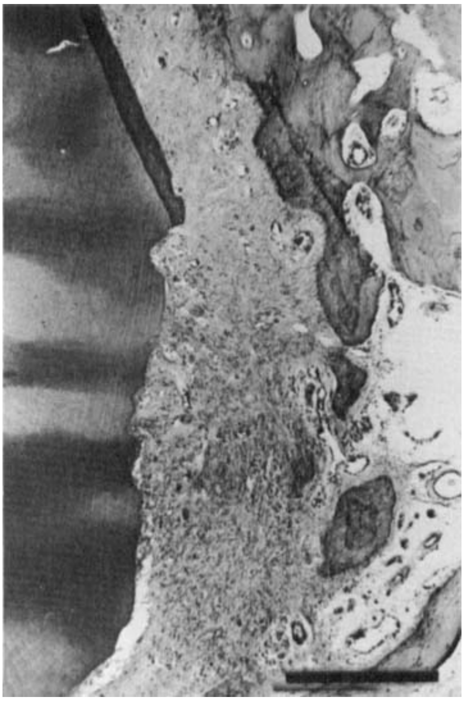

Fig. 7. Surface resorption showing signs of reversal. Tooth from group 4. Bar = 50 µm. (Magnification, ×40.)

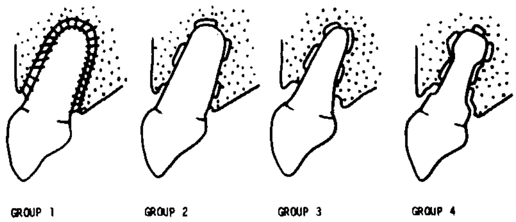

Fig. 8. Predominant healing patterns 21 days after replantation of experimentally treated teeth. Group 1: complete regeneration of periodontal ligament. Group 2: ankylosis involving 50% of the root surface. Group 3: various degrees of ankylosis. Group 4: moderate degree of ankylosis at mid-root level, more extensive apically.

by bone-like tissue, which was often associated with ankylosis.

The predominant healing patterns in groups 1–4 are illustrated schematically in Fig. 8.

Discussion

As expected, the results confirmed that the teeth that were replanted immediately after extraction (group 1) may show a complete periodontal healing within 3 weeks, with a minimal amount of root resorption and ankylosis. A layer of cementoid and a fine layer of cementum, which appeared to have been formed after the experiment, was generally present on the root surface of these teeth, indicating that the cementoblast layer had survived the trauma of the experimental procedure.

By contrast, teeth that were allowed to dry for 45 min before replantation (group 2) had a high frequency of postoperative sequelae. In these teeth ankylosis involved about half of the periodontal root surface, while another 10–20% of the root surface showed surface and inflammatory resorptions.

Removal of the adherent devitalized soft tissue by root planing, followed by topical application of citric acid (group 3), apparently did not change the healing response much. The only variable that showed a significantly more favorable result in group 3 than in group 2 was the frequency of inflammatory resorption in the apical region. This result was perhaps somewhat unexpected in view of the encouraging results achieved with the new periodontal attachment procedures in the same experimental animal (22, 23). However, the observations are in harmony with those of Nordenram et al. (18), who found no improvement of the healing response after treating a monkey incisor with 0.6N HCl for 2 h before replantation.

On the other hand, when root-planed and citric acid-treated teeth were replanted and the treated root surface protected from direct contact with the bony wall of the alveolus during and after reinsertion (group 4, periodontal region), a different healing response occurred. In this group there were no sites of inflammatory resorption, and in all specimens the amount of ankylosis was lower than in all but one tooth in groups 2 and 3.

The high degree of bone fill seen in the sockets of exfoliated teeth indicates that the lower frequency of ankylosis in the region of widened periodontal ligament in group 4 was not due to an inability of bone tissue to fill the available volume within the 21-day healing period. The tentative conclusion is that, in citric acid-treated root surface areas that had been protected from mechanical injury or direct contact with the alveolar wall, a fibrous attachment had become established at an early healing stage which had prevented the development of ankylosis.

Resorption defects, in particular inflammatory resorptions, tend to be progressive with time, whereas ankylosis, on the other hand, may show a peak activity at 2 weeks, followed by reversal (12). These considerations add support to the conclusion that group 4 had a more favorable healing result than groups 2 and 3. The extensive ankylosis apically in group 4 confirms, however, that citric acid treatment alone does not ensure favorable healing. Preventing subsequent damage to the root surface and to the periodontal ligament remnants in the alveolus during the healing period seems to be a crucial factor.

The question arises as to the origin of the tissue elements that had repopulated the periodontal ligament space in those instances when the adherent periodontal tissue had either been devitalized by drying (group 2) or mechanically removed (groups 3 and 4). Andreasen & Kristerson (25) have concluded that repopulation by supracrestal connective tissue may occur only in the most coronal 0.4–1.0 mm of the periodontal ligament. It seems safe to assume, therefore, that the regions showing favorable healing in group 4 had been repopulated by cellular elements lining the wall of the alveolus and adjacent bone marrow spaces. Earlier studies supporting this consideration have been reviewed by Proye & Polson (11).

The presence of new hard tissue on the root surface was recorded in many sites in several teeth. With the exception of group 1, this observation was unexpected, since earlier observations have indicated that it takes more than 21 days for cementum formation to get started on a root-planed surface, regardless of whether citric acid has been applied. The distribution and appearance of the newly formed hard tissue show, however, that this must be regarded as a bone tissue associated with sites of temporary or permanent ankylosis. The formation of a bone-like tissue on the root surface, often connected to the alveolar bone by calcified bridges, has been described in greater detail by Andreasen (14).

The 21-day observation period chosen was sufficient for the formation of a fibrous connective tissue attachment to instrumented, acid-conditioned root surfaces. To ascertain whether this attachment mechanism will eventually be reinforced by deposition of new cementum, an experimental period of, say, 6 weeks is required (15, 26).

In conclusion, this study suggests that the frequency of ankylosis after replantation of teeth that have been air-dried for 45 min can be reduced by removing nonvital periodontal ligament remnants from the root surface, exposing dentin matrix collagen by treatment with citric acid, and preventing direct contact between the alveolus and the root surface during the healing period. However, the small number of observations and the variations in healing response within each specimen group indicate that the results must be interpreted with caution. Further experimentation is indicated.

Acknowledgement.—This study was supported in part by a grant from the Meltzer Foundation, University of Bergen.

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Received for publication 19 December 1983