Healing of Free Gingival Grafts With and Without Periosteum*

Part II. Radioautographic Evaluation

by

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THE HEALING of free gingival grafts on periosteum and on denuded bone was evaluated histologically in Rhesus monkeys.[1] Although the healing was initially delayed when the periosteum was removed, by 28 days there was no histologic difference in the rate of healing between grafts placed on bone or on periosteum. These findings agreed with results of previous clinical[2, 3] and histologic[4, 5] studies.

The present study evaluated radioautographically the healing of free gingival grafts placed on periosteum and on denuded bone in Rhesus monkeys. These radioautographic findings correspond to, and supplement the histologic evaluation previously reported.[1]

MATERIAL AND METHODS

Free gingival grafts were placed on periosteum and on bone in five adult male Rhesus monkeys (Macaca Mulatta) by the method already described.[1] Three of the monkeys received 1 hour prior to sacrifice an intravenous injection of tritiated thymidine, 1 μCi per gram of body weight (specific activity 6.7 curies per millimole).** In these monkeys experimental periods of 1 hour, 24 hours, 2, 4, 7, 14, 28 and 45 days were covered. The animals were sacrificed by exsanguination. The heads were dissected, fixed in 10% buffered formalin, and decalcified in 20% formic acid. Paraffin embedded tissue blocks, including the grafts, were sectioned buccolingually at 6 μm. Every fifth section, on glass slide, was processed for radioautographic evaluation. Kodak's nuclear tract emulsion (type NTB-3) was applied to the slides using the dipping technique.[6] They were placed in light-proof boxes and exposed for four weeks at 4°C. They were then developed for 5 minutes in Kodak's D 19 solution and washed for one-half hour. These sections were stained with hematoxylin and eosin, and mounted with Permount.††

Radioautographs were examined using a binocular microscope at 100 × magnification. Labeled cells were counted in five different spatial areas of the grafted tissues (Fig. 1). Area 1 corresponded to the tissue immediately overlying the alveolar bone. Area 2 encompassed the connective tissue of the graft. Area 3 involved the epithelium of the grafted tissue. Area 4 represented connective tissue cells surrounding the recipient site. Area 5 corresponded to the epithelium surrounding the graft. For purposes of cell counting, Area 3 was further subdivided in three sub-areas: coronal, intermediate and apical. Similarly, Areas 4 and 5 were subdivided in two sub-areas each: coronal and apical. Consequently nine zones were considered on each slide. A total of 160 slides were evaluated, 80 on periosteum and 80 on bone. Ten slides from each time interval were examined.

One microscopic field on each zone was counted. This field represented at 100 × magnification a rectangle of 100 × 69 μm. In these fields, total labeled epithelial or connective tissue cells were counted, using a Veeder‡‡ hand tally counter, and the value was recorded. Only cells with 10 or more radioactive grains in the nucleus were counted. Each section was counted twice at intervals varying from 1 to several weeks to determine if a possible error in counting would influence the results. An exceedingly small discrepancy was found. Where sub-areas were considered, the average for the area in the individual section was found. A mean for each area was computed by averaging the individual values recorded in the ten sections examined for each time period.

The mean labeled cell values for each individual area at different time intervals were analyzed by computer and diagrams comparing grafts on periosteum and on bone were obtained.

Furthermore, a mean total labeling value (mean of all areas) for each time interval was determined and the results compared using pairwise *t* tests.

RESULTS

One-Hour and 24-Hour Specimens

There was no difference in tissue response whether the graft was placed on bone or on periosteum. The epithe-

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lium showed thymidine uptake, both in the graft and in the recipient tissues. The grafted epithelium (Area 3) maintained its vitality as shown by the labeling of its basal cells (Fig. 2). There was significant activity in the epithelium surrounding the graft—Area 5 (Fig. 3A). The connective tissue of the graft showed little activity, while labeling was seen in the connective tissue surrounding the graft—Area 4.

Two- and Four-Days

When the grafts were placed on periosteum, there was activity both in the bed (Fig. 3B) and to a minimal extent in the grafted connective tissue (Fig. 4A). The connective tissue (Area 4) and epithelium (Area 5) surrounding the graft also showed activity (Fig. 4B). Epithelial remnants from deep rete pegs in the grafted tissue showed minimal labeling uptake (Fig. 4A).

The grafts on bone showed a similar distribution of labeling, except for the area of the bed (Area 1). Since the periosteum was removed there was no activity on the surface of the bone, except when strands of periosteum were inadvertently left attached to the bone. The con-

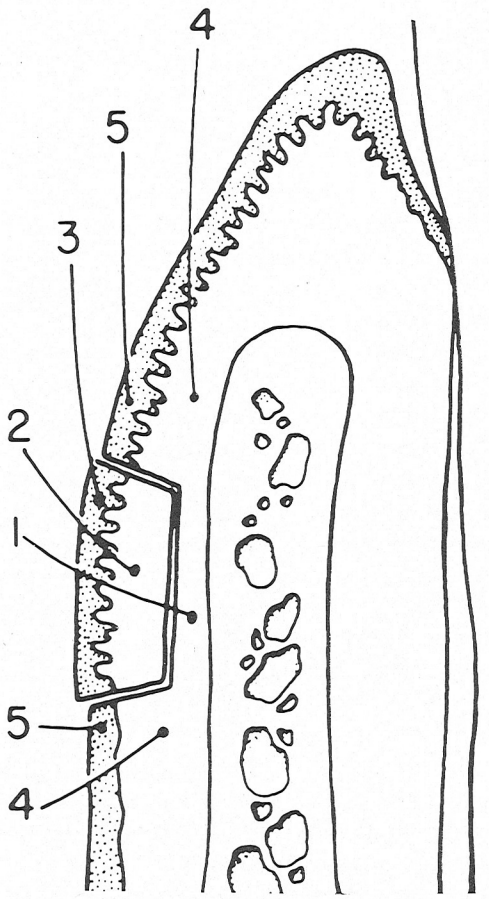


FIGURE 1. *Schematic diagram showing the areas selected for labeled cell countings.*

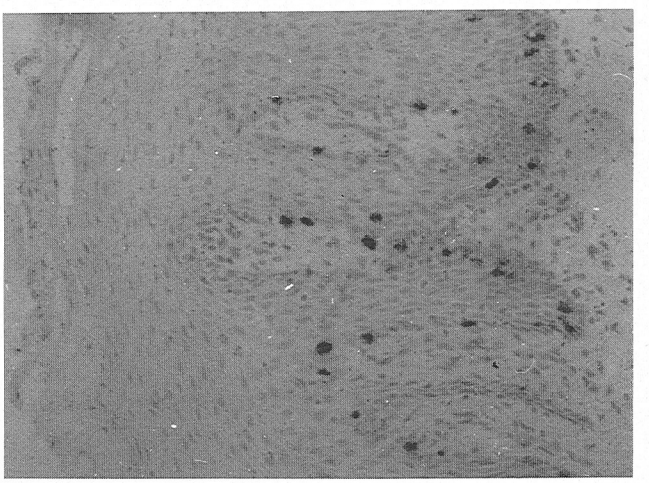


FIGURE 2. *Graft on bone, 1-hour specimen. Radioautograph showing labeling in the basal layers of the grafted epithelium (Area 3) (H & E, original magnification, × 125).*

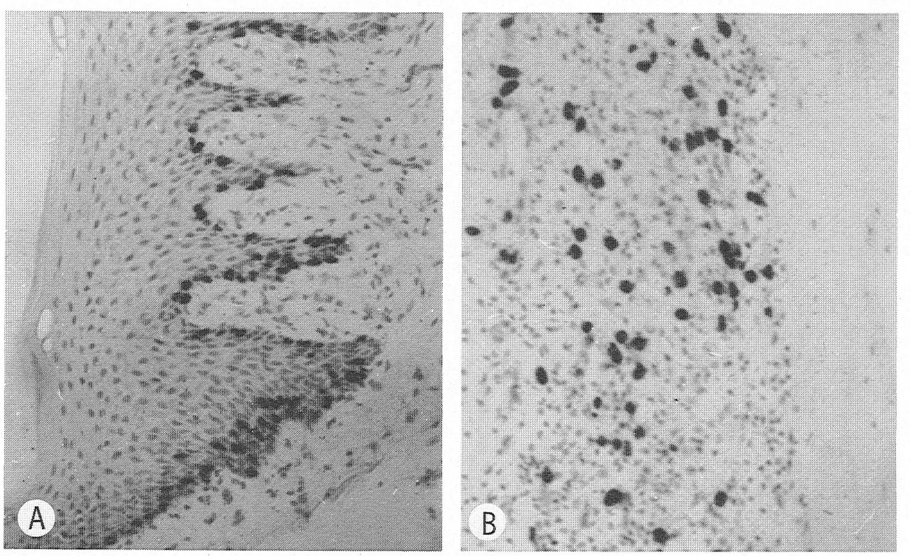


FIGURE 3A. *Graft on bone, 24-hour specimen. Radioautograph depicting cell labeling on the epithelium surrounding the graft (Area 5, coronal) (H & E, original magnification, × 125).* B. *Graft on periosteum, 2-day specimen. Radioautograph indicating labeling in the connective tissue of the bed overlying the alveolar bone (Area 1) (H & E, original magnification, × 125).*

nective tissue of the graft showed activity throughout (Fig. 5A).

Seven- and Fourteen-Days

All areas considered showed labeling with both procedures.

When grafts were placed on bone, the suprabony tissues (Area 1) as well as the grafted connective tissue (Area 2) revealed activity (Fig. 5B).

At 7 days, the epithelium surrounding the graft had started to migrate, showing increased activity. The migrating epithelium also showed thymidine uptake (Fig. 6A). The middle third of the graft was still devoid of epithelial coverage. However, labeling was evident in remnants of desquamated rete pegs (Fig. 6B). By 14 days, the epithelial coverage had been restored and the epithelium showed labeling throughout the deeper layers (Fig. 7A).

When the graft was placed on periosteum, by 7 days the connective tissue of the graft as well as the restored epithelial coverage showed labeling (Fig. 7B). Fibroblasts and angioblasts were the connective tissue cells showing proliferation.

The epithelial coverage was fully restored by 14 days, and the intensity of the labeling was decreased (Fig. 8).

Twenty-Eight and Forty-Five Days

At this stage repair was complete. However, when

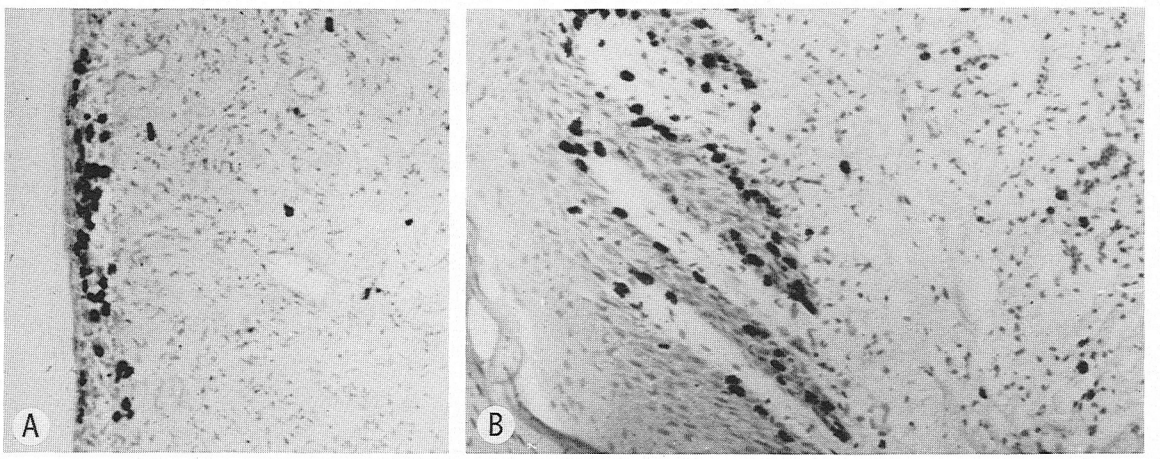


FIGURE 4A. *Graft on periosteum, 4-day specimen. Radioautograph of the grafted tissue. Minimal labeling is seen in the grafted connective tissue (Area 2). Remnants from deep rete pegs depict labeling uptake (Area 3) (H & E, original magnification, × 125).* B. *Graft on periosteum, 2-day specimen. Epithelial (Area 5) and connective tissue (Area 4) labelings are seen in these tissues surrounding coronally the area of the graft (H & E, original magnification, × 125).*

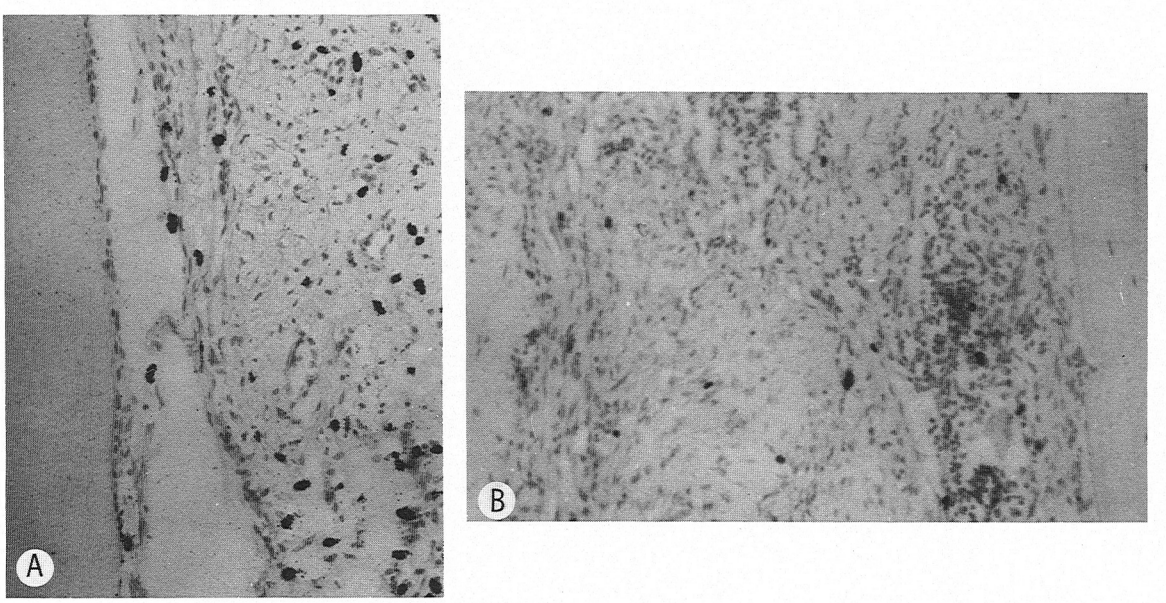


FIGURE 5A. *Graft on bone, 4-day specimen. The grafted connective tissue (Area 2) shows activity. A few strands of periosteum, left attached to the bone when the bed was prepared, depict labeling as well (Area 1) (H & E, original magnification, × 125).* B. *Graft on bone, 7-day specimen. Radioautograph showing labeling in the suprabony tissues that are undergoing organization (Area 1). The grafted connective tissue also depicts labeling (Area 2) (H & E, original magnification, × 125).*

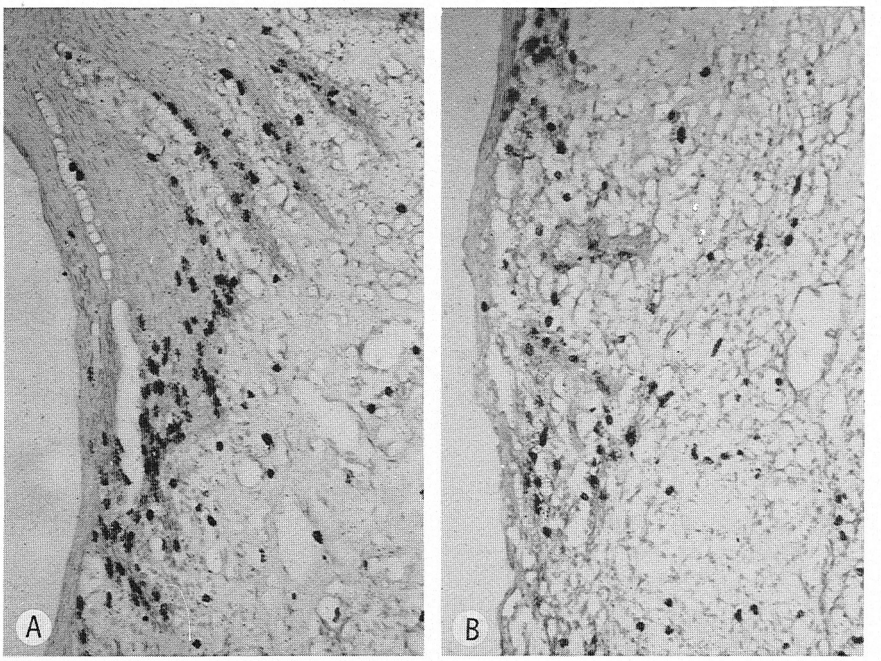


FIGURE 6A. *Graft on bone, 7-day specimen. Radioautograph showing labeling of the surrounding epithelium (Area 5), which has started to migrate towards the grafted area. The migratory epithelium also shows thymidine uptake (H & E, original magnification, × 125).* B. *Graft on bone, 7-day specimen. The middle third of the graft is still devoid of epithelial coverage. The migrating epithelium is seen depicting labeling, at the top of the picture. Labeling is also seen in the grafted connective tissue (Area 2) and in remnants of deep rete pegs (Area 3) (H & E, original magnification, × 125).*

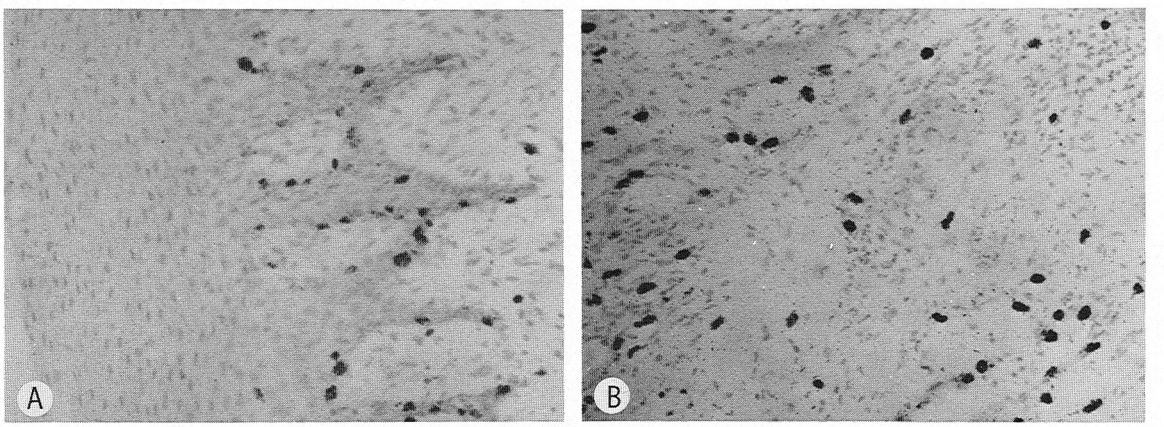


FIGURE 7A. *Graft on bone, 14-day specimen. The epithelial coverage has been restored on the grafted tissues (Area 3). Labeling is seen throughout the deeper layers (H & E, original magnification, × 125).* B. *Graft on periosteum, 7-day specimen. The epithelial coverage has been restored, and this radioautograph depicts labeling throughout its deeper layers (Area 3), as well as in the connective tissue (Area 2) (H & E, original magnification, × 125).*

grafts were placed on bone, very significant labeling was seen in the suprabony tissues (Area 1). This increased activity was seen both at 28 and 45 days (Fig. 9).

The epithelium of the grafted area also showed high thymidine uptake when the graft was placed on bone (Fig. 10A). This was not found with grafts on periosteum (Fig. 10B).

Labeled Cell Counts

Graphs 1 through 5 represent the time plots obtained from the five areas that were analyzed after the grafts were placed on periosteum and on bone.

Graph 1 represents the cell activity found over time in Area 1. Grafts *on periosteum* showed significantly increased activity in the suprabony tissues during the 1st week, reaching their peak between 2 and 4 days. After 7 days this area showed minimal cell labeling. With grafts *on bone*, the activity was minimal until 14 days. After 14 days it increased dramatically, reaching its peak at 45 days.

Graph 2 shows the activity in Area 2. Although cell labeling was generally more intense in the grafted connective tissue when placed on bone, both procedures showed a similar pattern of activity over time, reaching

a peak at 7 days. With grafts on bone, a stunting effect was initially detected in the connective tissue of the graft, which lasted for 4 days.

Graph 3 shows the activity in the grafted epithelium (Area 3). No activity was seen for the first 4 days. With both procedures the activity increased to similar levels at 7 days. Afterward, it became somewhat reduced with grafts on periosteum, while it further increased with those on denuded bone, reaching its peak at 45 days.

Graph 4 is a diagram of the activity encountered at different time intervals in the connective tissue surrounding the recipient site (Area 4), and Graph 5 contains corresponding data from the epithelium surrounding the graft (Area 5). Both areas exhibit a similar pattern in the distribution of labeled cells, and a significant increase in cellular proliferation during the first week after grafting.

Table 1 shows the results of the pairwise t tests comparing the mean total activity values for each time interval after the grafts were placed on bone and on

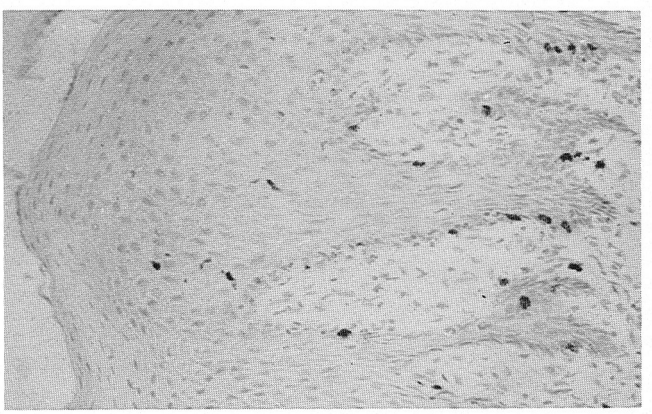


FIGURE 8. *Graft on periosteum, 14-day specimen. After the epithelial coverage is fully restored in the grafted area (Area 3) the intensity of labeling is reduced (H & E, original magnification, × 125).*

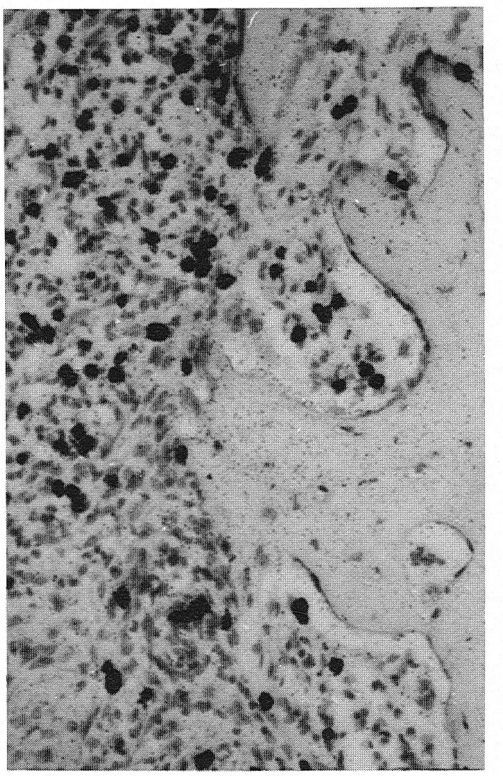


FIGURE 9. *Graft on bone, 45-day specimen. Persistent increased labeling in the suprabony tissues (Area 1). Activity is also seen in the bone marrow spaces (H & E, original magnification, × 125).*

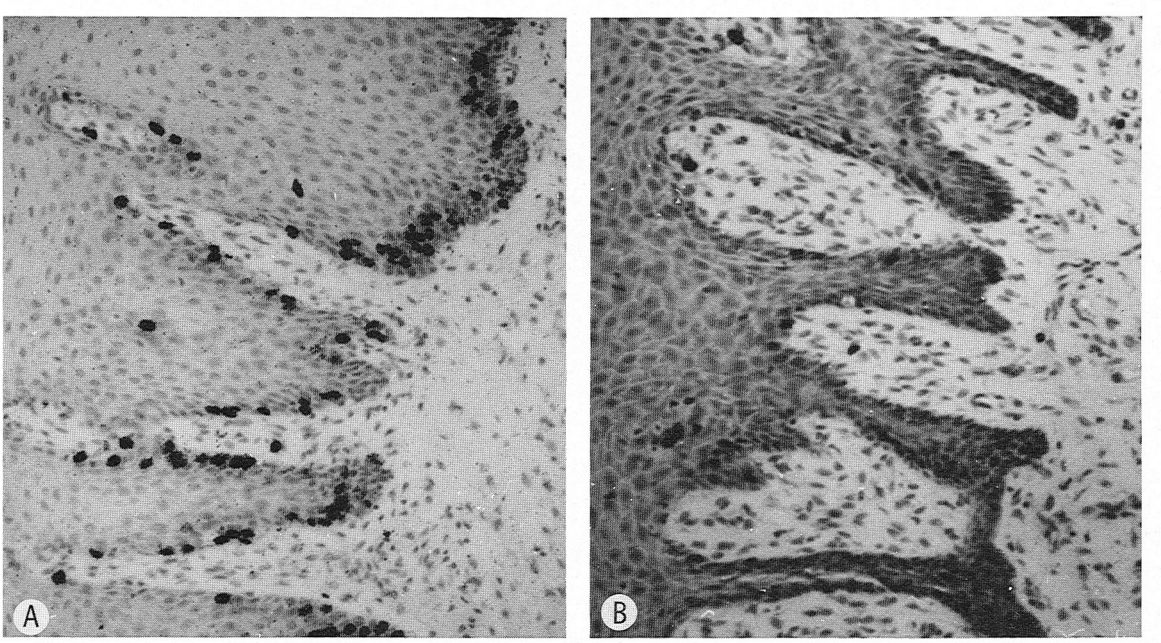
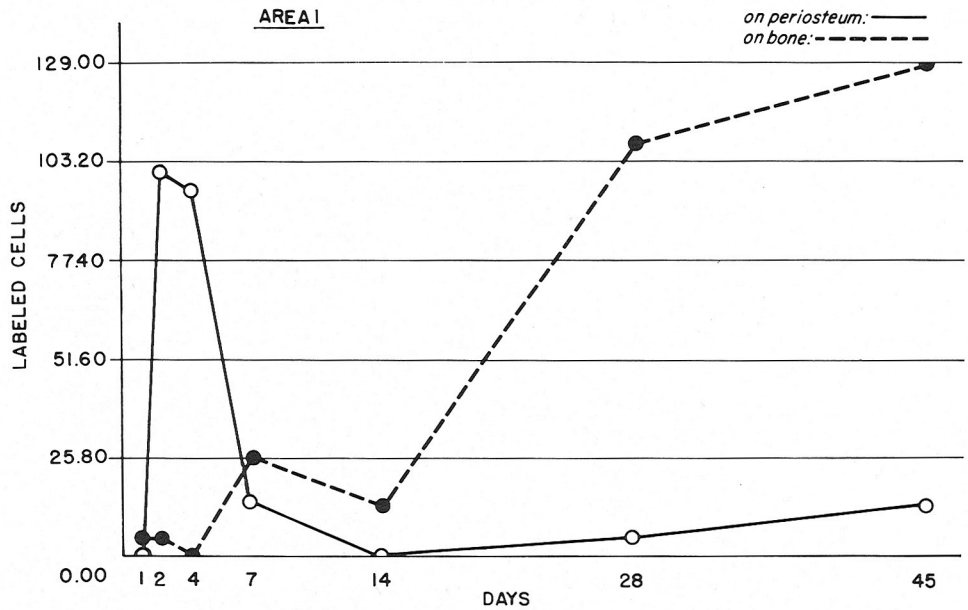
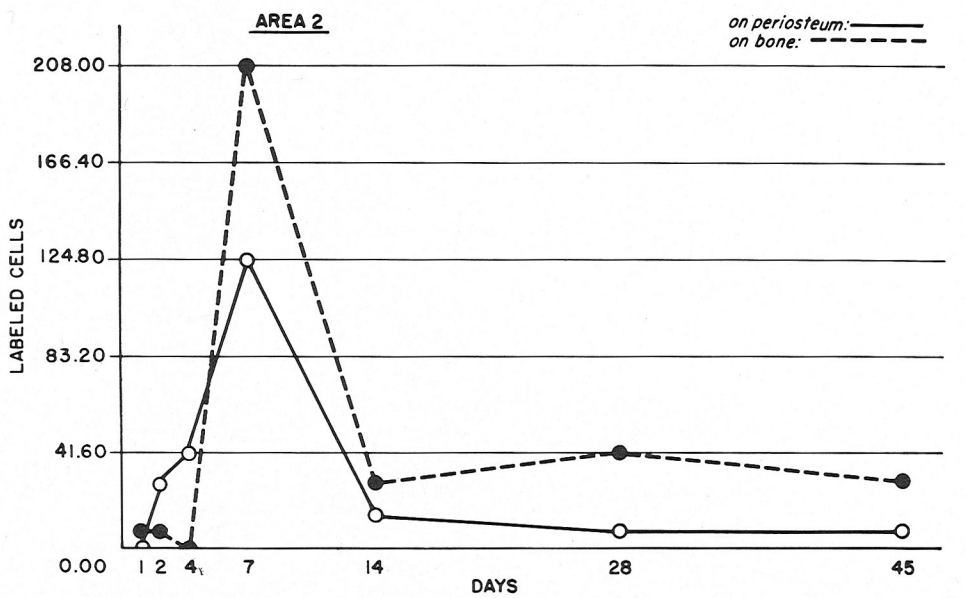


FIGURE 10A. *Graft on bone, 45-day specimen. High thymidine uptake is observed in the epithelium of the graft (Area 3) (H & E, original magnification, × 125).* B. *Graft on periosteum, 45-day specimen. Reduced thymidine uptake in the epithelium of the grafted tissues (Area 3) (H & E, original magnification, × 125).*



GRAPH 1. *Time plot showing the cell activity found over time after grafts on periosteum and on bone in Area 1: Tissues immediately overlying the alveolar bone.*

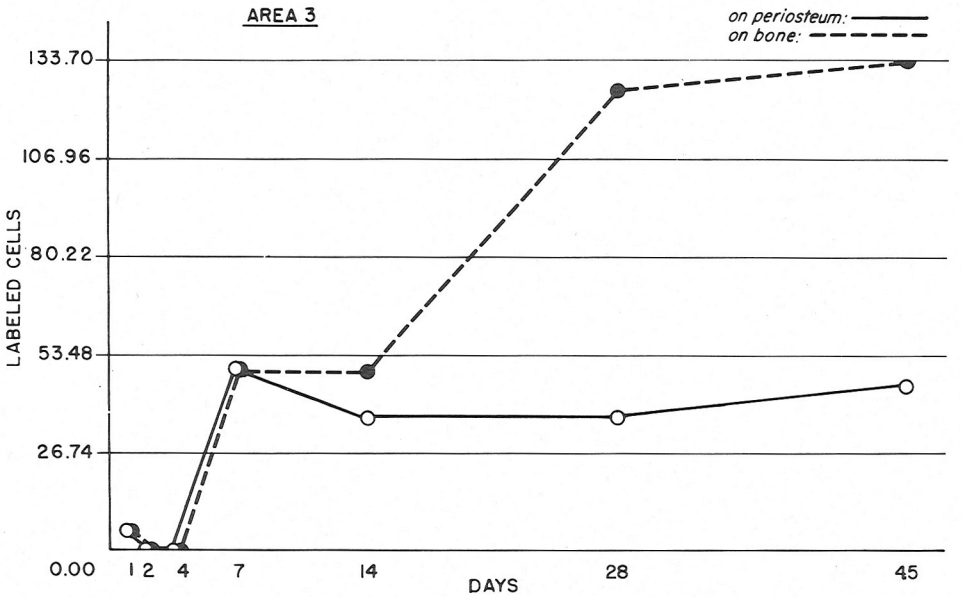


GRAPH 2. *Time plot showing the cell activity found over time after grafts on periosteum and on bone in Area 2: Connective tissue of the graft.*

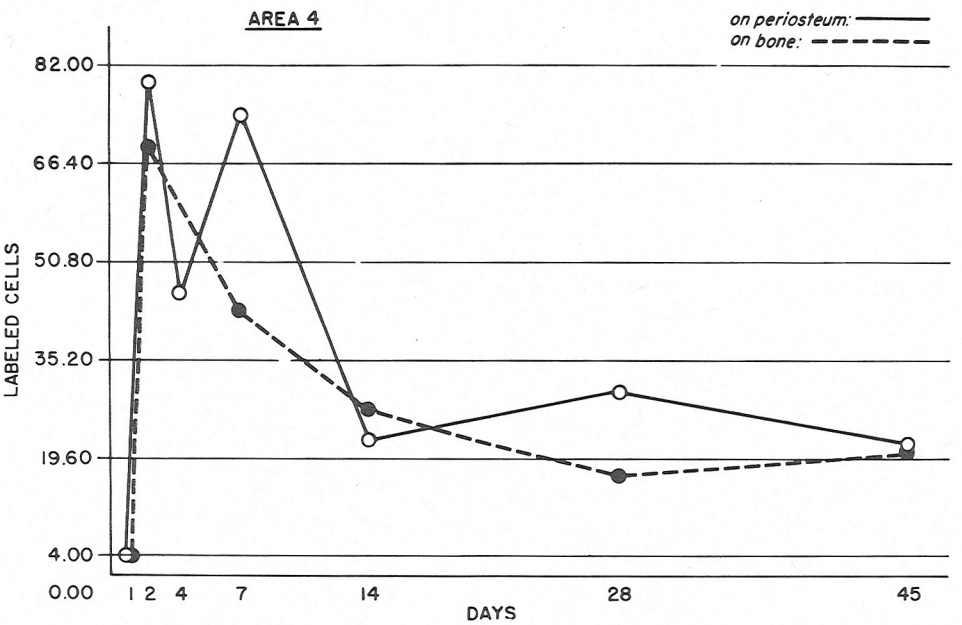
periosteum. No significant differences between both procedures were seen initially in the overall cell labeling, although radioactivity in both instances significantly increased when compared to the mean total activity recorded in areas where no surgery was performed (control = 11.209). At 14, 28 and 45 days there was a significant difference between grafts on bone and on periosteum, indicating a persistent increase in activity where grafts were placed on denuded bone.

DISCUSSION

Radioautographs provide an accurate assessment of the rate of healing after surgical procedures, and the results of this investigation agree with previous reports that the presence or absence of the periosteum in the bed prepared for a free gingival graft will not affect the final result of the procedure.[1-5] When the total healing activity is considered (Table 1) no difference is seen in the initial rate of healing between grafts on bone and on periosteum. The mean total labeling favored one procedure or the other during the first 2 weeks. However, a difference did exist after 14 days, being persistently high where grafts were placed on bone. This higher level of activity might be an indication of the need for further remodeling when grafts are placed on bone. Apparently there is a conflict between these findings and those from histologic[1] and clinical[2] evaluations showing initially delayed healing with grafts placed on bone. However, clinical and



GRAPH 3. *Time plot showing the cell activity found over time after grafts on periosteum and on bone in Area 3: Epithelium of the grafted tissue.*



GRAPH 4. *Time plot showing the cell activity found over time after grafts on periosteum and on bone in Area 4: Connective tissue surrounding the recipient site.*

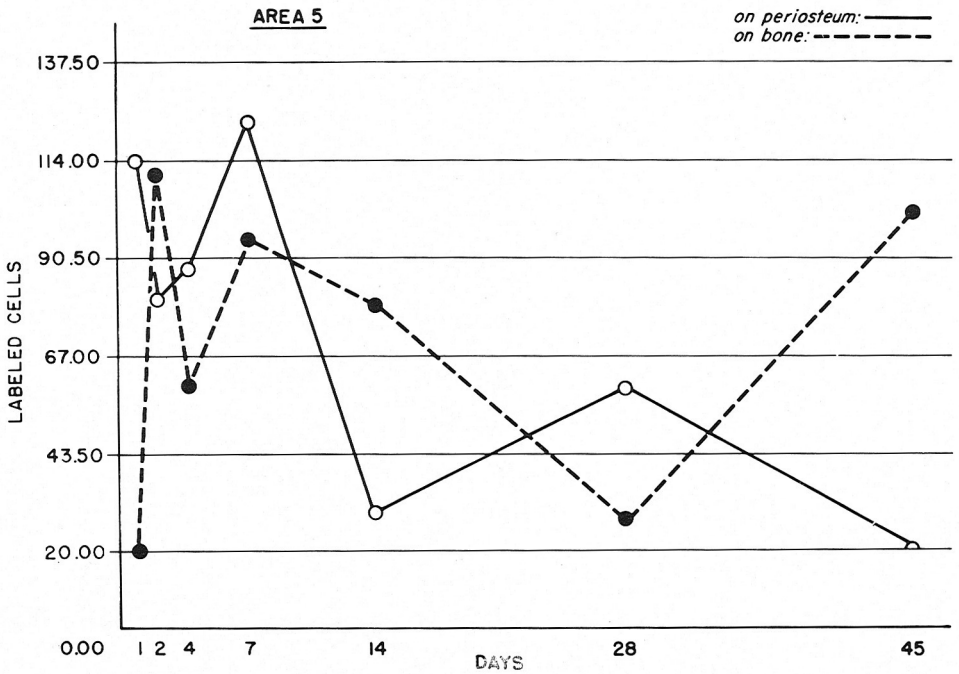
histologic evaluations are mainly based on the degree of epithelial coverage and tissue maturation. Increased labeling from all possible areas accounts for the lack of significant difference found initially when the mean total labeling values for both procedures are considered.

When individual areas are considered it is evident that the retention of periosteum on the bone favored an early start of healing at the level of the interface. When the grafts were placed on denuded bone, no activity was seen in this area until 7 days, except when strands of periosteum were inadvertently left. On the other hand, this delay in proliferation at the interface did not seem to impair the survival of the graft, especially of the grafted connective tissue. This area (Area 2) showed the same kind of activity whether on bone or on periosteum.

It seems that the nutrition received through the fibrinous interface, whether on periosteum or on bone, and from the neighboring tissues was sufficient to maintain the vitality of the graft. The surrounding areas showed initially high proliferative activity both in the epithelium and the connective tissue, and this high activity was maintained throughout the 1st week after grafting.

The epithelium of the graft certainly reflected the impact of the reduced nutrition received. Although in 1 hour it showed thymidine uptake, immediately afterward the epithelium was practically lost by desquamation.

J. Periodontol.
November, 1979



GRAPH 5. *Time plot showing the cell activity found over time after grafts on periosteum and on bone in Area 5: Epithelium surrounding the graft.*

TABLE 1. *Results of Pair-Wise t Tests Analysis Between Mean Total Labeling Values at Different Time Intervals Comparing Grafts on Periosteum and on Denuded Bone*

	On bone	On periosteum	Mean difference	P value
1 day	30.714	17.857	12.857	0.12
2 days	32.375	46.000	−13.625	0.32
4 days	29.750	40.000	−10.250	0.55
7 days	72.333	74.000	−1.667	0.90
14 days	43.778	24.556	19.222	0.05
28 days	72.125	28.500	43.625	0.05
45 days	89.111	26.889	62.222	0.01

However, remnants from deep rete pegs remained and showed activity after 2 days. As a consequence, they did help in the reepithelialization of the graft. The epithelial coverage was also restored by the migration of the surrounding epithelium. This migrating epithelium behaved similarly to that after gingivectomy,[5] wedging toward the grafted tissue due to increased proliferation of epithelial cells from the neighboring areas. The wedged epithelium itself showed proliferation to restore epithelial thickness.

An interesting observation was the persistent increase in turnover of the epithelium in the grafted area when the grafts were placed on bone even 45 days after the surgery. Whether this high proliferative activity was associated with remodeling of the restored periosteum is only speculative. However, histologically the epithelium showed already complete maturation.

SUMMARY AND CONCLUSIONS

Free gingival grafts were placed on periosteum and on bone in five adult male Rhesus monkeys. Three of the monkeys received an intravenous injection of tritiated thymidine 1 hour prior to sacrifice.

After processing, radioautographs covering postoperative periods from 1 hour to 45 days were obtained. Labeled cell counts were obtained in five areas: (1) bed, (2) grafted connective tissue, (3) grafted epithelium, (4) surrounding connective tissue, and (5) surrounding epithelium. A total of 160 slides were evaluated, 80 on periosteum and 80 on bone.

Diagrams were made comparing the mean labeled cell values for each individual area after both procedures. A mean total activity was also determined and analyzed statistically. Results showed that the total rate of healing was initially similar whether grafts were placed on bone or on periosteum, as indicated by the total cell labeling counts. However, after 14 days, cell proliferation became significantly higher with grafts on bone. The presence of the periosteum warranted early proliferation at the level of the bed, since with grafts on bone no activity was seen there until 7 days. Similar rates of proliferation were seen in the surrounding areas, including both connective tissue and epithelium, with increased activity confined to the 1st week. The epithelial coverage of the graft was restored by migrating epithelial cells from the surrounding areas, and also by proliferation of remnants of deep rete pegs in the grafted tissue. These remnants showed labeling at 2 days.

Within the limits of this study the following conclusions can be drawn:

1. The total rate of cell proliferation after a free gingival graft procedure is similar whether on periosteum or on bone.

2. Proliferation from the surrounding tissues will be sufficient to warrant the success of the graft.

3. The presence of the periosteum in the bed will provide a source for initial repair at the interface.

4. Remnants from deep rete pegs in the graft will contribute to its reepithelialization.

5. Delayed remodeling is expected when grafts are placed on bone. This affects mainly the newly formed periosteum.

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Abstracts

Pharmacotherapy of Masticatory System Dysfunction

Jagger, R. G.
J Prosthet Dent **40:** 183, August, 1978.

This survey of both the systematic pharmacotherapeutic and placebo-effect management of myofascial pain-dysfunction (MPD) syndrome stressed the beneficial and adverse consequences of various drug and dose schedules of administration. Excellent palliative rather than curative symptomatic relief of acute muscular symptoms was reported with ethyl chloride spray and intramuscular injections of local anesthetic agents. The significance of systemic treatment with minor tranquilizers, muscle relaxants, antidepressants and miscellaneous compounds upon the high anxiety and emotional components associated with MPD was set forth in order to provide specific clinical guidelines for rational drug therapy. Possible side effects must be explained to a patient, and treatment by drugs should be on a short term basis to prevent drug dependence. *Department of Restorative Dentistry, Heath Park, Cardiff CF4 4XY, South Wales, United Kingdom.*
Dr. Richard Singer

Oral Hygiene, Periodontal Health and Need for Periodontal Treatment Among Institutionalized Mentally Subnormal Persons in Norway

Svatun, B., and Gjermo, P.
Acta Odontol Scand **36:** 89, No. 2, 1978.

The study consisted of 328 persons ranging in age from 5 to 45 in 38 institutions. A clinical examination was given to each patient scoring plaque, calculus, inflammation, pocket depth, and treatment requirements. It was found that oral hygiene and gingival health decreased with increasing age, and factors which contributed to increased treatment requirements were epilepsy, Down's Syndrome, and severe mental deficiency. In institutions where nurses performed the toothbrushing, a higher level of gingival health was noted. Dental hygiene students in Norwegian Schools receive 250 hours of special training in the care of handicapped persons, therefore employment of dental hygienists in institutions for the retarded was recommended. *Department of Pedodontics, Dental Faculty, Geitmyrsveien 71, Oslo 4, Norway.*
Dr. Gary Galovic

The Effect of Topical Citric Acid Application on the Healing of Experimental Furcation Defects in Dogs

Crigger, M., Bogle, G., Nilvéus, R., Egelberg, J., and Selvig, K. A.
J Periodont Res **13:** 538, November, 1978.

Experimental through-and-through bifurcation defects were established by modifying the method of Ellegaard *et al.* (1973) as evaluated by Johansson *et al.* (1978) in eight adult Labrador retriever dogs' mandibular premolar teeth. Six months after chronic through-and-through defects were positively established, surgical procedures including elevation of buccal and lingual mucoperiosteal flaps, as described by Nilvéus (1978), were performed. After thorough instrumentation, cotton pledgets soaked with citric acid (pH 1) were placed in each furcation for 3 minutes, rinsed, then closed with Gelfoam Dental Packs on one side of the mouth. On the other (sham operated) side, two teeth were instrumented thoroughly and closed, also with Gelfoam, while one (control) was left untreated. Plaque control was administered daily postoperatively by topical tetracycline (3%) ointment. Sutures were removed in 1 week and healing was uneventful. Block sections were taken after 6 weeks and examined histologically and compared histometrically. Histologically the sham and control teeth all showed patent furca with bacterial plaque, continuous epithelial lining, and extensive inflammatory infiltrate in the interradicular connective tissue. The instrumented (sham) teeth showed some slight degree of regeneration of connective tissue attachment. Of the 23 acid-treated teeth, only two demonstrated patent defects, eight showed incomplete and 13 showed complete new attachment with connective tissue fibers and occasionally new cells, generally without evidence of a resorptive phase in healing. Ankylosis was observed in one section. Histometrically, the bone level was much higher on the acid treated side in each animal and more advanced opposite the root surface than at the center of the lesion. It was noted that the conventional reattachment procedure was unpredictable in this dog model in contrast to the dramatic success of the citric acid treatment regimen. The high healing capacity these animals have, underscored the validity of their results. Further, the citric acid did not seem to have any detrimental effect on the hard or soft tissues healing or on bone regeneration. A critical evaluation and literature review was included. *University of Bergen, School of Dentistry, 17 Årstadveien, N-5000 Bergen, Norway.* Dr. Leslie P. Racowsky