Healing of Free Gingival Grafts With and Without Periosteum.*

Part I. Histologic Evaluation

by

RAUL G. CAFFESSE, D.D.S., M.S., Dr. Odont.†

FREDERICK G. BURGETT, D.D.S., M.S.‡

CARLOS E. NASJLETI, D.D.S.§

WALTER A. CASTELLI, D.D.S., M.S.‖

A CONSIDERABLE NUMBER of publications have evaluated histologically,[1-8] clinically[2, 3] and biometrically,[13] the results of free gingival grafts in mucogingival surgery. Classically gingival grafts have been placed on a periosteal bed to promote tissue healing. However, Dordick, Coslet and Seibert[14] have reported good and predictable clinical results in humans with free gingival grafts placed on denuded bone. Bissada and Sears[15] later reported histologically similar results when grafts were placed on periosteum or on denuded bone in Rhesus monkeys. These findings recently have been confirmed clinically and histologically in humans.[16, 17]

No study dealing with gingival grafts has used radioisotopes to trace the dynamics of the healing process of both epithelium and connective tissue.

The present investigation evaluated histologically and radioautographically the healing of free gingival grafts placed on periosteum and on denuded bone in Rhesus monkeys, using radioautographic and standard histologic techniques.

Only histologic findings will be reported here, since radioautographic results are presented separately.

MATERIALS AND METHODS

Five adult male Rhesus monkeys (Macaca mulatta) weighing between 15 and 18 pounds were used. A mild chronic gingivitis with moderate amounts of calculus was present in all animals.

All teeth were scaled and polished 1 week to 10 days prior to the surgical appointment.

The animals were anesthetized with an intravenous injection of pentobarbital sodium, 30 mg/kg of body weight. Free gingival grafts were performed according to standard techniques, using the palatal mucosa as the donor area. The grafts were always split thickness in nature. Two beds were prepared on the facial aspect of each quadrant, by performing a split thickness procedure apically to the mucogingival line. On one bed the periosteum was left intact, while on the other the periosteum was stripped off, leaving the bone exposed. Dry foil templates, 3 by 6 mm, were used to standardize the size of the grafts. They were fixed to the recipient sites using 00000 atraumatic sutures. No periodontal dressing was placed.

Experimental periods of 1 hour, 24 hours, and 2, 3, 5, 7, 14, 28, 45 and 72 days were covered. Two quadrants, one on each monkey, were operated at each time interval.

The monkeys were kept in individual cages under controlled temperature and humidity, and were fed on a regular diet and water ad libitum.

On the established day the animals were sacrificed by exsanguination. Three of the monkeys, covering periods from 1 hour to 45 days, 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).¶ After sacrifice the heads were dissected, fixed in 10% buffered formalin solution, and decalcified in 20% formic acid. The tissue blocks including the grafts were embedded in paraffin, sectioned buccolingually at 6-μm intervals and mounted on glass slides. Every fifth slide was processed for radioautographic evaluation. The remaining slides were prepared by standard histologic techniques and stained with Ehrlich's hematoxylin and eosin, Mallory and Masson for connective tissue, Rhodamine B for keratin and Weigert's elastica for elastic fibers.

RESULTS

Of the 20 grafts placed on denuded bone, two were lost during the first week. None of the 20 grafts placed on periosteum were lost. From a clinical standpoint, all grafts healed at a similar rate, since it was impossible to determine from observation whether they had been placed on bone or on periosteum.

Histologic Findings

One-Hour and 24-Hour Specimens

The grafts were clearly detectable on the recipient bed. The tissue presented a stratified squamous epithelium

* Supported by the Medical Research Service of Veterans Administration.

† Professor of Periodontics, the University of Michigan School of Dentistry, Ann Arbor, Mich. 48109 and Consultant, Veterans Administration Hospital.

‡ Professor of Periodontics, The University of Michigan School of Dentistry.

§ Supervisory Research Biologist, Dental Research Section, Veterans Administration Hospital and Senior Dental Research Associate, the University of Michigan School of Dentistry.

‖ Professor of Anatomy, the University of Michigan Medical Center and Consultant, Veterans Administration Hospital.

¶ New England Nuclear Corporation, Boston, Mass.

with definite keratinization, as depicted by Rhodamine B (Fig. 1). The grafted tissue maintained its vitality.

The underlying connective tissue was composed of intermingling bundles of dense collagenous fibers, with some dilated blood vessels, few cellular elements and inflammatory cells (Figs. 2A, 2B).

When the graft had been placed *on periosteum*, the recipient bed clearly depicts a well defined and dense periosteum, firmly attached to the underlying bone. A layer of a more loosely arranged connective tissue, with fibers running parallel to the surface of the bone, covered the periostuem (Fig. 2A) at the level of the interface. A few inflammatory cells were found in the involved tissues. The bone showed vitality throughout, with viable osteocytes being present, and a layer of osteoid deposition depicted (Fig. 2A).

A very close adaptation at the level of the interface was apparent. Polymorphonuclear leukocytes and some lymphocytes were invading this area at 24 hours (Fig. 2A).

When the graft had been placed *on bone*, only isolated periosteal strands remained attached to it. There were very few lacunae in the superficial layers of the bone. The graft was anchored to the bone by a loosely arranged fibrinous clot showing scattered blood elements and inflammatory cells (Fig. 2B). The adaptation in this instance did not seem to be as good as with the grafts placed on periosteum.

Two to Five-Day Specimens

On periosteum, the adaptation of the graft to the bed was well maintained at the level of the interface. This area had been invaded by acute inflammatory cells, mainly polymorphonuclear leukocytes. These cells were also invading the connective tissue bed, and to a lesser degree the graft. In the bed the concentration of inflammatory cells was more pronounced closer to the interface, becoming less pronounced toward the bone (Fig. 3A). No changes in the bone were apparent. Engorged and dilated blood vessels could be seen both in the bed and in the graft (Figs. 3A, 3B). A significant inflammatory response involved both the graft and the bed.

The grafted epithelium showed signs of degenerative changes. The existing cells had lost their individuality, and there were indications of pyknotic degeneration. The epithelium was significantly thinner due to desquamation of the superficial layers, and in some instances was lost completely (Fig. 3B). However, at the periphery of the graft the superficial location of the interface was almost invisible. In 5 days the surrounding epithelium had already started to migrate on top of the grafted surface to cover the area (Fig. 3C). Remnants of the deeper portions of the rete pegs from the graft seemed to cooperate in this task.

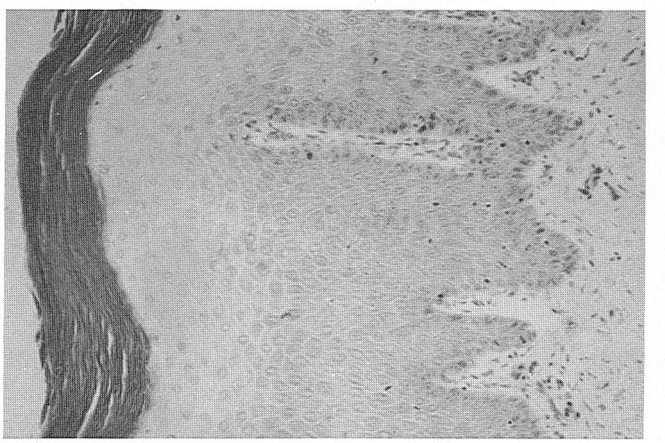

FIGURE 1. *One-hour specimen. The grafted tissue shows a stratified squamous epithelium displaying definite keratinization (Rhodamine B, original magnification, × 100).*

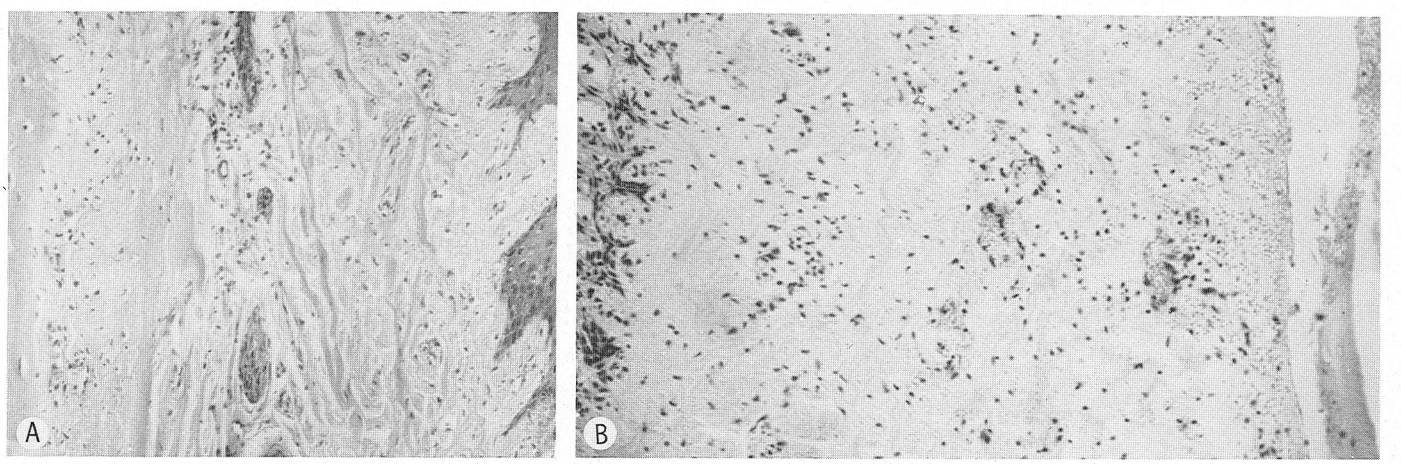

FIGURE 2. A. *Twenty-four hour specimen on periosteum. A close adaptation is seen between graft and periosteum at the interface. Minimal inflammation is present (H & E, original magnification, × 100).* B. *Twenty-four-hour specimen on bone. The graft is loosely attached to the bone. A few periosteal strands remain attached to it. A fibrinous clot is depicted at the interface (H & E, original magnification, × 100).*

On bone, the adaptation of the graft to the denuded bone could be seen. The interface was clearly detected and inflammatory cells were present in this area. Acute inflammatory cells were spaced throughout the grafted tissue coupled with dilated blood vessels (Fig. 4A). The grafted connective tissue showed signs of hyalin degen-

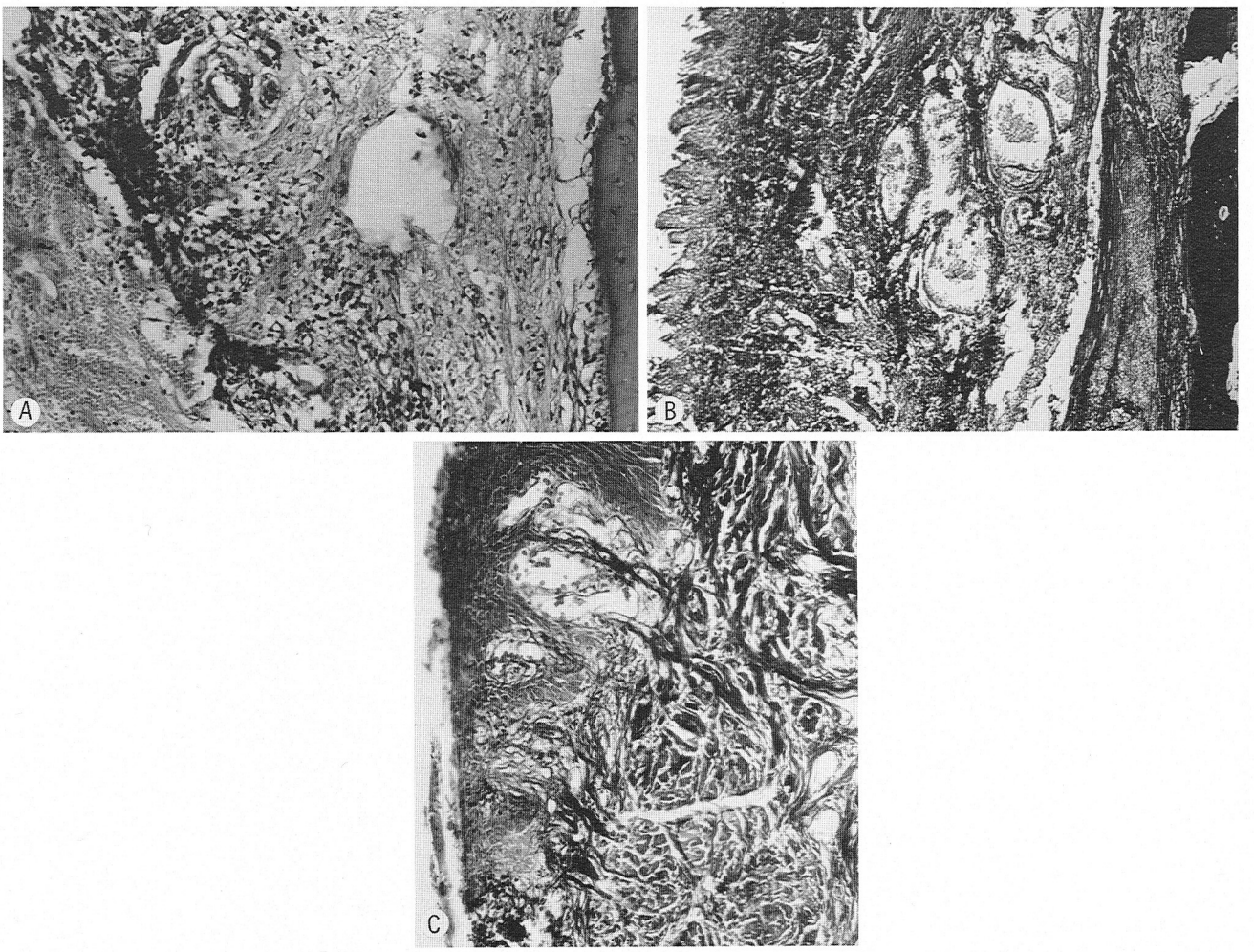

FIGURE 3. A. *Three-day specimen on periosteum. Acute inflammatory cells are invading the graft and the bed. This inflammation is heavier at the level of the interface (H & E, original magnification, × 100).* B. *Three-day specimen on periosteum: Dilated blood vessels are seen in the graft. The epithelium is lost completely (Masson, original magnification, × 100).* C. *Five-day specimen on periosteum: The surrounding epithelium starts to migrate over the grafted area (H & E, original magnification, × 100).*

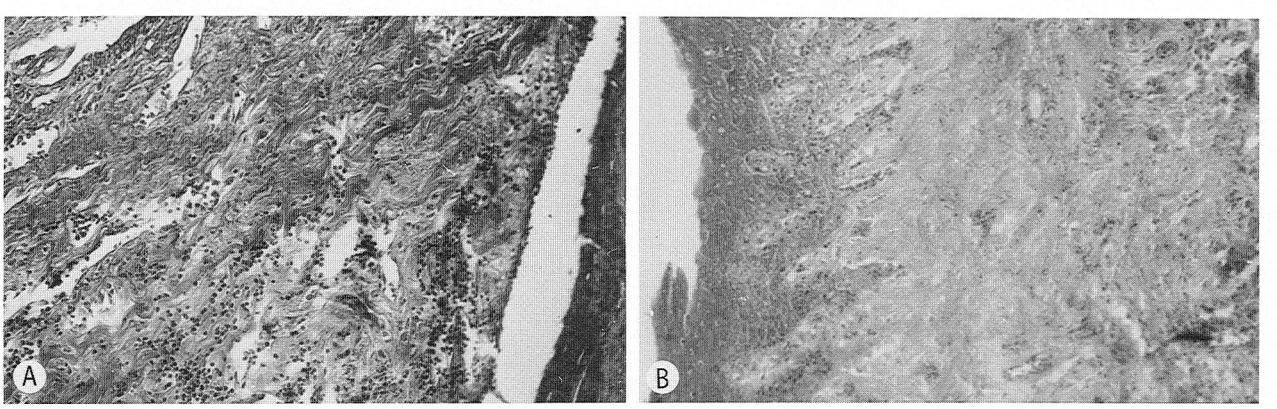

FIGURE 4. A. *Three-day specimen on bone. Acute inflammation is invading the graft. The graft/bone interface is clearly depicted (Masson, original magnification, × 100).* B. *Three-day specimen on bone: Desquamation and degenerative changes are seen affecting both the epithelium and the connective tissue of the graft (H & E, original magnification, × 100).*

eration, especially in the subepithelial area (Fig. 4B). Some lacunae were observed in the superficial area of the bone (Fig. 4A).

Changes in the grafted epithelium followed the same pattern previously described including desquamation and degeneration (Fig. 4B). However, no migration from the surrounding epithelium tending to cover the grafted area was apparent as yet.

Seven-Day Specimens

On Periosteum: It was almost impossible to localize the position of the interface. All the suprabony tissues showed persistent inflammation with dilated blood vessels (Fig. 5A). Cellular elements, mainly fibroblasts, predominated throughout the connective tissue (Figs. 5A, 5B). No reaction in bone was seen.

The migrating epithelium had completed the coverage of the grafted area. The peripheral areas were already showing a relationship between epithelium and connective tissue which was similar to that found in attached gingiva, with deep rete pegs and an undulating basement membrane. The central portion of the graft, however, was covered by a thin epithelium, approximately 10 to 15 cell layers thick and with a smooth basement membrane (Fig. 5B).

On Bone: The interface bone/graft was no longer detectable. New connective tissue fibers had been established in this area, apparently gaining "attachment" to the bone. Young connective tissue fibers were mainly parallel to the surface of the bone. The grafted connective tissue immediately overlying this area showed persistent inflammation and dilated blood vessels (Fig. 6A). The more superficial connective tissue showed very few inflammatory cells (Fig. 6B). The interface between the connective tissue at the sides of the graft and that of the bed (either attached gingiva or alveolar mucosa, depending on the border of the graft considered) could no longer be detected. Fibers had regained continuity at this level. The epithelium from these sides has started to migrate to cover the graft. Deep rete pegs from the grafted epithelium seemed to contribute to its reepithelialization (Fig. 6B).

Fourteen-Day Specimen

On Periosteum: There was some persistent inflammation in the suprabony tissue at the level of the interface (Fig. 7A). Complete epithelialization had occurred. The epithelium had developed the morphologic characteristics of attached gingiva. However, no keratinization was detected with Rhodamine B (Fig. 7B).

On Bone: New attachment of the graft to the bone was evident. Bone remodeling was still present with areas of active bone resorption throughout (Fig. 8). Connective tissue fibers immediately overlying the bone showed improvement in their organization, running parallel to the surface of the bone, and already resembling the pattern of the periosteum normally seen in attached gingiva. The healing of the epithelium was similar to that just described for the grafts on periosteum.

Twenty-Eight Day Specimens

It was very difficult to distinguish between the healing of grafts placed on periosteum and those placed on bone. In both instances connective tissue healing had taken place so that it was impossible to ascertain the location of the previous interface (Fig. 9). In grafts placed on

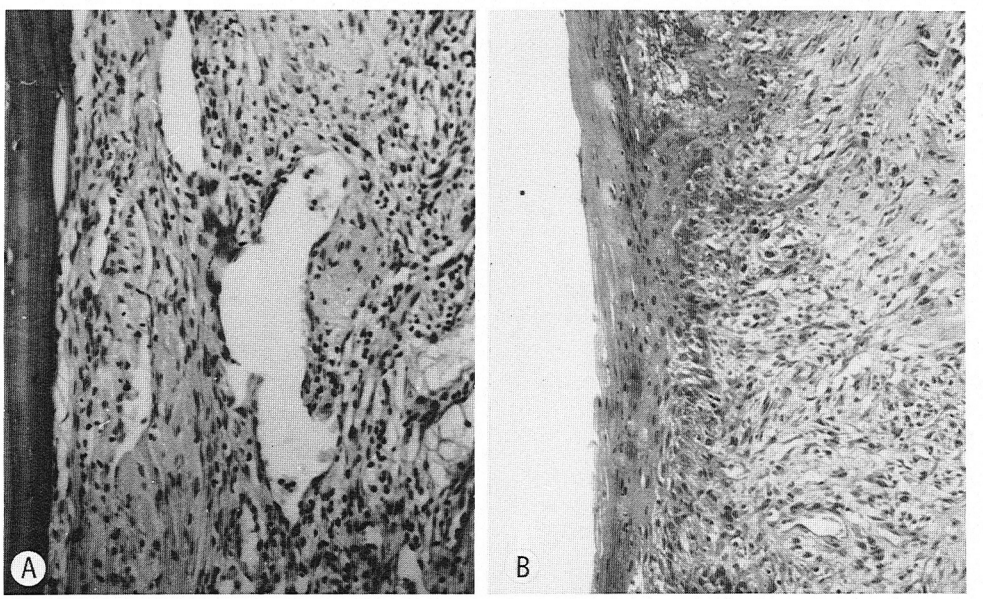

FIGURE 5. A. *Seven-day specimen on periosteum. Inflammation with dilated blood vessels is seen. The interface is no longer detectable (H & E, original magnification, × 100).* B. *Seven-day specimen on periosteum. The middle third of the graft shows complete epithelial coverage (H & E, original magnification, × 100).*

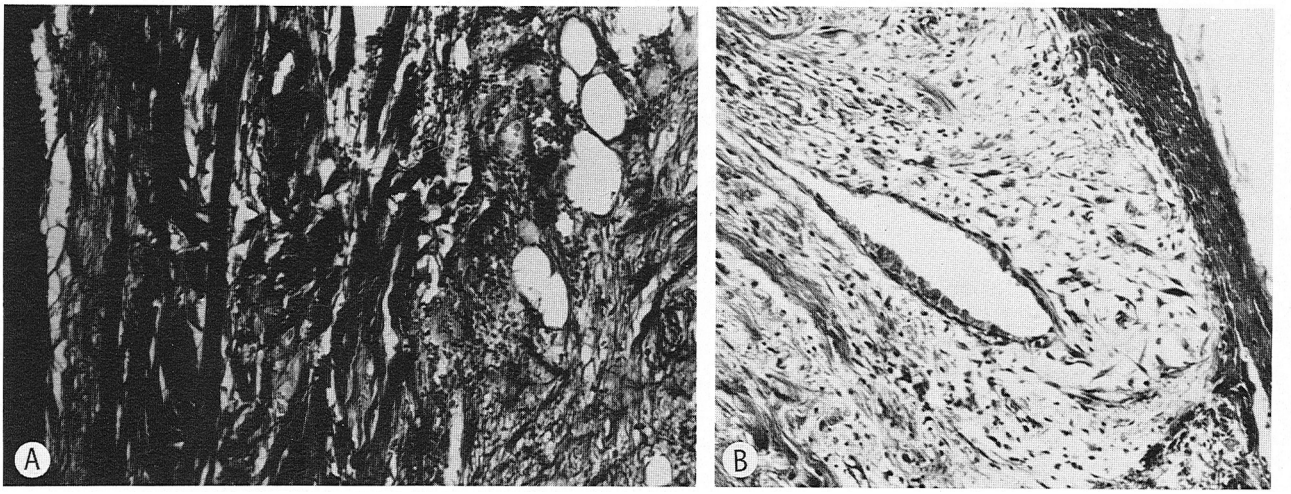

FIGURE 6. A. *Seven-day specimen on bone: Dilated vessels and inflammatory cells are seen in the graft. New connective tissue fibers parallel to the bone have been reestablished (Mallory, original magnification, × 100).* B. *Seven-day specimen on bone. The epithelium starts to migrate to cover the graft (H & E, original magnification, × 100).*

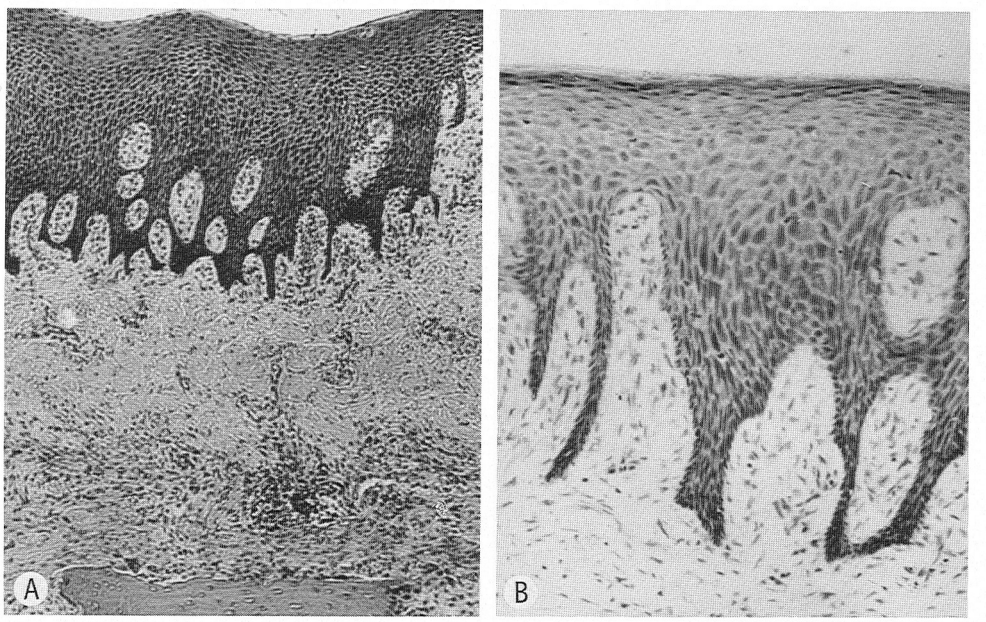

FIGURE 7. A. *Fourteen-day specimen on periosteum: Morphologic characteristics of attached gingiva are seen. H & E, original magnification, × 100).* B. *Fourteen-day specimen on periosteum: No keratinization is detected (Rhodamine B, original magnification, × 100).*

bone, the organization of the suprabony connective tissue increasingly resembled that of periosteum (Fig. 10). No areas of bone resorption were present. However, Elastica stain revealed certain differences. Grafts on periosteum showed the presence of elastic fibers in the deeper connective tissue, in an area corresponding to the previous interface (Fig. 10A). Similar distribution was seen in the alveolar mucosa (Fig. 10B). Conversely, grafts on bone showed virtually no elastic fibers (Fig. 10C).

In all cases the epithelium of the graft had completed its full differentiation, and Rhodamine B stain depicted a clear keratinization of its surface (Fig. 9).

Forty-Five and Seventy-Two Day Specimens

Essentially the same observations described at 28 days apply for these time intervals. Maturation of the connective tissue was completed after both experimental procedures (Figs. 11A, 11B). In grafts placed on bone, a new periosteum had developed. In both situations the epithelium showed the same arrangement that was described in 28-day specimens (Figs. 11A, 11B).

Elastica stain revealed the same difference described previously: persistence of elastic fibers in the connective tissue of grafts placed on periosteum (Fig. 12A) and the

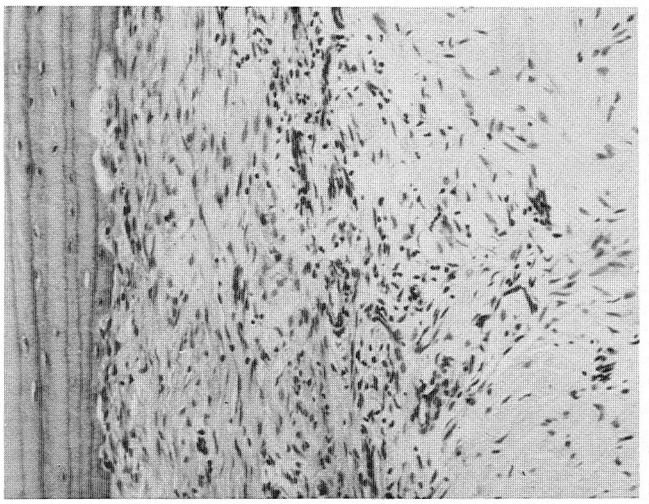

FIGURE 8. *Fourteen-day specimen on bone: Attachment to bone is seen. Persistent areas of superficial bone resorption are present. A new periosteum seems to be developing (H & E, original magnification, × 100).*

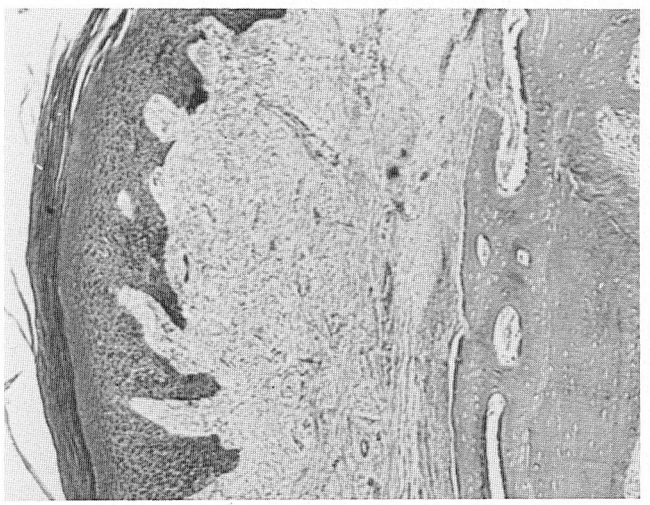

FIGURE 9. *Twenty-eight day specimen on bone: Tissues have repaired (H & E, original magnification, × 100).*

absence of these fibers when the graft was placed on bone (Fig. 12B).

DISCUSSION

The findings of the present study agreed with previous reports dealing with the healing of free gingival grafts placed on periosteum.[1-8] These findings also agreed with reports of clinical[14] and histologic[15] studies indicating that the presence or absence of the periosteum at the recipient site did not influence the final results obtained with free gingival grafts. Although there was an initial lag period of about 1 week for the grafts placed on bone, the stages of healing seemed to be similar for both grafting procedures.

The presence of the periosteum apparently favored a better initial adaptation of the graft to the bed. This in turn allowed for the formation of a fibrinous clot and hence the establishment of an initial plasmatic circulation. The lack of perfect adaptation at the interface of graft and bone probably accounted for the more severe degenerative changes initially seen within the graft. Although the epithelium was affected in a similar way whether the graft was placed on periosteum or on bone, in the latter instance the connective tissue showed signs of hyalin degeneration after 5 days. The degenerative changes affected mainly the superficial connective tissue. These changes were an indication that the diffusion of nutrients from the bed was more severely affected by the lack of periosteum. However, it must be remembered that, laterally a connective tissue contact was established between the graft and the surrounding recipient tissues. The healing in these areas was similar for both procedures, and at 7 days it was impossible to determine the location of these interfaces, whether on periosteum or bone. Nutrition could certainly come from these lateral sources until organization took place at the level of the bone.

The graft/periosteum interface at the bottom allowed for a faster reorganization. This area was rapidly invaded by inflammatory cells trapped in a fibrinous mesh. However, by 7 days attachment seemed to be restored at the level of the interface whether the graft was placed on periosteum or on bone. When on bone, a new periosteum seemed to develop early in healing. At 7 days young connective tissue fibers were seen parallel to the bone. Some Sharpey fibers were evident. The process of bone remodeling might be related to this new fiber attachment. In the longer time intervals observed, complete maturation of this tissue could be ascertained by specific connective tissue stains.

The epithelium underwent significant changes in both procedures. Degeneration and desquamation were evident. However, deep rete pegs seemed to survive. Keratinization disappeared early during the healing process. It reappeared only after 28 days, as detected by Rhodamine B stain. The degree of keratinization was similar, whether on bone or on periosteum.

Epithelial proliferation started earlier from the recipient site to restore epithelial continuity when grafts were on periosteum. In 5 days epithelial migration had already started and at 7 days a thin epithelium was already covering the graft. It should be remembered that the grafts were only 3 mm wide in the occlusal apical direction. When on bone, epithelial coverage was not restored until 14 days.

There was some difference in the response of bone to both procedures. When it was protected by periosteum, there was no discernible impact from the procedure. When it was exposed, superficial necrosis in isolated areas was evidenced by the presence of lacunae. Bone resorption was seen even 14 days after the procedure. However, no permanent damage was found.

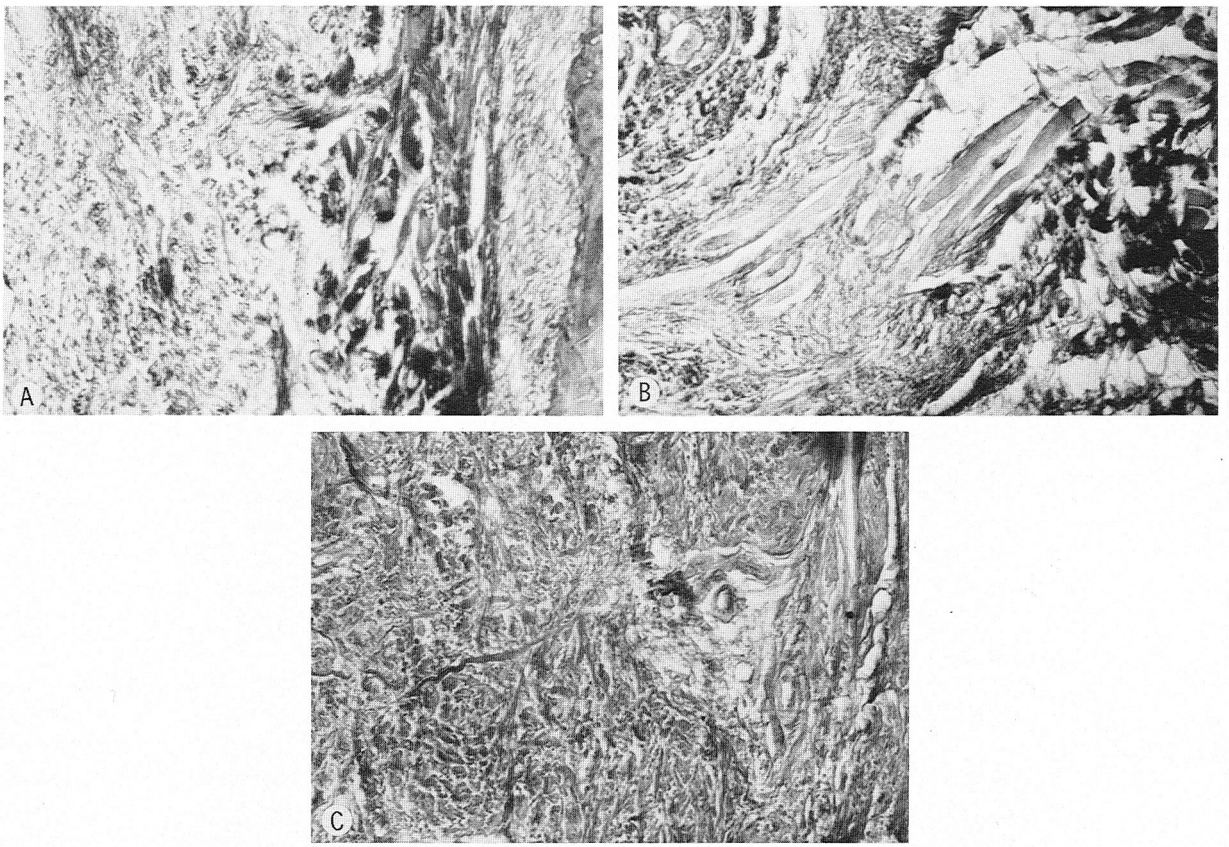

FIGURE 10. A. *Twenty-eight day specimen on periosteum. Elastic fibers are seen at the level of the interface (Weigert's elastica, original magnification, × 175). B. Elastic fibers in alveolar mucosa in an unoperated area (Weigert's elastica, original magnification, × 175.* C. *Twenty-eight day specimen on bone. No elastic fibers are detected (Weigert's elastica, original magnification, × 100).*

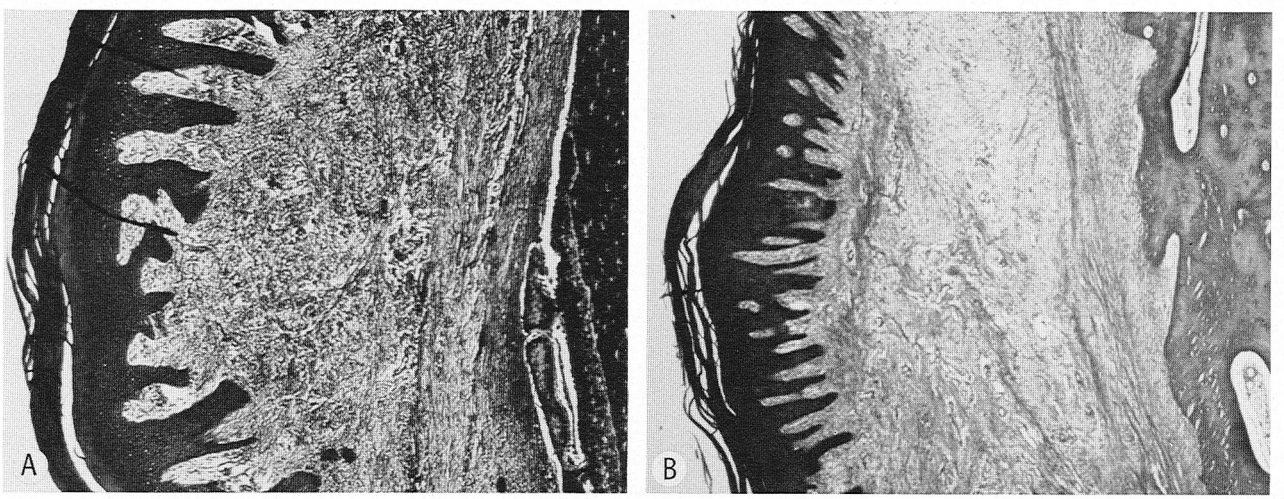

FIGURE 11. A. *Seventy-two day specimen on periosteum. Normal tissues have developed (Masson, original magnification, × 100).* B. *Seventy-two day specimen on bone. All tissues have regained normal characteristics (H & E, original magnification, × 100.)*

An interesting finding was the persistence of elastic fibers in the connective tissue of grafts placed on periosteum, while they were not found in grafts placed on bone. They may have been left there during preparation of the bed, since the bed was prepared apically to the mucogingival line. This could have been the case if a thick layer of connective tissue was left covering the periosteum. However, during preparation of the bed, care was taken to assure that the bed did not show any clinical mobility and that only a thin soft tissue layer remained exposed. Besides, elastica stain failed to show the presence of elastic fibers earlier than 28 days in the grafted areas. It may also be that elastic fibers differentiated again in this area during healing, or that they

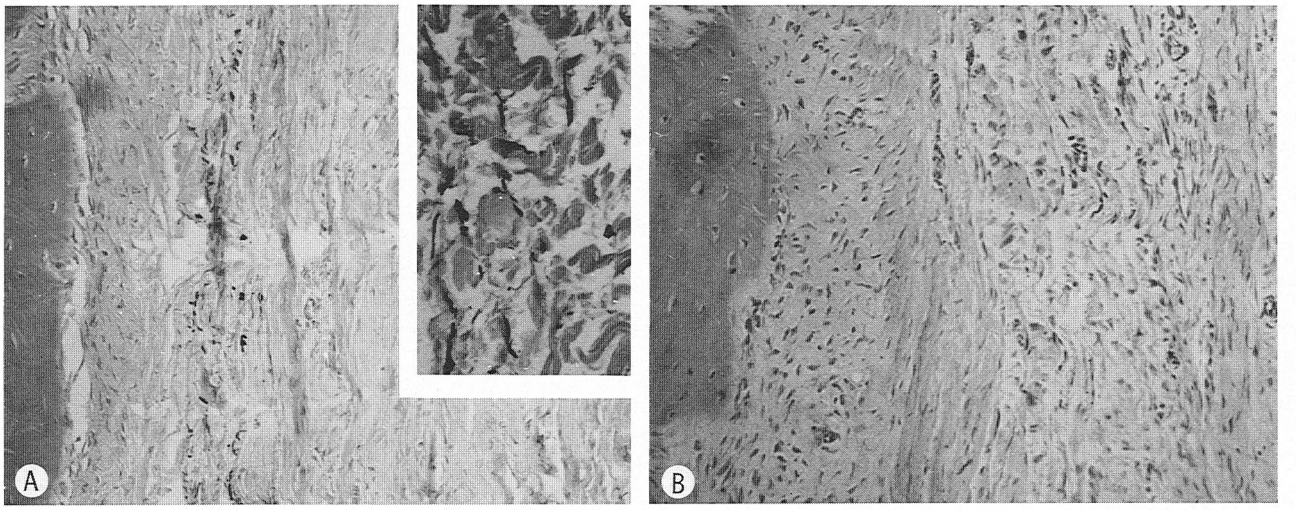

FIGURE 12. A. *Seventy-two day specimen on periosteum. Persistent elastic fibers are seen in the suprabony tissues (Weigert's elastica, original magnification, × 100; Insert, higher magnification, × 275).* B. *Seventy-two day specimen on bone. No elastic fibers are seen (Weigert's elastica, original magnification, × 100).*

"invaded" the tissues of the bed from the neighboring alveolar mucosa. Furthermore, this finding might be related to the degree of scar tissue formed as a result of the grafting procedure, which is more pronounced when it is done on denuded bone. This may explain the lack of elastic fibers in the area of the graft. At any rate, this finding correlates with the clinical results reported,[14] indicating that grafts on bone showed less mobility than those on periosteum. Mobility refers to lateral displacement of the graft and not to graft attachment.[15]

The findings of the present study showed that where grafts are used to increase the amount of attached gingiva, they do not need to be placed on periosteum to succeed. The predictability and the healing are very similar whether the grafts are placed on a bed with periosteum or on denuded bone.

These findings should not be extrapolated to the treatment of localized gingival recessions using free gingival grafts, and they do not conflict with previous reports.[18-20] The bone and the tooth are completely different in their behavior as a recipient site. A "bridging" effect need not occur under the experimental conditions tested in the present study.

Summary and Conclusions

The healing of free gingival grafts placed on periosteum or on denuded bone was evaluated histologically in five adult male Rhesus monkeys. Forty gingival grafts were performed, covering observation periods from 1 hour to 72 days.

When grafts were placed on bone there was an initial delay in healing. However, by 28 days the rate of healing for grafts placed on bone was similar to that for grafts on periosteum.

The periosteal bed seemed to favor a better initial adaptation at the level of the interface, which might have accounted for a better nourishment of the grafted tissue. As a consequence, grafts on bone showed initially more significant degenerative changes involving the superficial connective tissue, and a delay in the starting of the epithelial migration. The epithelial coverage was restored in 7 days when the grafts were on periosteum and in 14 days when they were placed on bone. Keratinization was found in both instances after 28 days.

The bone was undisturbed when the grafts were on periosteum. However, when grafts were placed on bone, the bone showed initially empty lacunae followed by bone resorption and repair.

Specific stain showed the persistence of elastic fibers in the connective tissue when the grafts were on periosteum. This was not observed when they were placed on bone.

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

1. The maintenance of the periosteum on the recipient site does not affect the success of a free gingival graft.
2. The initial healing is delayed when grafts are placed on bone.
3. After 28 days, there is no difference in the rate of healing whether a graft is placed on bone or on periosteum.
4. Grafts on bone produce superficial bone remodeling.
5. Elastic fibers may persist when grafts are placed on periosteum.

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Announcements

FEDERACION ODONTOLOGICA DE CENTRO AMERICA y PANAMA (FOCAP)

"The Federación Odontológica de Centro América y Panamá (FOCAP) will celebrate its XVII Congress from 21–25 January, 1980, in the City of Panama, Dr. Rodrigo Eisenmann, President. The main theme will be: Oral Diseases—Aethiology, Prevention and Treatment. Further inquiries should be addressed to Dr. Hernán de J. Ramos M., Apartado Postal 6677, Panamá 5, República de Panamá."

PANAMERICAN ASSOCIATION OF PERIODONTOLOGY

The Panamerican Association of Periodontology is sponsoring a Panamerican Congress from April 15–20, 1980 in San Juan, Puerto Rico. An interesting and timely program featuring a number of internationally known speakers will be presented.

For further information contact: Executive Committee, Panamerican Congress, GPO Box 70175, San Juan, Puerto Rico 00936.

FRENCH SOCIETY OF PERIODONTOLOGY

The French Society of Periodontology will hold its annual meeting in Monte Carlo (Monaco) from April 29th to May 1st 1980.

The scientific chairman is Dr. Jan LINDHE (Gothenburg), the meeting chairman is Dr. Maurice TREVOUX (Paris). During the 3 days, three subjects will be discussed: "Research and Prevention," with DRS. R. ATTSTROM, I. BAY, I. MANDEL and A. SHEIHAM; "New Concept in Periodontal Surgical Therapy," with: DRS. ABJEAN, AINAMO, BENQUE, ELLEGAARD, FISSORE, FONTENELL, KORBENDAU, LINDHE, PALLANCA and VALENTIN; "Gingival Behavior and its Relationships to Surgical Success and Failure," with DR. C. OCHSENBEIM (Dallas); "Interdisciplinary Approach to Periodontal Therapy with DR. H. CORN (Philadelphia).

The French Society of Periodontology presents following the 6th Annual Meeting in Monte Carlo (Monaco) a 2 day course, May 2nd and May 3rd 1980, with: DR. C. OCHSENBEIN (Dallas), on "Management of Periodontal Pockets Associated With the Maxillary Tuberosity and Mandibular Retro Molar Areas," and "Rationale and Technical Aspects of the Lingual Approach to Mandibular Osseous Surgery," and DR. H. CORN (Philadelphia) on: "Update Reconstructive Mucogingival Surgery."

For further informations, please contact: Société Française de Parodontologie, 57 rue d'Amsterdam 75008 Paris, France.