06.8516

J Oral Maxillofac Surg
43:359–364, 1985

Alveolar Wound Healing and Ridge Remodeling After Tooth Extraction in the Rat:

A Histologic, Radiographic, and Histometric Study

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Healing of extraction wounds in rats was analyzed by histologic, radiographic and histometric methods at 0, 7, 14, 30, 60 days after tooth removal. Total alveolar volume, volume density of bone, percentage of bone formation, bone resorption areas, and height of both vestibular and lingual crests were analyzed. Total alveolar bone volume and bone density in the apical third increased from 0 to 60 days. Maximum bone formation was observed at 14 days, whereas the greatest bone resorption was observed seven days after extraction. The height of the lingual crest was lowest 14 days postextraction and then increased progressively to day 60.

The process of healing after tooth extraction is of continuing interest for the oral and maxillofacial surgeon. Although radiographic and histologic studies of the reparative process have been described in some detail,[1–12] to our knowledge no histometric studies have yet been performed. The purposes of this investigation were to develop an atraumatic surgical technique for the complete extraction of rat molars (since root remnants delay and alter the healing process); to perform a histologic, radiographic, and histometric study of the extraction wound healing; and to study remodeling of the ridge by histometric methods.

Material and Methods

Fifty male Wistar rats weighing 80–90 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (0.025 g/kg body weight). Young rats about 30 days old were used, since they show little cementum apposition and thus allow complete tooth extraction.

The anesthetized animal was placed on its back on an operating table to immobilize the head; special lip and cheek retractors were used, and the tongue was retracted to one side of the mouth with a mosquito hemostat. Two enamel hatchets were used to perform the extractions: one with a cutting edge was used to detach the surrounding gingiva, and the other was used as an elevator for luxation of the molars (Fig. 1). Final removal of the molars was done with cotton pliers. The three left mandibular molars were removed in this manner. Postoperative bleeding was insignificant. After surgery no suturing was performed, and the animals were given neither a special diet nor antibiotics.

The rats were killed in five groups 0, 7, 14, 30, and 60 days postoperatively by ether inhalation. The mandibles were dissected, fixed in 10% formalin solution, and divided along the midsaggital plane. Each half mandible was radiographed by use of mammagraphic film, decalcified in ethylenediaminetetraacetate (*p*H 7.2) and embedded in paraffin. Sections were made at the level of the mesial socket of the first molar in a buccolingual orientation and were stained with hematoxylin and eosin.

Histometric measurements based on standard stereologic methods[13–15] were made by use of an image analyzing system (Kontron MOP AM 03). Tracings were made of projections of the sections, and zones of bone formation and bone resorption observed microscopically were then transferred to the corresponding zones of the tracings.

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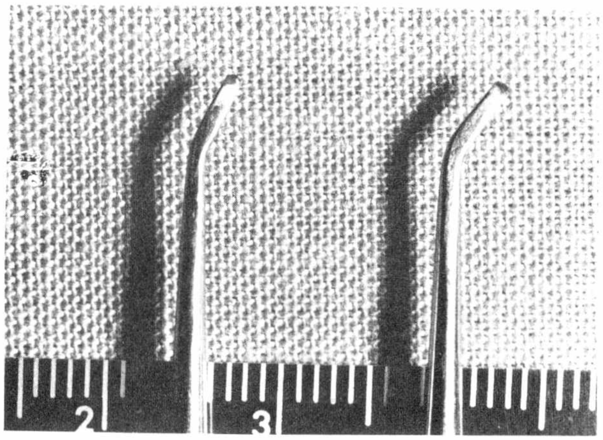


FIGURE 1. Enamel hatchets used to perform the extractions. *Left*, Sharp hatchet used for the syndesmotomy. *Right*, Hatchet used as a dental elevator.

The following histometric determinations were made: total alveolar volume (TAV), volume density of bone (VDB), percentage of bone formation (BF) and bone resorption areas (BR), and height of both vestibular and lingual crests. Total alveolar volume was considered as the bone tissue and its marrow spaces situated above line *a* drawn tangential to the upper cortical border of the mandibular canal (*A*) and perpendicular to the external surface of the buccal plate (Fig. 2). Volume density of bone was considered as the ratio between the trabecular area and bone area measured in rectangle ACFI, outlined on the apical third of the socket (Fig. 3). This rectangle and other lines of measurement were determined as follows:

A: upper cortical border of the mandibular canal.

C: the point on line *a* equidistant from A and B (intersection of line *a* and the external buccal plate).

F: the point one third of the distance from C to the top of the vestibular crest (D).

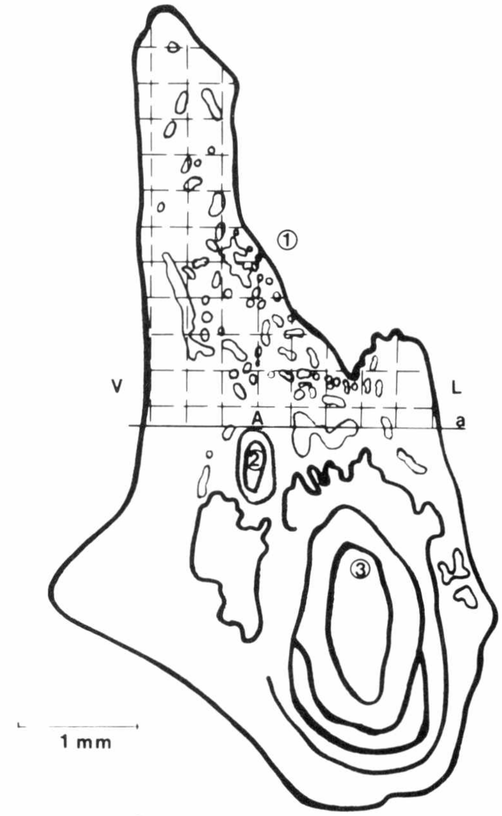
E: two thirds the same distance.

G: the top point of the lingual crest.

H: the midpoint of the distance DG (HC coincides with the middle of the socket).

I: the point of intersection of a line perpendicular to segment CD, drawn from F to the line HC.

The percentage of bone formation and bone resorption was measured both on the bony surface of the marrow spaces and on the bony surface of the alveolar ridge situated above line *a*. Areas covered by osteoid tissue and hypertrophic osteoblasts were considered as bone formation areas, while erosive zones were considered as bone resorption areas. The height of vestibular crests was represented by the segment CD (Fig. 3). Lingual crest height was represented by a perpendicular line drawn to line *a* from G, the segment GJ (Fig. 3).



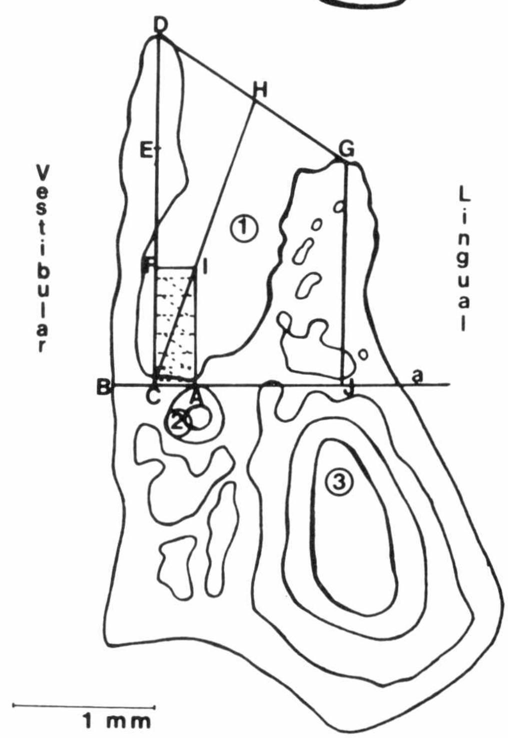


FIGURE 2 (*above*). Alveolus 14 days after surgery. The dotted zone represents the area measured for the total alveolar volume above line *a*. V, vestibular plate; L, lingual plate; 1, alveolus; 2, mandibular canal; 3, growing incisor.

FIGURE 3 (*below*). Alveolus immediately after surgery. Points ACFI form the rectangle used for the measurement of the volume density of bone. V, vestibular plate; L, lingual plate; 1, alveolus; 2, mandibular canal; 3, growing incisor.

FIGURE 4. Microscopic section of mesial first molar socket immediately after surgery. Notice the socket filled with blood coagulum. (Hematoxylin and eosin, ×40.)

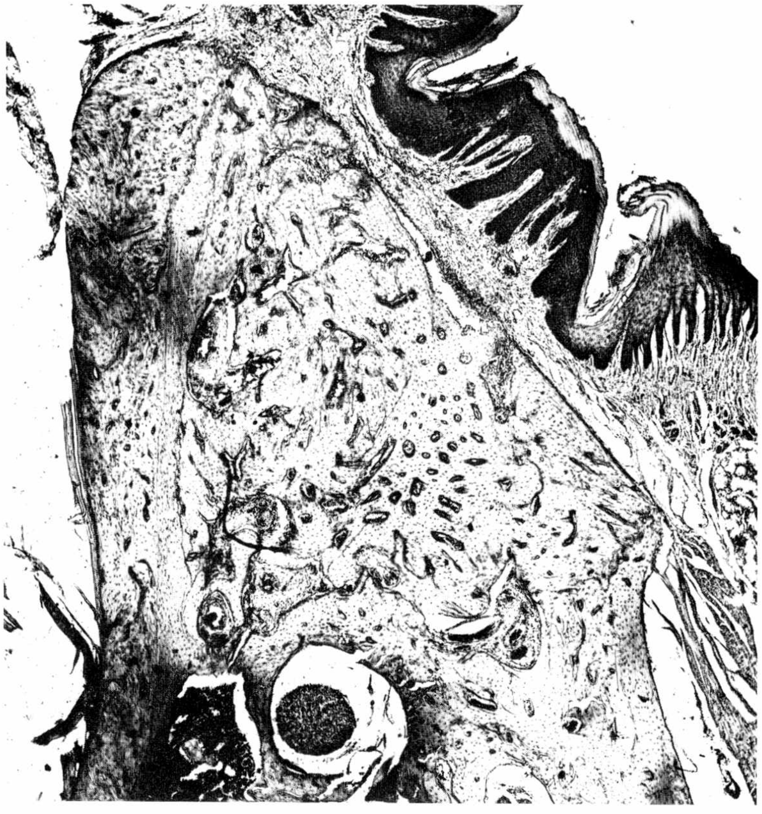
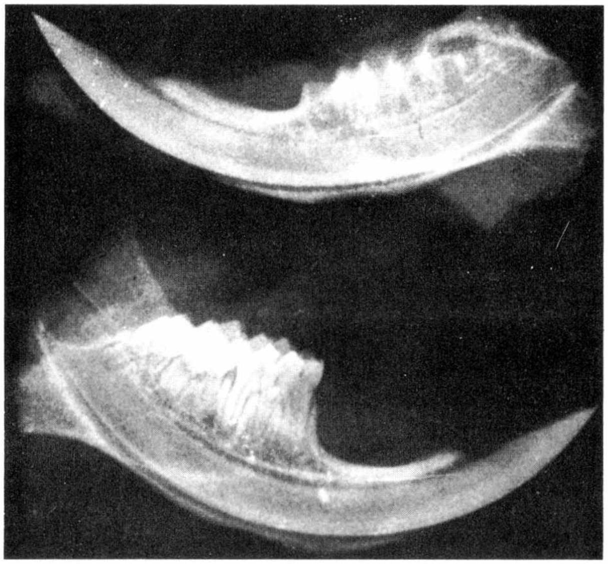


FIGURE 5. Microscopic section of mesial first molar socket 60 days after surgery. Notice the socket filled with laminar bone tissue. (Hematoxylin and eosin, ×32.)



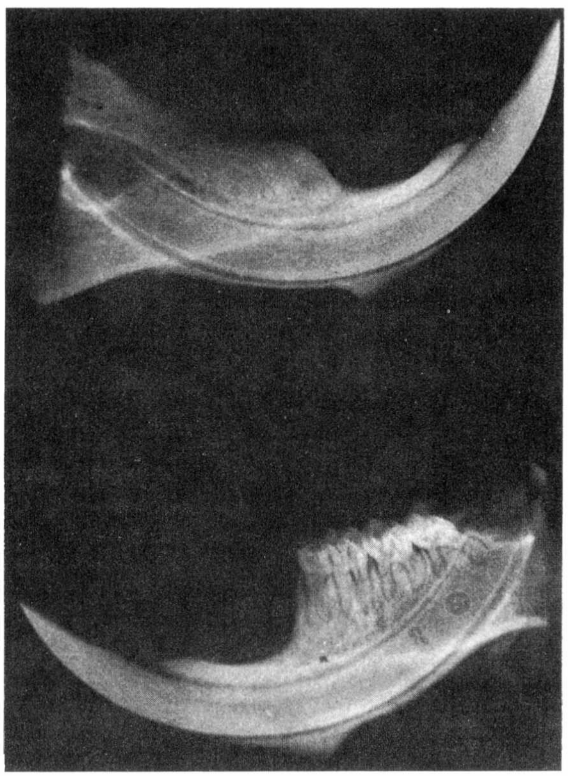


FIGURE 6 (*above*). Radiographic image immediately after surgery. *Upper*, Edentate ridge. Notice radiopaque line defining the sockets. *Lower*, Dentate ridge.

FIGURE 7 (*below*). Radiographic image 60 days after surgery. *Upper*, Edentate ridge. Notice that it is occupied by radiopaque tissue having the same characteristics as that of the adjacent alveolar bone. *Lower*, Dentate ridge.

Results

Histologic Findings

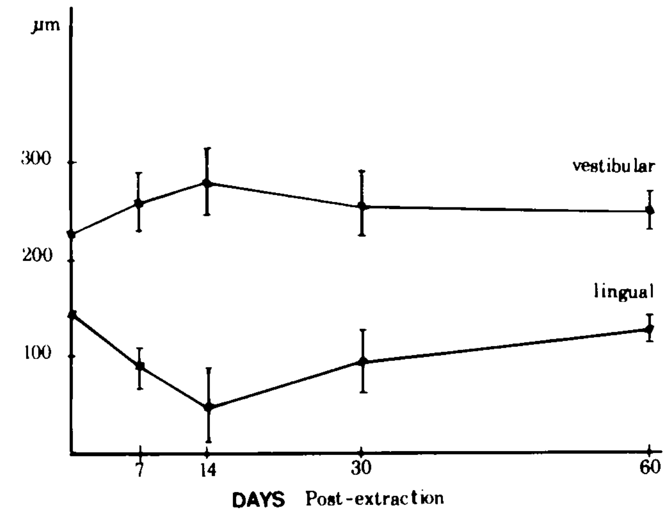
Sections removed from the socket immediately postoperatively showed it to be filled with blood coagulum and debris of the periodontal membrane attached to the bone (Fig. 4). At seven days, delicate trabeculae of bone were observed in the fundus of the socket and in the apical third of the internal surface of the vestibular and lingual walls. The top of the vestibular and lingual crests showed signs of resorption, and so did both internal surfaces. The rest of the socket was occupied by connective tissue, and the wound surface was incompletely covered by new epithelium. On the 14th day, in most specimens, the socket was almost totally occupied by reticular bone. The trabecular surfaces showed intense bone activity. The wound surface was completely covered by new epithelium. At 30 days young bone filled the socket. Active osteoblasts were seen mainly on the alveolar ridge. The epithelium over the socket was well keratinized. At 60 days the entire socket was filled with laminar bone tissue. The structure of the socket area showed an almost homogeneous continuity with the adjacent area of the mandible (Fig. 5).

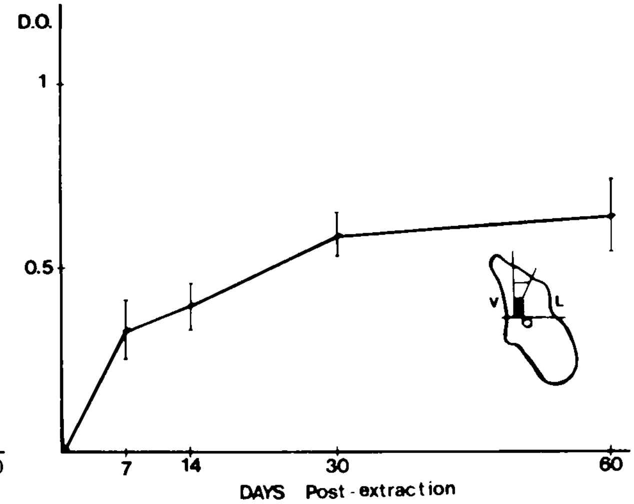
Radiographic Observations

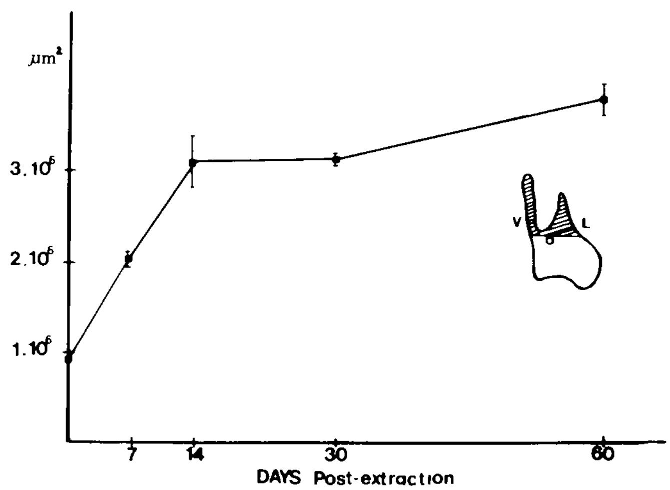
Immediately after surgery the lamina dura was seen as a radiopaque line defining the sockets, which appeared uniformly radiolucent (Fig. 6). At seven days the alveolar cortex appeared less distinct and the fundus of the sockets showed a radiopaque image. On the 14th day the sockets were occupied almost completely by radiopaque tissue. At 30 days the edentulous areas were replaced by radiopaque tissue, and it was not possible to distinguish the socket walls. At 60 days all the edentulous areas were occupied by radiopaque tissue having the same characteristics as those of the adjacent alveolar bone (Fig. 7).

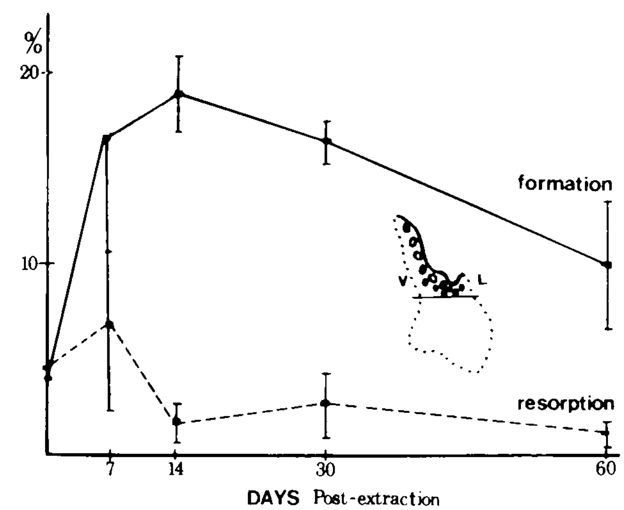
Histometric Determinations

Results obtained immediately postoperatively were used as starting point for comparative analysis with the other four groups, since there was no bone response. The TAV was 9×10^5 μm^2, and the VDB in the apical third was zero, since there was no bone formation. The percentage of BF and BR were 4% and 4.4%, respectively. At seven days an increase in the TAV (20×10^5 μm^2) and VDB in the apical third (0.33) was observed. An increment in the percentage of BF (17%) and BR (6%) was also observed. On the 14th day an increment was observed in all parameters considered, except in the percentage of BR (1.8%): TAV was 31×10^5 μm^2, VDB in the apical third (0.40), and percentage of BF was 19%. At 30 days TAV (32×10^5 μm^2) and the VDB in the apical third (0.58) were greater. The percentage of BF (16%) was lower than at 14 days, and









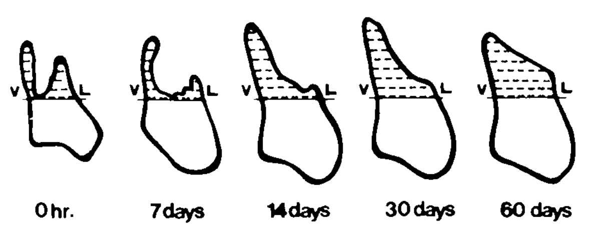


FIGURE 8 (*left, top*). Mean height of vestibular and lingual crests from 0 to 60 days.

FIGURE 9 (*left, center*). Mean total alveolar volume from 0 to 60 days.

FIGURE 10 (*left, bottom*). Ridge remodeling after tooth extraction. The dotted zones represent the total bone volume variations from 0 to 60 days.

FIGURE 11 (*right, top*). Mean volume density of bone in the apical third from 0 to 60 days.

FIGURE 12 (*right, center*). Percentage of bone formation and bone resorption from 0 to 60 days.

the percentage of BR (2.7%) was higher than that at 14 days. At 60 days the TAV (38×10^5 μm^2) and VDB (0.64) increased. However, the percentage of BF (10%) and BR (1%) were lower than at 30 days.

Variations of both buccal and lingual crest height at the different postoperative intervals from zero to 60 days are shown in Figure 8. The lowest height of lingual crest was observed 14 days postoperatively, increasing progressively to the 60th day.

Histometric determinations showed that TAV increased from 0 to 60 days (Fig. 9). Figure 10 summarizes total bone volume variations and the ridge remodeling. The VDB in the apical third of the socket also increased progressively with time (Fig. 11). Maximum values for bone resorption areas and bone formation areas were observed at seven and 14 days, respectively (Fig. 12).

Discussion

The study, by radiographic, histologic, and histometric techniques, of normal healing of extraction wounds in rats was made possible by the development of an atraumatic surgical technique, which avoids producing retained root remnants and bone fragments that alter and delay the healing process.[4,10,11] The uniformity of our surgical results was demonstrated in the histometric measurements.

The determination of the rectangle ACFI in the apical third of the socket was useful, since this area is occupied only by newly formed bone. This histometric analysis, together with the surgical technique for the study of alveolar wound healing, permitted us to accurately assess bone activity at each stage considered. Future investigations may now evaluate modifications in bone activity by comparison with the histometric parameters established in the present study.

The maximum percentage of bone resorption activity seven days postoperatively reflects active remodeling of the alveolar crests, principally in the internal and the top surfaces of the buccal crest and in the top of the lingual crest. At 14 days postoperatively the lingual crest reaches its minimum height, the socket being completely filled with new bone, and maximum bone formation occurring.

The remodeling process at the alveolar crest has been described by Pietrokovsky,[9] who reported that two weeks after tooth extraction both the buccal and the lingual alveolar bony crests had resorbed and disappeared: "the mylohyoid and the buccinator muscle attachments divided the bony socket wall into two well-demarcated regions: one, that resorbs after tooth extraction, and another that remains and forms the residual bony ridge." In our histometric study no significant difference was found in the height of the alveolar crests between 0 and 60 day groups, in spite of the modifications in the height of the alveolar crests during the intermediate stages.

This control study of normal alveolar wound healing may provide the basis for future comparative studies under experimental conditions.

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