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Alveolar Wound Healing After X-Irradiation:

A Histologic, Radiographic, and Histometric Study

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Healing of extraction wounds in rats following cephalic irradiation was studied by histologic, radiographic, and histometric methods 14 days after tooth extraction. Irradiation was given at 0, 3, and 7 days after surgery in doses of either 15, 20, or 30 Gy. No significant differences were seen with the different doses given seven days post-extraction. However, socket healing was delayed when irradiation was given immediately and three days after extraction. On the basis of these observations, it is recommended that radiation not begin until at least one week after the extraction of teeth.

There is general agreement that teeth with doubtful prognoses, either due to caries or periodontal disease, should be removed prior to irradiation.[1–4] However, the interval between extraction and the onset of radiotherapy is a subject of controversy. A waiting period of one to two weeks has been adopted arbitrarily by many therapists.[4,5] To our knowledge no histometric studies have been reported concerning the best time for the administration of radiation therapy following dental extractions. The present study was carried out to determine the bone tissue response during the alveolar socket wound healing after different doses of x-irradiation applied at varying intervals after tooth extraction. The evaluation was performed by radiographic, histologic, and histometric methods.

Material and Methods

One hundred male Wistar rats weighing 80–90 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (0.025 g/kg body weight), and the three left mandibular molars were removed by use of an atraumatic technique.[6] No suturing was performed, and the animals were given neither special diet nor antibiotics. Ten rats had surgery only and served as controls (group I). The remaining rats were divided into three groups of 30 animals each according to the time lapse between the extractions and radiation: immediately after surgery (group II); three days after surgery (group III); and seven days after surgery (group IV). Groups II, III, and IV were subdivided into three subgroups (A, B, and C) of 10 animals each according to the dose of radiation administered: 15 Gy (A); 20 Gy (B), and 30 Gy (C). Cephalic radiation consisted of a single dose with the rest of the body shielded by a 3-mm sheet of lead. The radiotherapy apparatus (Philips) was operated at 250 kV(p) and 14 mA with a 25-cm target–skin distance. There was 0.25-mm copper and 1-mm aluminium filtration, and a dose rate of 3.30 Gy/min. The irradiation dose was calculated from a phantom model with a P.T.W. dosimeter.

Rats were killed 14 days post-extraction by ether inhalation. The left hemimandibles were dissected, fixed in 10% formalin, radiographed with mammographic film, decalcified in EDTA (pH 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 stained with hematoxylin and eosin.

Histometric measurements based on standard stereologic methods[6–10] were made by use of an image analyzing system (Kontron MOP-AM 03). Tracings were made of projections of the sections,

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and zones of bone formation and bone resorption observed microscopically were then transferred to the corresponding zones on the tracings. The following histometric determinations were made: total alveolar volume (TAV) considered as the bone tissue and his marrow spaces measured on the tracings in mm^2; volume density of bone (VDB) considered as the ratio between the trabecular area and bone area; percentage of bone formation (BF), and percentage of bone resorption (BR) areas. TAV, BF, and BR were measured on the area situated above a line tangential to the upper border to the mandibular canal; VDB was measured in a rectangle outlined on the apical third of the socket as previously described.[6]

Results

All the animals in group I survived to the end of the experiment. The animals that received 15 Gy also survived except for the three animals of group II, which died on the 8th day post-irradiation. All the animals of groups II and III that received 20 and 30 Gy died before the end of the experiment. All the animals in group IV survived at the end of the experiment.

Radiographic Observations

At 14 days the sockets in animals of group I were occupied almost completely by radiopaque tissue (Fig. 1, *top*). Group IIA showed distinct radiolucency in some portions of the alveolus while the fundus of the sockets had slightly radiopaque appearance (Fig. 1, *middle*). In group III A the alveolar cortex had disappeared completely, but the apical portion of the alveolus showed radiopaque tissue. In animals of group IV A the alveolar cortex could not be distinguished, the apical portion of the alveolus was notably radiopaque and the alveolar ridge showed an irregular surface (Fig. 1, *bottom*). Similar changes were observed in groups IV B and C.

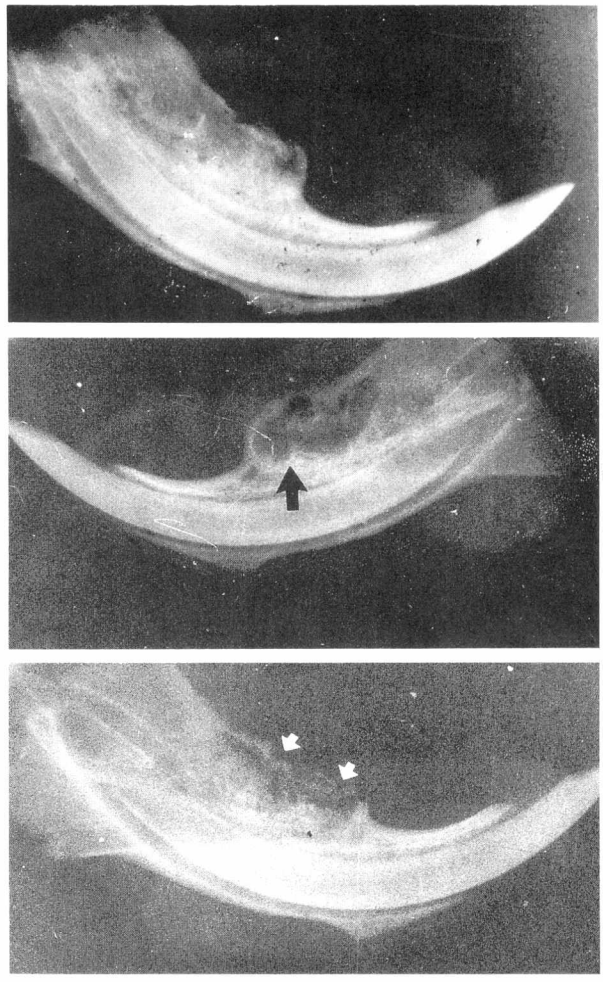


FIGURE 1. *Top*, radiograph taken 14 days after surgery. Notice that the alveolar ridge is occupied by radiopaque tissue. *Middle*, radiograph taken 14 days after surgery in an animal irradiated with 15 Gy immediately after surgery. Notice that the alveolar cortex appears distinct in some portions and the fundus of the sockets has a slightly radiopaque image (*arrow*). *Bottom*, radiograph taken 14 days after surgery in an animal irradiated with 15 Gy seven days after surgery. Notice that the apical portion of the alveolus is notably radiopaque. An irregular surface is seen on the ridge (*arrows*).

Histologic Findings

The sockets of animals of group I were almost totally occupied by thick woven trabeculae lined by cuboidal osteoblasts. Erosive zones were observed at the top of the lingual crest. Epithelium completely covered the socket (Fig. 2, *top left*). In animals of group II A a few bony trabeculae were seen only in the fundus of the sockets. The socket wall showed signs of bone resorption on the top of the vestibular and lingual crests as well as on both internal surfaces (Fig. 2, *top right*). Delicate bone trabeculae were observed in the apical portion of the sockets in the animals of Group III A and IV A, B, and C; it was most marked in group IV A (Fig. 2, *bottom*). In all cases, the rest of the alveolus was occupied by connective tissue. Epithelialization was incomplete in all the experimental groups.

Histometric Determinations

The histometric findings are summarized in Table 1. The TAV was lower in all the experimental animals as compared to the control group (Fig. 3). In rats of groups II and III a remarkable decrease in TAV was seen (Fig. 4). The percentage of BF was

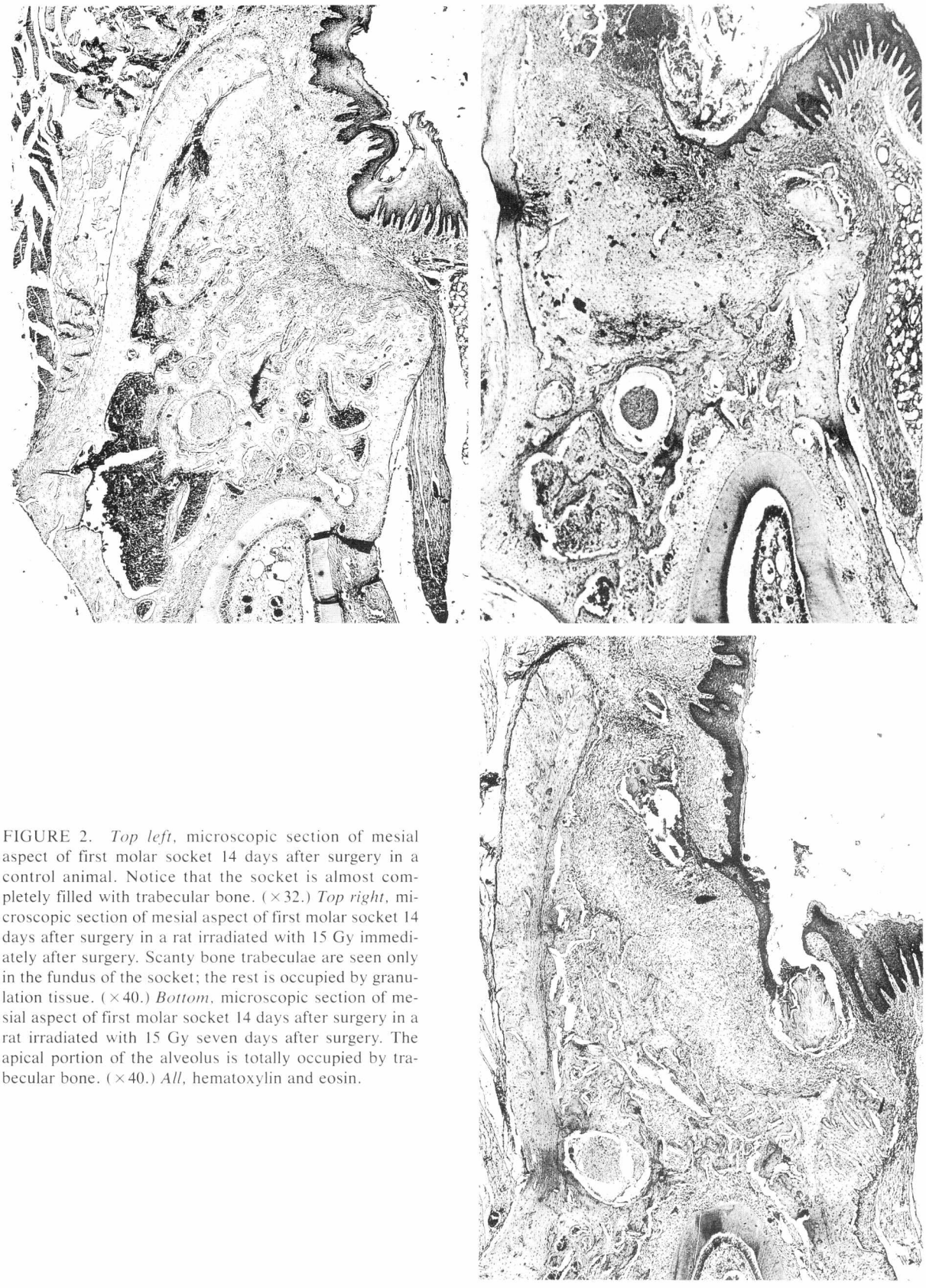
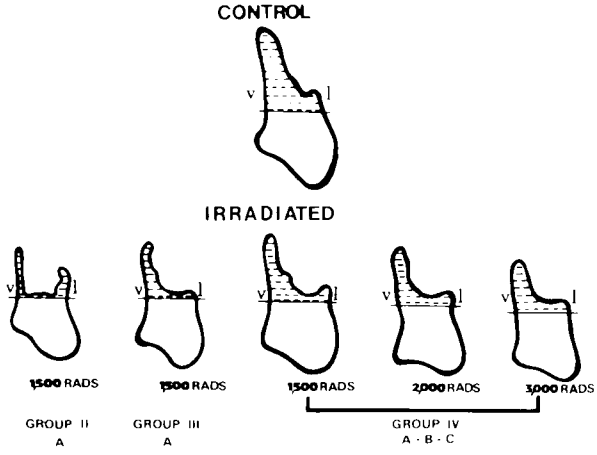


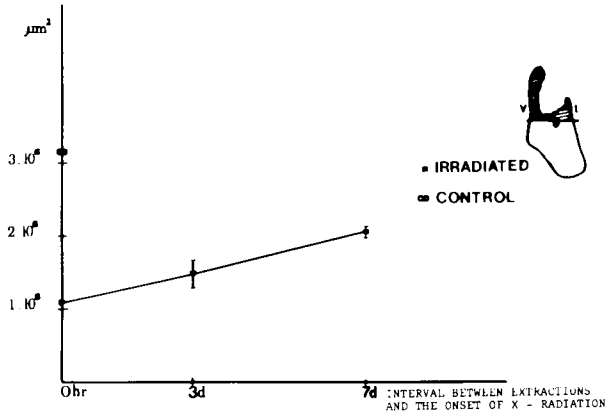
FIGURE 2. *Top left*, microscopic section of mesial aspect of first molar socket 14 days after surgery in a control animal. Notice that the socket is almost completely filled with trabecular bone. (×32.) *Top right*, microscopic section of mesial aspect of first molar socket 14 days after surgery in a rat irradiated with 15 Gy immediately after surgery. Scanty bone trabeculae are seen only in the fundus of the socket; the rest is occupied by granulation tissue. (×40.) *Bottom*, microscopic section of mesial aspect of first molar socket 14 days after surgery in a rat irradiated with 15 Gy seven days after surgery. The apical portion of the alveolus is totally occupied by trabecular bone. (×40.) *All*, hematoxylin and eosin.

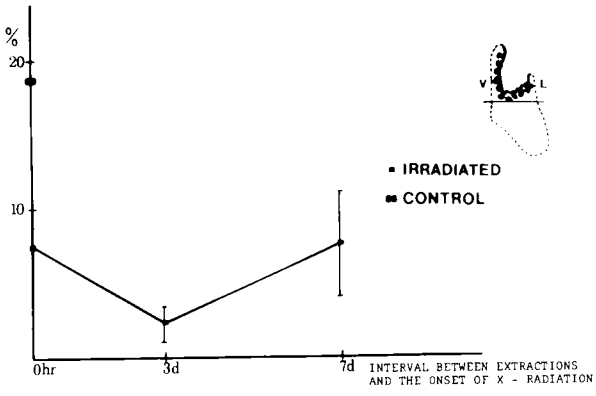
Table 1. Histometric Determinations

	X-Radiation Dose (grays)	TAV	VDB	BF	BR
Group I		$31.8 \times 10^5 \pm 3 \times 10^5$	0.40 ± 0.06	19.00 ± 0.3	1.81 ± 1.05
Group II	15	$11.6 \times 10^5 \pm 0.7 \times 10^5$	0.11 ± 0.09	7.47 ± 0.2	3.07 ± 2.30
Group III	15	$15.4 \times 10^5 \pm 1.8 \times 10^5$	0.28 ± 0.11	2.35 ± 1.2	2.55 ± 1.22
Group IV	15	$20.1 \times 10^5 \pm 0.5 \times 10^5$	0.38 ± 0.16	7.52 ± 3.0	1.67 ± 1.42
Group IV	20	$19.5 \times 10^5 \pm 0.5 \times 10^5$	0.34 ± 0.10	1.81 ± 1.0	1.19 ± 0.87
Group IV	30	$19.5 \times 10^5 \pm 0.6 \times 10^5$	0.25 ± 0.08	1.57 ± 0.6	2.64 ± 1.48

± SD
TAV: total alveolar volume: in mm^2 measured on tracings.
VDB: volume density of bone: ratio between trabecular area and bone area.
BF: percentage of bone formation areas.
BR: percentage of bone resorption areas.







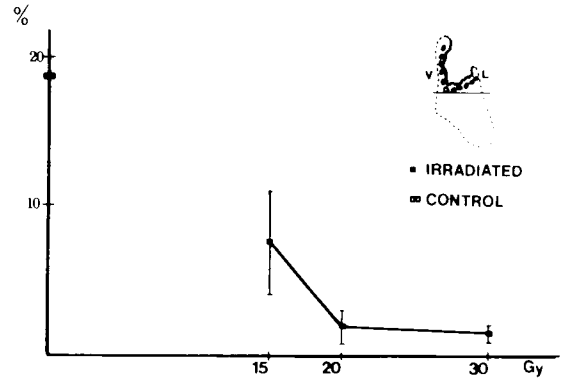


FIGURE 3 *(top left).* Schematic representation of the alveolus 14 days after surgery in a buccolingual orientation. The dotted zones represent total bone volume.

FIGURE 4 *(top right).* Mean values of total alveolar bone volume 14 days post-surgery in animals irradiated with 15 Gy in different stages of alveolar wound healing.

FIGURE 5 *(bottom left).* Percentage of bone formation 14 days after surgery in animals irradiated with 15 Gy at different stages of alveolar wound healing.

FIGURE 6 *(bottom right).* Percentage of bone formation 14 days post-surgery in animals irradiated with different doses of x-irradiation at the same stage (seven days) of alveolar wound healing.

diminished in all experimental animals when compared with the control group. The difference was more evident in those animals irradiated on the third day after surgery (Fig. 5), the stage at which the alveolus is occupied by granulation tissue. Moreover, the percentage of BF decreased with the dose of radiation (Fig. 6).

Discussion

This study confirms that a marked delay in socket healing is provoked by x-irradiation as reported by other authors.[11-13] However, there are definite differences when the healing stage at which irradiation is given is considered. A previous study[6] using histometric methods showed that under normal conditions maximum bone formation occurs on the fourteenth day after tooth extraction. Thus, animals were sacrificed 14 days postextraction in this study. Furthermore, using a technique that resulted in complete tooth extraction[6] enabled us to study the effect of x-irradiation on the healing process without interference. It is important to emphasize this point because a frequent complicating factor is the presence of root fragments, which delay the healing process.[12-18]

The delay in socket healing was most remarkable when the rats were irradiated immediately or on the third day after surgery. Quantitative studies on wound healing of rat skin have shown that the vascular response diminished when x-irradiation was applied after three days of healing, increasing on the fourteenth day.[20] The healing of wounds requires a sufficient amount of blood vessel development.[20] A reduction is associated with a decrease in alkaline phosphatase activity that may possibly be related to fibroblastic depression.[21] This could produce a decrease in osteoblastic differentiation, which could explain the reduced percentage of bone formation that was found. Moreover, histometric measurements demonstrated an increase in bone resorption in irradiated animals, corroborating the histologic descriptions made by others.[13,22,23]

Analysis of the histometric parameters showed that only those animals receiving 30 Gy or x-irradiation had an increase in the percentage of bone resorption. The TAV was lower in these animals than in controls, but no variations were seen among them. Our results show that a given dose of x-irradiation causes the most noticeable delay in alveolar wound healing when it is delivered shortly after surgery, possibly because at this time the mechanism of bone formation has not yet been initiated.

An interval of one to two weeks between tooth extraction and radiation therapy has been widely recommended.[3-5,11-13] Some of these authors based their recommendation on the fact that epithelial closure would occur in this interval, which to the best of our knowledge is a clinical, subjective parameter. Based on this study, radiation therapy should be initiated at the time the apical third of the socket starts to fill with bone. This was probably at about seven days in the experimental animals. Although it is possible to detect such early bone formation radiographically in the excised rat mandible, it may not be possible to do so in the human jaw. However, it can be assumed that a similar period would probably apply.

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