

# **<sup>99m</sup>Tc-Diphosphonate Bone Imaging and Uptake In Healing Rat Extraction Sockets**

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***Clinically positive bone scans of the jaws may result from a variety of benign dental conditions. An experimental system for studying radionuclide imaging and uptake in the jaws of rats was developed. Sequential <sup>99m</sup>Tc-diphosphonate bone scans and radionuclide uptake determinations were performed on rats after standardized extractions of their mandibular left first molars. Positive bone scans were seen 4–16 days after molar extraction, and increased radionuclide uptake was found in the healing extraction wounds 4–42 days after the extraction. Conventional radiography and histology fail to show unusual bony architecture in extraction sockets at such times. These results correlate with clinical findings in patients and suggest that human beings may have positive bone scans for several months after dental extraction.***

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Due to the sensitivity but nonspecificity with which bone-imaging agents concentrate in areas of altered bone metabolism (1–5), it is essential to recognize and identify benign conditions that may cause positive bone scans. Such conditions include postoperative healing, fractures, arthritis, osteomyelitis, Paget's disease, fibrous dysplasia, and osteoid osteoma (6–8). A variety of benign dental conditions (including healing extraction wounds, dental abscesses, periodontal abscesses, pericoronitis, and osteomyelitis) have been also shown to produce positive bone scans (7,9,10). Figure 1 shows a representative example of a positive bone scan resulting from a benign dental condition. This patient, who was suspected of having recurrent Hodgkin's disease, had his right mandibular first molar extracted 2 months before scanning. Osseous repair in the extraction socket was shown to have caused the increased uptake appearing in the bone scan.

These observations suggested that an experimental system for the systematic study of radionuclide uptake and imaging in the jaws could provide data useful for interpreting bone scans. Since increased radionuclide uptake has been found in experimental animals with benign dental disease (11–13), the

present study sought to develop an animal model for the controlled study of <sup>99m</sup>Tc-diphosphonate uptake and bone imaging during osseous repair and healing in the jaws.

## METHODS

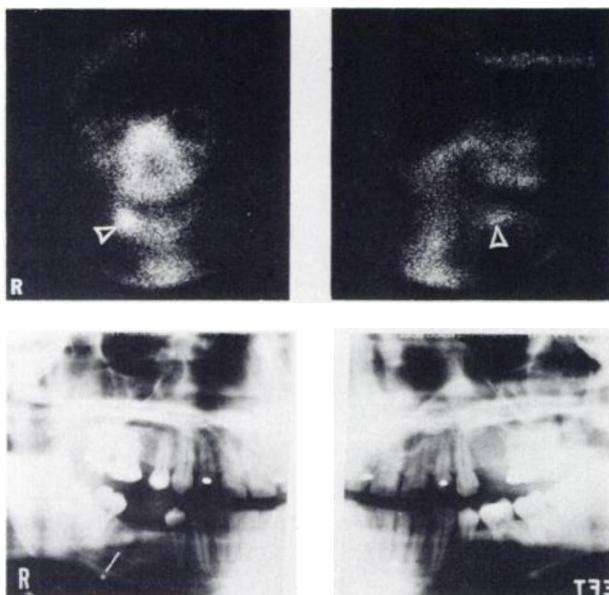
Sixty-three male Sprague-Dawley rats were used. The rats were maintained two to a cage in a temperature-controlled room with a 12-hr alternating light-dark cycle. At the start of the study, the rats were all 14 weeks old and weighed an average of 343 gm (s.d. 8.2 gm).

With the rats under sodium pentobarbital anesthesia (intraperitoneal Nembutal, 4.5 mg/100 gm body weight), the mandibular left first molar of each rat was extracted using the elevator and forceps technique, and the bone wounds were standardized by removing the root tips and inter-radicular septa and

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**FIG. 1.** (Top) Anterior and right lateral  $^{99m}\text{Tc}$ -diphosphonate images taken with scintillation camera. (Bottom) Panoramic radiograph of same patient. Asterisk on left radiograph indicates 2-month-old healing wound from extraction of mandibular right first molar; arrows on scans indicate corresponding area of increased radiolucency uptake in posterior right mandible.

enlarging the socket with a No. 2 round burr driven by a portable dental engine. The resulting wound was a spherical defect, approximately 2–3 mm in diameter, at the alveolar crest. Groups of 3–5 rats received  $^{99m}\text{Tc}$ -diphosphonate bone scans at the following times after extraction: 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 26, and 34 days.

The  $^{99m}\text{Tc}$ -diphosphonate solution was made with technetium from a standard  $^{99}\text{Mo}$ - $^{99m}\text{Tc}$  generator (Minitec, Squibb, Princeton, N.J.) and a diphosphonate bone-imaging kit (Diagnostic Isotopes, Upper Saddle River, N.J.). With rats under ether anesthesia, 0.3–0.5 ml of the tracer was injected into the exposed saphenous vein using a 1-cm<sup>3</sup> tuberculin syringe and a 27-gauge needle. The mean dose for all rats was 0.65 mCi, with a range of 0.23–0.97 mCi. The injected dose was determined by inserting the syringe into a Radx dose calibrator before and after the injection and calculating the difference. Three hours after injecting the tracer, the rats were killed and scanned. External counting and imaging were done with a Pho/Gamma IV camera system (Searle Radiographics, Des Plaines, Ill.) and a microdot film recorder with RP50 mammography film. A pinhole collimator provided the sensitivity and resolution needed for the small subjects under study. Scintigrams containing 40,000 counts were taken in the left lateral and ventral positions.

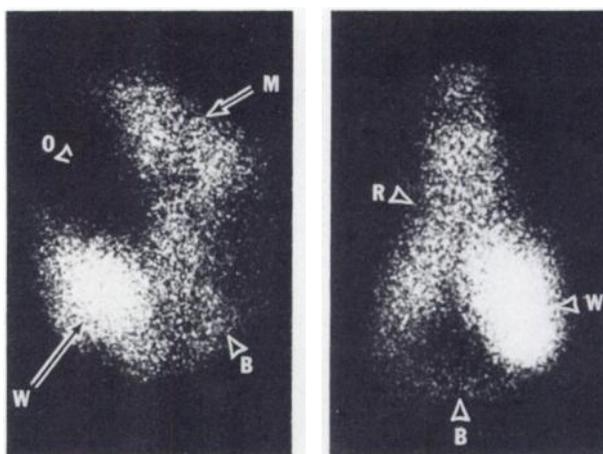
Immediately after scanning, the left hemimandible

was excised. The extraction wound area was cleaned and, using a separating disk in a slow-speed dental engine, a 1–2-mm-wide coronal section of mandible containing the wound was removed. This specimen was then sectioned transversely, immediately superior to the inferior alveolar canal, into an upper and a lower piece. The upper piece contained the wound (W), and the lower piece was used as control (C). The  $^{99m}\text{Tc}$  activity in each bone sample was then determined using a Searle Radiographics Model-8725 NaI(Tl)-crystal well counter. Counts were also taken of two 1:10,000 dilutions of the tracer used that day, and the injected dose was determined by averaging them and subtracting the background.

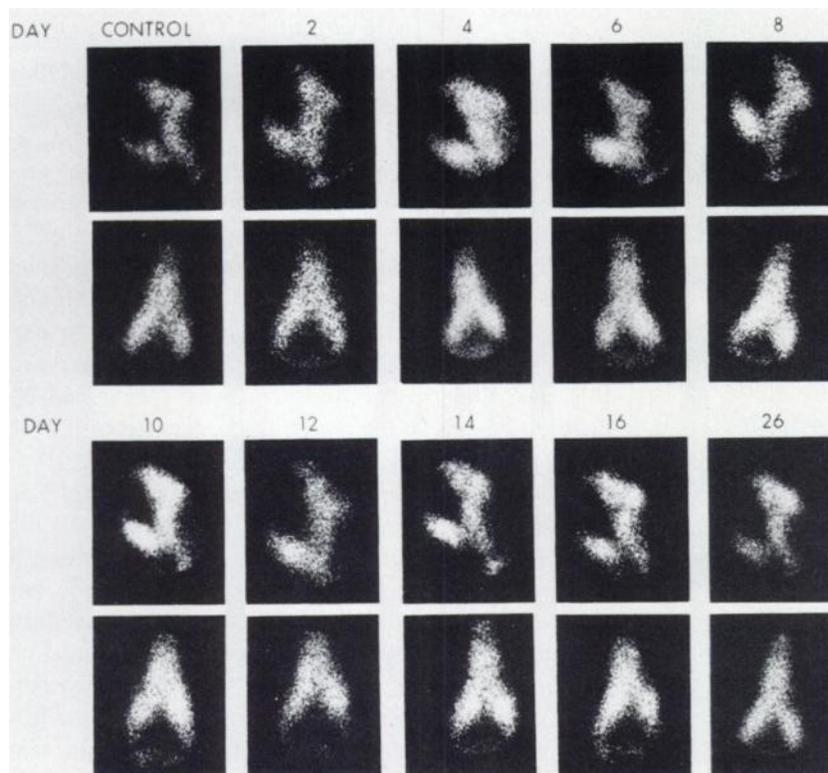
All bone samples were then dehydrated at 110°C for 24 hr and weighed to the nearest 0.01 mg. The average weights were 14.06 mg (W) and 17.10 mg (C). The activity of each sample was then expressed as a percentage of 1/10 of the injected activity per gram of dry bone. Mean uptake values and standard deviations were determined for each experimental and control group, and Student's t-test was used to determine the statistical significances between the following group means: wound and control values at each time interval; wound values at 8 days and all other wound values; wound values at 16 days and all other wound values; and control values at 16 days and all other control values.

## RESULTS

With the pinhole collimator placed 0.5 in. from the rat skull, scintigrams were obtained in both the left lateral and base positions that clearly showed the bones of the skull and the area of the wound (Fig. 2). Healing extraction wounds were also visi-



**FIG. 2.** Left lateral view (left) and base view (right) from  $^{99m}\text{Tc}$ -diphosphonate scintigram of rat skull 12 days after extraction of mandibular left first molar: (M) maxilla; (B) area of junction between cervical vertebra and base of skull; (O) entrance to oral cavity; (R) right mandible; (W) extraction wound in left mandible. This animal had localized osteomyelitis around extraction wound.



**FIG. 3.** Representative series of  $^{99m}\text{Tc}$ -diphosphonate scintigrams of rat skulls from before extraction through 24 days after extraction of mandibular left first molars. Left lateral views are on top, with base views of same rat below. Anatomic orientation and landmarks are same as in Fig. 2.

ble with a parallel-hole collimator, but anatomic structures were then much less well defined. Representative bone scans of rat heads, performed at regular intervals after molar extraction, are shown in Fig. 3. Visual evaluation of the scans revealed little, if any, increased activity in the wound area until 4 days after extraction. Increasingly greater activity was then observed in the extraction area. A first peak of activity was seen at 8 days after extraction, and it remained at approximately the same intensity at days 10 and 12. Intensity then increased again, reaching a maximum at 16 days after extraction, after which the activity decreased. Three animals showed more intense activity at the wound site than the others. At necropsy, a dental abscess was found around a retained root tip in one rat, and osteomyelitis of the operated hemimandible was found in the other two rats (Fig. 2).

The results of the well counting of technetium in the prepared mandible sections are shown in Table 1. During the first 3 days after extraction, there were no significant changes in radionuclide uptake in the wound site. Uptake of radioactivity in the wound area increased steadily from 4 to 8 days after extraction and then remained at relatively the same uptake level until day 14, at which time uptake again increased. Maximal concentrations of radioactivity in the wound area were observed from 14 to 18 days after extraction. Although concentrations decreased

through the remainder of the experiment, they remained significantly greater than those of the corresponding controls. Mean values during days 8–14 were significantly higher ( $p < 0.01$ ) than those of days 0–6 and significantly lower ( $p < 0.05$ ) than those of days 14–18.

Radioactivity in the control pieces showed changes similar in nature to, but quantitatively much less than those seen in the wound pieces. Statistically significant increases in uptake were observed at days 4–8 and 12–16; however, uptake in the wound pieces was substantially greater from day 3 on.

#### DISCUSSION

The present study showed that areas of healing osseous wound as small as 2 mm in diameter may be visible in a  $^{99m}\text{Tc}$ -diphosphonate bone scan if a pinhole collimator is used. Patients undergoing bone scans frequently have areas of increased radionuclide uptake in the jaws. Although primary malignant tumors and metastatic lesions do occasionally occur in the jaws, they are unusual, and many of the areas of increased uptake can be attributed to a variety of benign dental conditions (6,7,9,10). The healing extraction wounds studied here illustrate one common dental condition that may result in a positive bone scan.

Healing of extraction wounds in patients and experimental animals has been studied by numerous

TABLE 1. UPTAKE OF  $^{99m}\text{Tc}$ -DIPHOSPHONATE IN WOUND (W) AND CONTROL (C) AREAS OF RAT MANDIBLES

Days after extraction	No. of rats	%ID $\times 10^{-3}$ /gm W (mean $\pm$ s.d.)*	Wound (range)	%ID $\times 10^{-3}$ /gm C (mean $\pm$ s.d.)†	Control (range)
Control	4	0.53 $\pm$ 0.09	0.40-0.62	0.50 $\pm$ 0.03	0.46-0.54
1	5	0.27 $\pm$ 0.05	0.19-0.33	0.29 $\pm$ 0.06	0.20-0.38
2	5	0.46 $\pm$ 0.15	0.28-0.70	0.57 $\pm$ 0.11	0.42-0.69
3	5	0.62 $\pm$ 0.10	0.51-0.71	0.71 $\pm$ 0.14	0.55-0.91
4	4	0.98 $\pm$ 0.19‡	0.84-1.26	0.63 $\pm$ 0.07	0.54-0.70
6	4	0.94 $\pm$ 0.44	0.37-1.45	0.73 $\pm$ 0.44	0.22-1.24
8	5	1.48 $\pm$ 0.91	0.92-3.11	1.05 $\pm$ 0.32	0.57-1.49
10	5	1.03 $\pm$ 0.17	0.79-1.24	0.57 $\pm$ 0.10	0.53-0.74
12	5	1.05 $\pm$ 0.31	0.80-1.55	0.67 $\pm$ 0.30	0.51-1.22
14	5	1.21 $\pm$ 0.40‡	0.83-1.81	0.64 $\pm$ 0.20	0.47-0.98
16	4	1.90 $\pm$ 0.68‡	1.08-2.66	0.96 $\pm$ 0.18	0.75-1.20
18	5	1.57 $\pm$ 0.40	1.07-2.11	0.78 $\pm$ 0.21	0.50-1.03
26	3	0.77 $\pm$ 0.06‡	0.69-0.82	0.49 $\pm$ 0.10	0.40-0.60
34	4	1.07 $\pm$ 0.15‡	0.88-1.20	0.78 $\pm$ 0.15	0.56-0.92

\* Mean percentage of 1/10 injected dose per gram bone in the piece of mandible containing the wound ( $\pm$  s.d.).

† Mean percentage of 1/10 injected dose per gram bone from the piece of mandible beneath that containing the wound ( $\pm$  s.d.).

‡ W > C at the corresponding time period ( $p < 0.05$ ).

|| W > C at the corresponding time period ( $p < 0.01$ ).

investigators using histologic (12,14-16), autoradiographic (17), and fluoroscopic (18-20) techniques. The sequence of events that characterizes alveolar bone healing after tooth extraction is qualitatively similar in humans, dogs, and rats, varying primarily in the duration of the various phases. After the formation of a fibrinous coagulum, clot organization proceeds with the formation of young connective tissue. The initial bone formation after tooth extraction occurs in areas adjacent to, but outside of the socket proper, a phenomenon termed "extra-alveolar repair" (18-20). Bone formation within the socket begins at the periphery and proceeds inwards, gradually filling the socket with coarse immature bone. This new bone then matures and remodels until it is indistinguishable from the surrounding alveolar bone (12,14,15,17).

Few experimental studies of osseous activity related to disease or surgery in the jaws have appeared using radionuclide imaging or uptake techniques. Increased uptake and positive bone scans have been shown in rabbits during tooth reformation and eruption after extraction (18) and in dog mandibles with healing osseous allografts (13). Green et al., studying  $^{85}\text{Sr}$  uptake in healing maxillary molar extraction sockets in the rat, found that tracer uptake increased from 7 to 14 days after extraction, after which time values steadily decreased through 36 days (12). Our data on  $^{99m}\text{Tc}$ -diphosphonate uptake agree with those of Green et al. (12) concerning peak uptake periods and the relation between extraction and control areas, but they did not observe the biphasic nature of uptake with time. This suggests that either

$^{99m}\text{Tc}$ -diphosphonate is more sensitive than  $^{85}\text{Sr}$  in the evaluation of subtle bone changes or there is some difference between osseous healing in the maxilla and mandible.

The apparently biphasic nature of the increased uptake of radioactivity in the left mandible appears to correlate with two relatively distinct phases of wound healing after tooth extraction. The first phase, occurring over days 4-12, consists of uptake in areas immediately surrounding the extraction socket and possibly results from extra-alveolar bone formation. The second phase consists of the deposition of new bone in the socket proper, accounting for the large nuclide uptake that reaches its maximum 16 days after extraction. Undoubtedly, these phases overlap considerably. Subsequent remodeling and maturation of bone formed within the socket, as well as remodeling of adjacent bone, would account for the persistent but steadily decreasing uptake seen after day 16.

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