INVESTIGATIVE NUCLEAR MEDICINE

A Study of Irradiated Bone. Part II: Changes in Tc-99m Pyrophosphate Bone Imaging

Michael A. King,* David A. Weber, George W. Casarett, Francis A. Burgener, and Ovide Corriveau

University of Rochester School of Medicine and Dentistry, Rochester, New York

Quantitative Tc-99m pyrophosphate bone imaging was carried out in locally irradiated and control areas of New Zealand albino rabbits to determine the potential role of bone imaging in assessing the time course of radiation effects in bone and surrounding tissues. In vitro Tc-99m tissue assays, and serial radiographs, from the irradiated and contralateral limbs were obtained at regular intervals over the first 12 mo following irradiation for comparison with quantitative results from the camera studies. The autoradiographic localization of TcPPi was also studied in the x-irradiated and contralateral bones of the rabbits. The results show that TcPPi bone imaging is a sensitive in vivo indicator of early radiation effects upon vasculature and bone remodeling. The findings suggest that the quantitative bone-imaging technique may be useful in the evaluation of the effects of treatment modalities on the skeleton.

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The need for screening methods to differentiate tumor and disease processes from the uncomplicated course of radiation bone atrophy poses an important problem in the clinical management of patients who have undergone radiation treatment involving bone. Radiography has been the method of choice for doing this, but the sensitivity of this technique is such that postirradiation alterations may take years to become appreciable (*1*). Changes in the local accumulation of bone-imaging agents at sites of previous radiation therapy have been reported (*2–13*) and could provide an earlier indication of postirradiation alterations in bone. However, the postirradiation time course and the significance of these changes have not been investigated for the newly introduced Tc-99m bone-imaging agents.

Using the rabbit as the animal model, this study was designed to evaluate the potential use of quantitative bone imaging with Tc-99m pyrophosphate (TcPPi) to assess the time course of postirradiation changes in bone and surrounding tissues. Bone images before and after irradiation, along with in vitro specimen assays and Tc-99m contact autoradiograms, were compared with serial radiographic findings and with the studies of changes in bone remodeling and blood flow that were reported in the first paper of this series (*14*).

MATERIALS AND METHODS

A total of 86 adult, male New Zealand albino rabbits were used. Of these, 41 received a single x-ray dose of 1756 rads, 33 received a fractionated dose of 4650 rads delivered over 3 wk to the left hind limb, and 12 rabbits served as sham-irradiated controls. The procedure for irradiation of the left hind leg was described previously (*14*).

Imaging studies. Conventional analog (Polaroid) and computer-processed quantitative images were obtained using a gamma camera with a 15,000-hole high-resolution, low-energy collimator and a 20% analyzer window. Quantitative images from the standard-field gamma camera were stored in a 64 × 64 array on mag-

* Present address: Dept. of Nuclear Medicine, Univ. of Massachusetts Medical Center, 55 Lake Ave. North, Worcester, MA 01605.

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For reprints contact: George W. Casarett, Dept. of Radiation Biology and Biophysics, Univ. of Rochester Medical Center, Rochester, NY 14642.

netic disk for quantitative analysis and display.

Animals were imaged following the i.v. injection of 2 mCi (in 100–150 μl) of TcPPi. Images were obtained with the animals immobilized (unanesthetized) on an imaging platform; static images of the hind limbs were recorded at 5 min (the 0-hr image), 1.5, and 3 hr following injection. Each sampling time included five animals from an irradiated group and one sham-irradiated control. Each rabbit was imaged before being irradiated to check for pre-irradiation differences in TcPPi uptake between one leg and the other. Changes in the deposition of tracer in irradiated bone and surrounding tissues were evaluated in vivo by measuring uptake within regions of interest (ROIs) in the irradiated areas of the diaphysis of the left tibial shaft (cortical bone) and the left distal femoral and proximal tibial head (mostly trabecular bone), and comparing these with the nonirradiated contralateral areas on the computer-processed images (Fig. 1).

The time-dependent changes in TcPPi uptake in bone and bone marrow, as compared with skin and muscle in vivo, were measured with the gamma camera. Three animals were injected with TcPPi intravenously and killed with a 260-mg i.v. dose of sodium pentobarbital at 10, 70, or 195 min after tracer injection. Quantitative images were recorded immediately following the death of each animal. The muscle and skin were removed from each hind leg and repeat quantitative images were made.

In vitro tissue assay. At the postirradiation times listed in Table 2, tissue specimens were obtained from three rabbits for in vitro assay of TcPPi accumulation at 3 hr after i.v. administration. Sampling sites (on both legs) included the tibial shaft, tibial marrow, skin, muscle, and the distal femoral and proximal tibial heads. Tissues were evaluated for changes in the relative uptake of TcPPi. The level of significance of the deviation in the relative uptake ratio from 1.0 was calculated using Student's t-test (*15*).

Macroautoradiography. Macroautoradiograms were made from the tibial shaft and the femoral head from both hind legs of three rabbits from each dose regimen at 1, 3, 6, and 12 mo after irradiation. The animals were injected intravenously with 2 mCi/kg body weight of TcPPi 2 hr before killing. Bone specimens were placed in 95% ethanol for fixation, and dehydration was carried out using acetone under vacuum. Monitoring of fixing and dehydrating solutions showed negligible loss of activity. The specimens were then embedded in Ward's Bioplastic (*16*) to preserve the soft tissues (marrow). Sections of bone and plastic, 2 mm thick, were cut with a thin-sectioning machine (*17*), cleaned with 95% ethanol, and air-dried. The sections were attached to labeled 3- by 5-in. cards to maintain their identity, covered with plastic wrap to prevent chemography (*18*), and placed in contact with x-ray film† for 12 hr. The films were developed by hand. The total time from death of the animal to contact with the x-ray film averaged 8 hr.

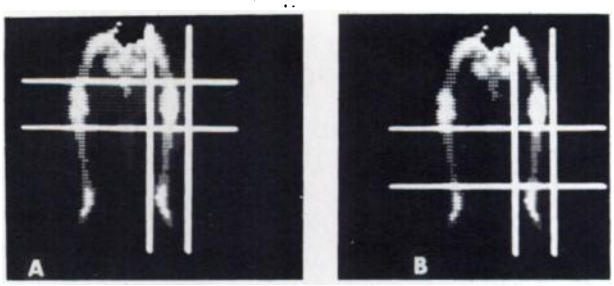

FIG. 1. Regions of interest (ROIs) selected on computer-processed scintiphotos, as used to quantify in vivo TcPPi deposition. Shown are: (A) knee-joint ROI; (B) tibial-shaft ROI.

Microautoradiography. In two control rabbits, and in one of the single-dose irradiated rabbits killed at each of the times used for the macroautoradiographic studies, a more detailed analysis of the autoradiographic localization of TcPPi was made. In these animals, smaller specimens of cortical bone, with the soft tissues removed, were embedded in Ward's Bioplastic. Sections of embedded bone, 200 μ thick, were cut with the thin-sectioning machine, attached to glass microscope slides with 910 adhesive,‡ and ground to 50 μ with dry sandpaper. The slides were cleaned in 95% ethanol, dipped in emulsion‖ (*19*), stored in a refrigerator for 12 hr, and developed (*19*).

Radiographic studies. Radiographs were made of all animals before irradiation and at 3, 6, 7.5, 9, 10.5, and 12 mo after irradiation. A final radiograph of the excised tibiae and femora was also made at the time of death. The radiographs of each animal maintained in the study for 12 mo were scored for the following changes: patchy bone mineralization, endosteal scalloping, cortical thickening, cortical thinning, and trabecular thickening or blurring. The scoring of the radiographs by the radiologist associated with this study (F.B.) was made

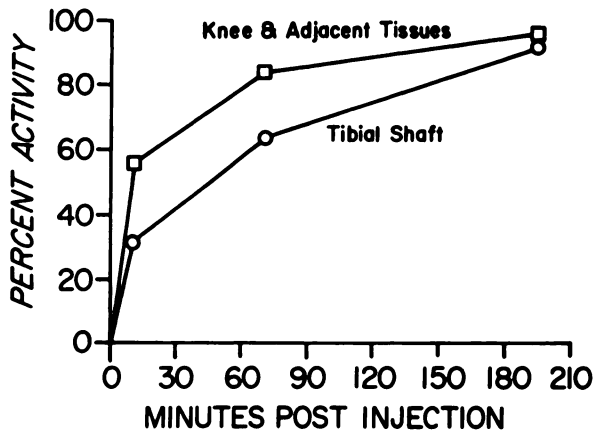

FIG. 2. Graph showing relative contribution of activity (percentage) in bone and bone marrow to total activity seen in vivo with ROI selection over knee joint and tibial shaft.

TABLE 1. MEAN (WITH RANGE) Tc-99m PPi RATIOS BETWEEN SHAM-IRRADIATED AND CONTRALATERAL ROIs IN CONTROL RABBITS, AT LISTED TIMES AFTER DOSE

	Imaging time (hr)	Tibial-shaft ROI	Knee-joint ROI
Single-dose irradiated	0	1.02 (0.93–1.12)	0.95 (0.83–1.06)
	3	1.02 (0.93–1.12)	0.99 (0.93–1.09)
Fractionated-dose irradiated	0	1.02 (0.86–1.11)	0.97 (0.86–1.11)
	3	1.02 (0.91–1.08)	0.98 (0.92–1.09)

without knowledge of which leg was irradiated, the time after exposure, and whether the radiographs were of control or irradiated animals.

RESULTS

Bone imaging studies. For each ROI the contribution of the TcPPi activity present in bone and marrow, compared with surrounding tissues, can be estimated as a function of time after tracer injection from the data presented in Fig. 2 for nonirradiated rabbits.

Table 1 lists the mean and range of values for the single nonirradiated rabbit imaged with the irradiated rabbits. These rabbits were used as indicators of camera uniformity at each time point and, as a group, they defined the variation in measurement of uptake differentials likely to occur with this technique.

Figure 3 illustrates the changes in the 0- and 3-hr bone images found at various times after irradiation with one of the single-dose irradiated rabbits. The results from the quantitative camera studies are presented graphically in Fig. 4. A complete listing of data—including levels of significance of the t-test and data for a number of early time points (which for the sake of clarity are not shown in Fig. 4)—is available (*20*). Qualitatively, a similar biphasic response (early and later increases) in TcPPi deposition was observed with both irradiation schedules, in terms of irradiated-to-control ratios for leg activity. In trabecular bone, a decrease in relative uptake of TcPPi was observed near the end of the study, especially in the fractionated-dose rabbits.

In vitro specimen assays. The data obtained from these assays for TcPPi uptake at 3 hr after administration are given in Table 2 in terms of the irradiated-to-nonirradiated uptake ratio. The absolute uptake of the tissues of each leg in terms of the percentage dose per gram of tissue, normalized for rabbit weight, were also calculated (*20*), but the uncertainties were such that no comment can be made regarding the presence of an indirect effect of irradiation upon the uptake of TcPPi by the bone of the nonirradiated leg.

Microautoradiography. In the sections of bone from the rabbits used in the microautoradiographic studies, TcPPi was found to be concentrated mostly near bone surfaces (endosteal, periosteal, and Haversian) at 2 hr after injection. This is illustrated in Fig. 5, where two photomicrographs from the irradiated tibia of a rabbit, 3 mo after the single dose, show the pattern of uptake near the surfaces of resorbing and forming osteons, respectively, these being determined by histological criteria for undecalcified bone sections (*21*) and tetracycline labeling of forming surfaces (*14*). We noted that at 2 hr after injection, accumulation was about the same on both types of remodeling bone surfaces.

Macroautoradiography. The differences in TcPPi deposition between irradiated and nonirradiated tibial shaft in the single-dose rabbits, as observed in contact autoradiograms, are illustrated in Fig. 6. Similar differences, but less marked, were observed in the frac-

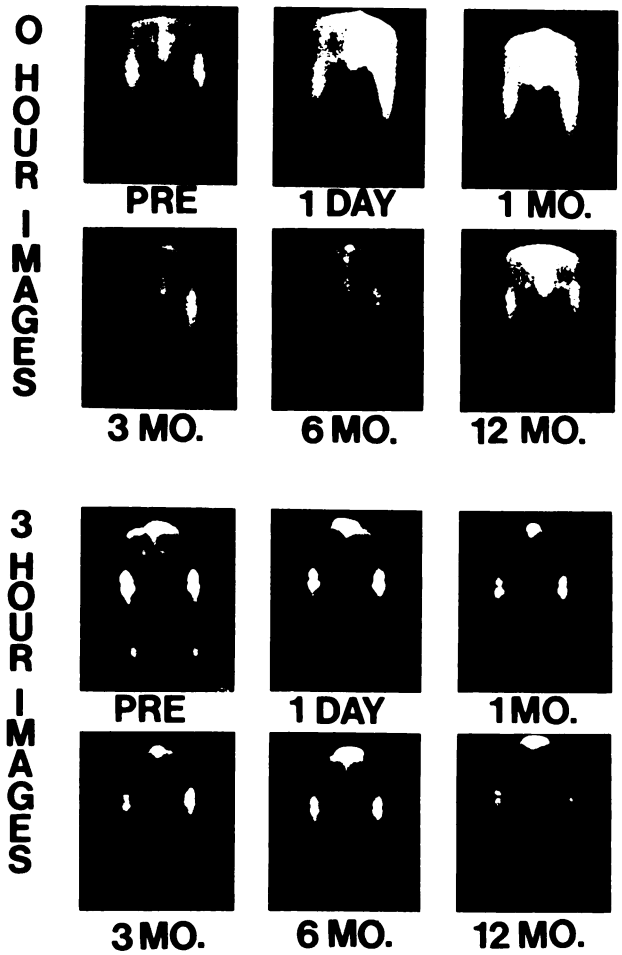

FIG. 3. Composite set of 0- and 3-hr TcPPi camera images for rabbit given single dose of 1756 rads left hind leg. Animal is supine; irradiated leg is at observer's right.

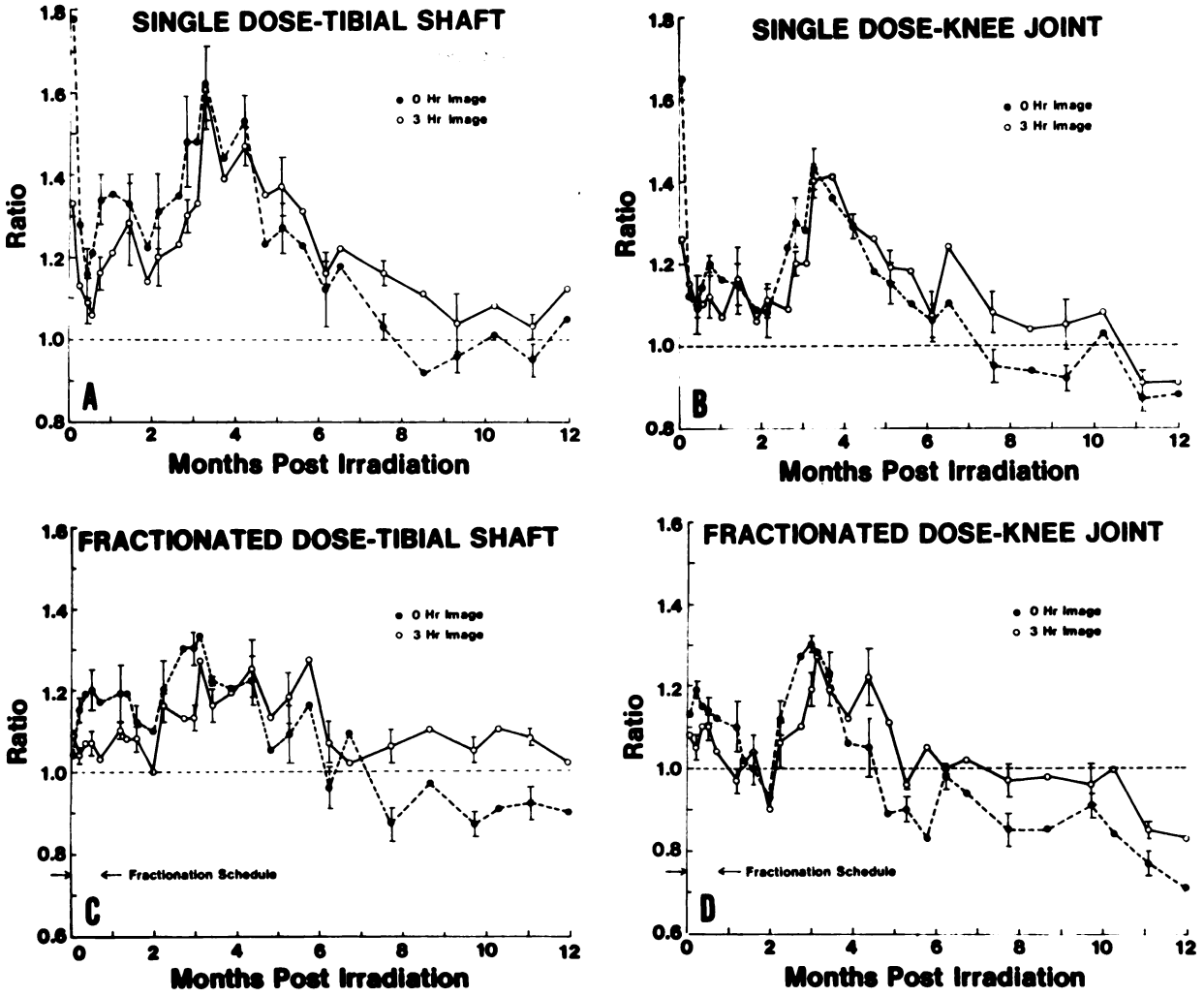

FIG. 4. Plot of mean irradiated-to-control uptake ratios for TcPPi, determined from camera images of five irradiated rabbits studied at each time point. Standard error of mean is indicated at selected points. Rabbits were imaged at zero time and 3 hr after injection. Regions of interest as indicated.

tionated-dose rabbits. The most significant finding was a markedly greater number of discrete concentrations of TcPPi in the irradiated than in the nonirradiated tibial cortex at 3 and 6 mo after irradiation, with lesser differences observed at 1 and 12 mo. No significant differences between irradiated and contralateral bone were observed in the periosteal or endosteal concentration of TcPPi, except when the plastic had shrunk away from the bone. This resulted in reduced attenuation of Tc-99m's high-energy internal-conversion electrons and low-energy x-rays, and a correspondingly greater impression of the apparent Tc-99m activity on the surface.

Relative changes in TcPPi accumulation were difficult to evaluate in trabecular bone of the distal femoral head due to poor infiltration of the plastic into this region in the time allotted for the process. Nevertheless an increase in the irradiated bone at 3 mo, and a decrease at 12 mo were suggested by inspection. In the fractionated-dose rabbits at 12 mo, the relative decrease in uptake by the bone of the irradiated side was readily apparent (Fig. 7).

Radiographic studies. Table 3 gives a summary of the time sequence of radiographic changes observed for the fractionated-dose animals, as determined in the study of the radiographs of the rabbits maintained to 12 mo following irradiation. Except in the rabbits that developed osteosarcomas (*20*), the alterations in the bone of the irradiated leg were not dramatic and were slow to develop. The radiographic findings were qualitatively similar in both irradiated groups of animals (*20*). In general, however, the fractionated-dose group showed less change in the irradiated legs, as compared with the nonirradiated contralateral, than did the single-dose rabbits.

DISCUSSION

The first objective of this investigation was to determine the time course of the alterations in TcPPi depo-

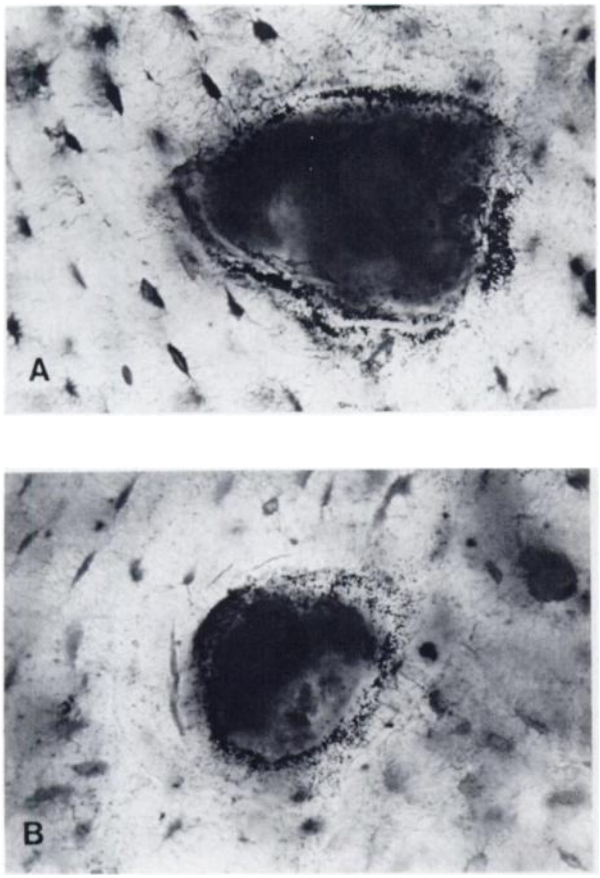

FIG. 5. Photomicrographs of undecalcified bone sections from irradiated tibia of rabbit 3 mo following single-dose x-irradiation. Note TcPPi localization around: (A) resorption cavity as determined by presence of Howship's lacunae, and (B) forming osteon as determined by tetracycline fluorescence (X 500 before reproduction).

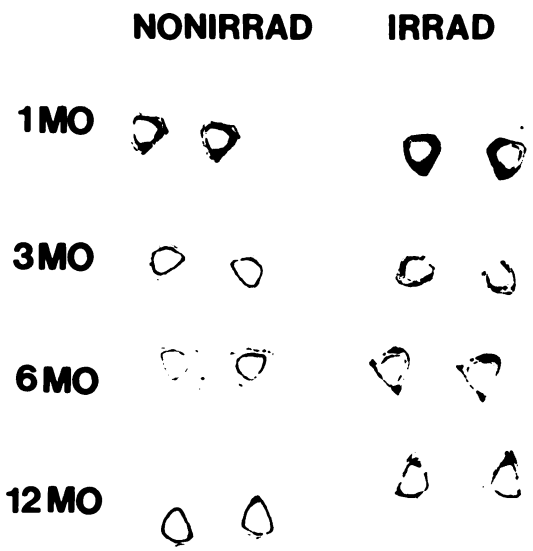

FIG. 6. Composite TcPPi contact autoradiograms of transverse sections of tibial shafts of rabbits at various times after 1756-rad single dose to the left leg. Note greater number of discrete concentrations of TcPPi in irradiated than in nonirradiated bone, especially at 3 and 6 mo.

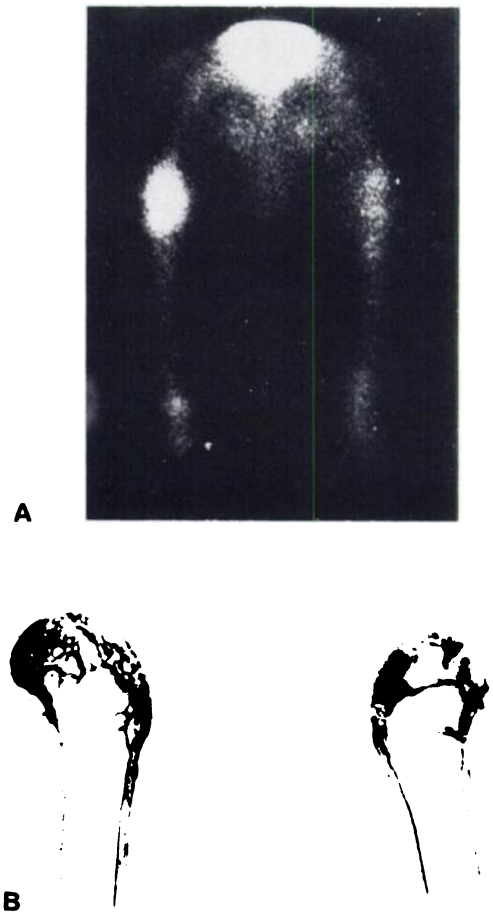

FIG. 7. (A) Camera image of hind quarters of rabbit at 3 hr after injection of TcPPi; made 12 mo after 4650-rad fractionated irradiation to left hind leg. (B) TcPPi contact autoradiograms of longitudinal sections from right and left distal femora of same rabbit as in A. In A and B irradiated leg is at right. Note relatively less uptake of TcPPi in epiphysis of irradiated as compared with nonirradiated bone.

sition after irradiation in the bone of adult rabbits, in the hope that this information could be extrapolated to human bone. For both radiation regimens used in this study, an early increase in uptake was observed. The literature on human exposure in radiotherapy reports effects ranging from increased to no change in uptake of F-18- or Tc-99m-labeled bone-imaging agents by irradiated nontumorous bone during and immediately after the fractionated irradiation schedule (*9,12,13*). This corresponds with our results for the fractionated-dose rabbits, where the early changes in the 3-hr camera images were observed quantitatively by computer analysis, although not qualitatively in the camera images. The magnitude of the initial relative increase in TcPPi uptake was found to be dependent on dose in preliminary studies of single-dose rabbits given smaller doses. Initial increases have also been observed by other investigators for Sr-89 and Ca-47 uptake in mice (*22*) and dogs (*23*) after single-dose irradiation.

The later phase of increased TcPPi uptake, as discussed below, is due to bone repair by remodeling. Increased uptake of imaging agents has been observed clinically for months after irradiation (*9*), but is not commonly reported. At comparable times following irradiation, increased Ca-47 activity ratios measured in vivo have been found in the tibias of dogs given single-dose irradiation equivalent to that used in this study (*23*). With larger single doses, this phase of increased bone uptake [anabolism (*23*)] did not occur. Instead, Ca-47 uptake was depressed below control by 1 to 2 mo after irradiation and remained low throughout the period of observation. Thus the later phase of increased uptake is probably dose dependent for its existence.

The late effect of radiation in radiotherapy patients is invariably reported as one of decreased uptake of imaging agents in the irradiated bone (*7–13*). This is in accord with the decreased uptake in trabecular bone near the end of the study period, especially with the fractionated-dose rabbits. Thus the effects of irradiation upon tracer localization in otherwise normal bone are dependent upon the time of imaging after irradiation, the total dose delivered, the fractionation schedule, and the type of bone irradiated.

Another objective of this study was to compare the postirradiation changes in the deposition and distribution characteristics of TcPPi with the alterations of bone pathophysiology observed in selected assays conducted in the same group of animals (*14*). Such an approach, it was hoped, would allow the time sequence of change in the postirradiation scintiphotos to be interpreted in terms of the mechanisms responsible. The following discussion is an attempt to do so.

The initial response to irradiation seen in the camera images was an increase in the irradiated-to-control ratios for leg uptake of TcPPi (Fig. 4). For both irradiation groups, the increase was found to be larger in the 0-hr than in the 3-hr images. The analog camera images (Fig. 3) showed that the increase occurred in both soft tissues and bone, and this was verified in the in vitro assays (Table 2). Significant relative increases in blood flow and erythrocyte volume were found in various tissues of the single-dose rabbits killed 1 day after irradiation (*14*). These could explain the differential in increased relative uptake of TcPPi between the 0-hr and 3-hr camera images and the increased relative uptake itself (*24*).

It is possible, however, that these were not the only mechanisms responsible for the increased TcPPi uptake ratios initially following irradiation. Changes in vascular permeability also occur after irradiation (*25–27*). The

TABLE 2. MEAN (WITH RANGE) OF IRRADIATED-TO-NONIRRADIATED RATIOS FOR Tc-99m PPI PER GRAM TISSUE

Tissue	Control	1 Day	1 Mo	3 Mo	6 Mo	12 Mo
			Single-dose rabbits			
Skin	0.97 (0.88–1.06)	1.44* (1.32–1.52)	1.29† (1.14–1.38)	1.30 (0.96–1.62)	1.14 (0.90–1.49)	1.12† (1.09–1.17)
Muscle	0.96 (0.90–1.05)	1.26† (1.15–1.37)	1.15 (1.03–1.21)	1.58† (1.33–1.88)	2.10 (1.18–3.13)	1.60† (1.30–1.93)
Epiphyses	0.97 (0.92–1.04)	1.21 (1.12–1.37)	1.16 (1.06–1.29)	1.13† (1.08–1.16)	1.12* (1.07–1.14)	0.95† (0.92–0.97)
Tibial marrow	1.04 (0.89–1.38)	1.46* (1.27–1.55)	0.85 (0.72–0.97)	0.93 (0.89–0.98)	0.75 (0.37–1.01)	0.75† (0.67–0.89)
Tibial shaft	1.00 (0.96–1.07)	1.20* (1.15–1.27)	1.10† (1.06–1.15)	1.22† (1.12–1.31)	1.31† (1.19–1.45)	1.08 (0.91–1.18)
			Fractionated-dose rabbits			
Skin			1.11 (1.01–1.17)	1.14 (0.91–1.27)	1.04 (0.96–1.10)	1.06 (1.00–1.15)
Muscle			1.11 (1.04–1.19)	1.15 (1.02–1.40)	1.29* (1.20–1.35)	1.26† (1.19–1.39)
Epiphyses			1.04 (1.01–1.07)	1.15† (1.06–1.23)	0.92 (0.86–1.02)	0.85† (0.80–0.93)
Tibial marrow			0.82† (0.75–0.93)	0.97 (0.87–1.10)	1.06 (0.71–1.34)	0.90 (0.71–1.03)
Tibial shaft			1.05 (0.98–1.12)	1.10* (1.07–1.13)	1.15 (0.97–1.42)	1.07 (0.96–1.16)

* $p < 0.05$ by two-tailed t-test.
† $p < 0.10$ by two-tailed t-test.

possibility that permeability plays an important role in the deposition of the Tc-labeled bone seekers has been suggested because these agents are complex molecules rather than simple ions (*28*). Thus the early phase of increased relative TcPPi uptake in the irradiated bone is thought to be due to changes in the vasculature (blood volume and flow, and possibly capillary permeability) during the inflammatory response to irradiation.

During the second phase of increased TcPPi uptake ratio, blood flow was not found to be increased (if anything it was decreased) in the irradiated bone and marrow of the rabbits (*14*). The time course for radiation-induced changes in bone remodeling in cortical and trabecular bone (*14*) corresponded remarkably well with the changes in TcPPi uptake during this second phase of increased uptake ratio measured by the 3-hr camera image. In the autoradiograms of the tibial shaft during this time interval, the only consistent and significant change between irradiated and control bone was a marked increase in the number of discrete concentrations of TcPPi in the cortex (Fig. 6), which appeared to be on the surfaces of remodeling osteons (Fig. 5). It is thus proposed that bone remodeling was responsible for the better part of the second phase of increased TcPPi accumulation ratio in the irradiated legs.

The soft tissues of the hind limb also played a role in the second phase of camera-measured increased relative uptake. In the in vitro studies (Table 2), muscle was found to be the major contributor to soft-tissue changes. This may be the site of the diffuse area of increased TcPPi activity observed in radiotherapy patients (*11,29*). Extraosseous accumulation of the Tc-99m-labeled bone seekers seems to be a common finding after soft-tissue inflammation and cell damage. Because muscle blood flow and erythrocyte volume were not increased (*14*), it does not appear that hyperemia was responsible for the increased soft-tissue uptake of TcPPi in the irradiated leg during this time period. Other possible mechanisms are increased vascular permeability and an increased tissue affinity for TcPPi, perhaps as a result of an increased calcium concentration (*30*).

The relative uptake of TcPPi was found to decrease in images following the phase of increased trabecular bone remodeling. The larger and earlier depression in TcPPi uptake ratios in the fractionated compared with single-dose rabbits corresponded with an earlier and greater depression in blood-flow ratio for the fractionated-dose rabbits (*14*). King et al. (*24*) found experimentally that a decreased blood flow would decrease TcPPi uptake in rabbit bone. Their model for the local accumulation of bone-imaging agents predicted that the effects of a change of blood flow on tracer localization would decrease with time after agent administration. This agrees with the earlier (postirradiation) and larger differential uptake in the knee joint ROI being observed in the 0-hr rather than the 3-hr image. Other factors such as a decrease in erythrocyte volume (*14*) and vascular permeability (*25*) could also have played a role in the depressed relative accumulation of TcPPi in the irradiated distal femoral and proximal tibial heads.

The general distribution of TcPPi observed microautoradiographically in this study was qualitatively similar to that described by Rowland (*31*) for Ca-45 in the cortical bone of rabbits and dogs at about the same time after injection. Both of these agents were concentrated mainly on bone surfaces having access to the vasculature, not uniformly distributed throughout the matrix, and not necessarily concentrated in areas of bone where calcification was in progress. A few Haversian canals were observed not to be labeled by TcPPi. This was also observed by Rowland (*31*) and is probably a reflection of the nonuniformity of capillary blood flow within bone (*32*), especially in the presence of canal plugs (*14*). A light, diffuse pattern of TcPPi uptake away from bone surfaces was also observed in cortical bone (Fig. 5) which did not appear to be necessarily localized

TABLE 3. SUMMARY OF CHANGES IN BONE RADIOGRAPHS* 4650-rad FRACTIONATED DOSE

Changes†	Time Post Start of Irradiation					
	3 Mo	6 Mo	7.5 Mo	9 Mo	10.5 Mo	12 Mo
Patchy bone mineralization (mixture of demineralization and sclerosis)	1	4	6	7	7	7
Endosteal scalloping	0	2	3	5	6	7
Cortical thickening	0	2	2	2	2	2
Cortical thinning	0	0	1	2	2	2
Trabeculation:						
Thickening	1	3	4	5	6	6
Unsharply defined	1	4	7	7	8	8
Distorted/disorganized	1	3	5	6	6	6

* Changes were determined by comparison between contralateral bones in a "blind" study of radiographs.

† Numbers to right of changes show number of rabbits, out of eight reviewed, that showed positive changes at indicated time.

near the lacunae of osteocytes or other structures (*33*). It may represent scatter from localization on such structures within the thick sections of bone used, or an artifact of the grinding technique.

In the present study, bone imaging with TcPPi has been found to be a sensitive monitor of bone remodeling. Radiographic changes, on the other hand, were found to become apparent only after the remodeling process had been under way for some time [Table 3 and (*14*)]. This difference in sensitivity was predicted by Greenberg et al. (*34*), on the basis of the difference in the basic parameters of bone change evaluated by the two techniques. Radiographs were, however, found to be better indicators of subsequent accumulated structural bone changes. That is, bone imaging showed greater deviation from control than did the radiographs during the period of active bone change, but there was a reversal in sensitivity of the techniques as the process of bone remodeling slowed and structural change accumulated. This pattern is that described by Charkes et al. (*35*) for the detection of soft-tissue metastasis to bone.

CONCLUSION

Quantitative imaging with TcPPi was found to be an excellent monitor for the reaction of both bone and soft tissue to irradiation. It was found to detect radiation-induced changes on both bone vasculature (blood flow and permeability) and remodeling. In the animal model it was observed to be more sensitive than conventional radiography for detecting early radiation effects in bone. The findings indicate that quantitative bone imaging may provide an important procedure for monitoring and investigating the effects of different treatment schedules and treatment modalities in experimental animals, and perhaps in patients.

FOOTNOTES

† Kodak No-Screen Medical X-Ray Film, Kodak Co.,
‡ Eastman 910 adhesive,
‖ Kodak NTB-2,

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