RELATIVE ACCRETION OF ^{99m}Tc-POLYPHOSPHATE BY FORMING AND RESORBING BONE SYSTEMS IN RATS: ITS SIGNIFICANCE IN THE PATHOLOGIC BASIS OF BONE SCANNING

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The relative roles of osteogenesis and osteolysis in the production of positive radionuclide images of skeletal lesions were investigated. The uptake of ^{99m}Tc-polyphosphate (Tc-PP) by each process was measured in an animal model that permitted bone formation and resorption to be studied independently. Ten rats received intramuscular implants of bone-forming demineralized matrix (DM) and resorbing devitalized bone (DV). Radiographs and Tc-PP scintiscans were made each week thereafter. At 6–10 weeks, the implants and normal bone samples were removed, counted for ^{99m}Tc, and examined histologically. The uptake of Tc-PP by DM implants was first detected on images made 3 weeks after implantation, and by DV implants, 1–2 weeks later. Serial radiography showed progressive calcification of DM and resorption of DV implants. Microscopic examinations of undecalcified sections, stained with a modified Goldner preparation, revealed vital-bone formation in the DM implants and osteoclastic resorption in the DV. Activity counts per gram of DM and DV implants were, respectively, 200% and 90% that of normal bone. Since only the bone-forming system (DM) accumulated Tc-PP at greater than normal concentrations, this study indicates that positive bone images of osteolytic lesions solely reflect compensatory osteogenic responses.

The current view on scintigraphic detection of bone lesions regards the osteogenic process as the major, if not the sole factor responsible for tracer uptake. Thus, the detection by imaging of malignant bone diseases, which are often characterized by focal osteolysis on radiographs, is believed to result from the selective accumulation of bone-seekers by compensatory bone-forming responses in normal tissues adjacent to osteolytic lesions (*1–3*). The contribution of resorbing bone in the production of positive images has not been delineated. This study was designed to examine the relative role of each process in the uptake of ^{99m}Tc-polyphosphate (Tc-PP) in an animal model in which bone formation and resorption could be studied independently. Our intention was to measure Tc-PP accretion by each of these biologic processes in comparison with normal bone and to extrapolate the results to the interpretation of positive bone images.

METHODS

The animal model consisted of intramuscular implants of two types of bone preparations in the hind legs of rats. One type was a bone-forming system and the other, a resorbing system. The bone-forming system was demineralized allogeneic bone matrix (DM), a substance shown by Urist et al (*4,5*) to possess osteogenic properties. When implanted in either osseous or several extraosseous tissues, the donor matrix is eventually totally replaced by newly synthesized host collagen that mineralizes to form vital bone. The matrix was prepared from various rat bones with 0.6 *N* HCl using the modified methods of Narang and Wells (*6*). The resorbing system was devitalized autogenous bone (DV), prepared from freshly excised scapulae by freezing and thawing as outlined by Irving and Handleman (*7*). This preparation is progressively resorbed by multinucleated giant cells which appear to be osteoclasts.

Albino rats of either sex, weighing approximately 250 gm, were used. Ten animals were implanted with the materials described above, one type in each leg, and an additional five animals were implanted with two types of control substances. These were polyethylene tubing (outer diameter, 0.017 in.) and non-

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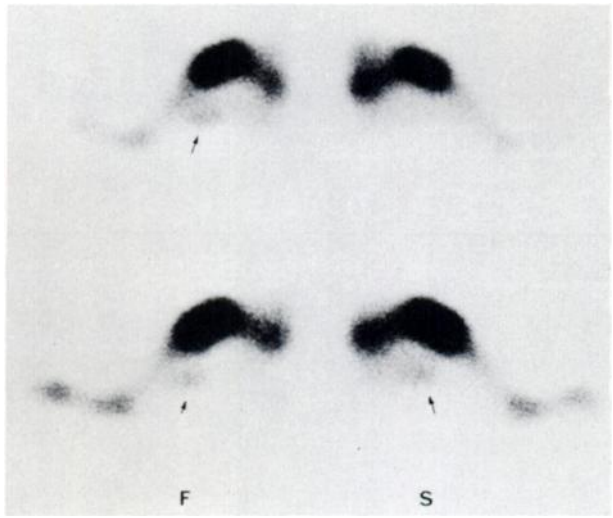

FIG. 1. Technetium-99m-polyphosphate scintillation camera images of hind legs of rat made at 3 weeks (top) and 5 weeks (bottom) after implantation. Arrows indicate radionuclide intensities overlying a demineralized femur implant (F) at 3 weeks (top), and overlying both the femur and a devitalized scapular implant (S) at 5 weeks (bottom). The DV implant was not visualized at 3 weeks.

osteogenic bone matrix. The latter material, prepared with 0.6 *N* HCl in 70% ethanol, is replaced with fibrous tissue rather than host collagen and does not induce bone formation (*8*). All implants measured 7–10 mm in length at the time of implantation. The sites of implantation were located at least 1.5 cm from the adjacent tibial shaft; this allowed separate camera images of each structure.

The rats were imaged with Tc-PP and radiographed at weekly intervals after implantation. Imaging was performed with a scintillation camera 4–6 hr after intravenous administration of 10 mCi of Tc-PP per kilogram of body weight. The imaging agent was prepared immediately before use by adding pertechnetate from a molybdenum generator to a commercial preparation of stannous polyphosphate (New England Nuclear Corp., Boston). One million counts were collected using a low-energy Div/Con collimator (Searle Radiographics, Des Plaines, Ill.) in the converging mode, and lead shielding was used to block radiation from the bladder and the remainder of the skeleton. Weekly imaging was continued until definitive radiographic evidence was obtained indicating calcification of DM and resorption of DV implants (6–10 weeks after implantation). At this time, the animals were injected with Tc-PP and killed 4 hr later. The implants and samples of their normal counterparts were removed, rinsed twice for 5 min in saline, dried with sponges, and counted for ^{99m}Tc activity in a well counter. Thereafter, 10-micron-thick sections were cut from undecalcified specimens of the bone-forming and resorbing implants and treated with a modified Goldner stain (*9*). In histologic sections stained with Goldner preparation, calcifying tissues, which stain green in color, can be differentiated from uncalcified matrix or osteoid, which stains red, while cellular structure is preserved. An estimation of calcified and osteoid matrix can be made for the histologic evaluation of metabolic bone diseases (*10*).

RESULTS

Uptake by the bone-forming implants was detected as a positive image 3 weeks after implantation, while that by the resorbing implants was not apparent until 1–2 weeks later. Polyethylene tubing and the nonosteogenic matrix never produced scintillation images. Positive detection of either DM or DV implants appeared on scintillation images in intensities comparable to the nearby tibia (Fig. 1).

Examination of serial radiographs indicated that DM implants were clearly mineralizing by 3 weeks after implantation in all instances (Fig. 2) and that the radiodensity of these implants increased progressively with time. Resorption of DV implants was first discernible 3–6 weeks after implantation (Fig. 2), but the extent of resorption of individual implants varied somewhat in the weeks that followed.

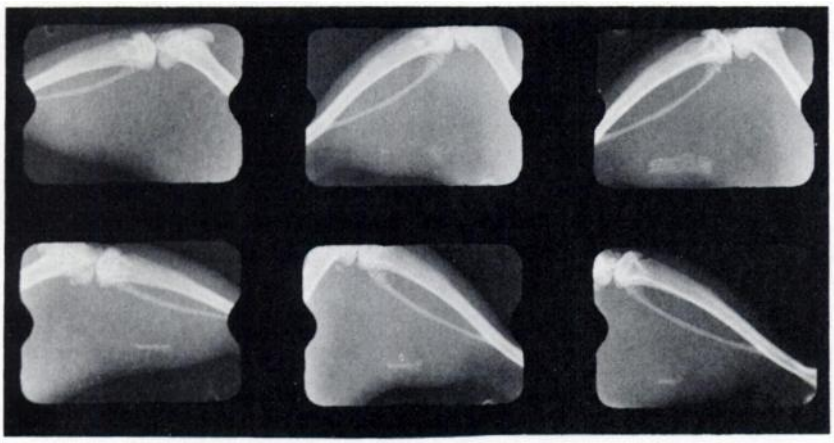

FIG. 2. Serial radiographs of rat hind legs made at (left to right) 1, 3, and 6 weeks after implantation of demineralized allogeneic rat femur (top) and devitalized autogenous scapular fragment (bottom).

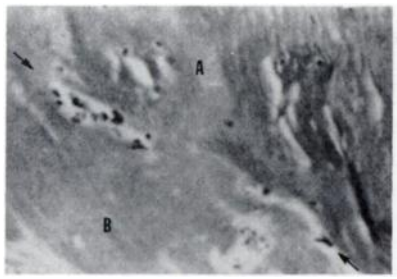

FIG. 3. Low-power view of 10-micron-thick undecalcified section from demineralized bone matrix implant (DM) treated with modified Goldner stain. This implant, removed 10 weeks after implantation, contains both calcified (A) and uncalcified (B) tissues. Calcified tissues containing osteocyte-like cells represent vital-bone formation which occurred during implantation. Cellular band between calcified and uncalcified tissues (arrows) contains osteoblast-like cells and resembles an osteoid seam. Acellular undecalcified matrix is still-unreplaced donor matrix.

No radiographic changes were observed in the sites containing implants of polyethylene tubing or non-osteogenic matrix. As mentioned previously, the time selected for the removal of the implants depended on radiographic confirmation of calcification of DM and resorption of DV implants. It required from 6–10 weeks after implantation to show radiographically that each process was firmly established. Note, however, that calcification of DM was always visualized before resorption of DV implants. Also, each animal had shown Tc-PP uptake on serial images by both types of implants (Fig. 1, bottom) before being killed. When harvested, all the DV and DM implants were enveloped in fibrous tissue and firmly attached to the adjacent muscles. All DM implants were mineralized throughout.

Figure 3 is a Goldner stain of a microscopic section from a bone-forming implant that had been decalcified before implantation. Both calcified and uncalcified tissues are visible. Numerous osteocyte-like cells were embedded in the calcified tissues. A number of empty osteocytic lacunae were seen in the uncalcified matrix; these appeared to represent still-unreplaced donor matrix. The uncalcified matrix was separated from the calcified portion by a band in which there were numerous nucleated osteoblast-like cells. This cellular band appeared to be an osteoid seam. The overall histologic picture was consistent with the gradual replacement of the donor matrix with host collagen and its subsequent calcification to form vital bone. There was no histologic evidence of a foreign-body reaction even though the implant was allogeneic.

In contrast, the resorbing implant (Fig. 4) contained calcified tissues exclusively, and these were devoid of osteocytes. The surfaces of the implant were scalloped and the invaginations were occupied by multinucleated giant cells. In general, the overall histologic finding was that of an ongoing osteoclastic-type resorption of nonvital bone.

The uptakes of Tc-PP by the implants and normal bone at the time of death (6–10 weeks after implantation) are listed in Table 1. The values are expressed as ^{99m}Tc counts per minute per gram of body weight. Table 2 contains similar data for the nonosteogenic matrix and polyethylene tubing implants of the five control rats.

DISCUSSION

Milch and Changus (*11*) have shown that primary and metastatic bone tumors usually undergo a variety of osteolytic and osteogenic processes. The pathologic basis of bone scanning has since been examined in several laboratories by correlating histologic, radiographic, and autoradiographic analyses of biopsy specimens (*2,3,12*). Based primarily on these correlations, the hypothesis has been formulated that the osteogenic component is responsible for the increased accumulation of bone-seekers, but this has not been substantiated experimentally (*1*).

Since the two coexisting processes in an osteolytic lesion cannot be uncoupled and examined separately, our experimental model offers a convenient method of comparing the accumulation of bone-seekers by each process relative to normal bone. The identity of each of the two implant systems, i.e., bone formation and resorption, was reasonably validated by the histologic data and shown by serial radiography. The viability and evolution of the two processes were confirmed by the changing concentrations of ^{99m}Tc activity in amounts greater than the surrounding musculature over a period of 6–10 weeks as shown by serial scintiscanning.

The imaging of nonvital resorbing bone implants required 4–5 weeks of implantation before they could bind sufficient Tc-PP to be visualized (Fig. 1). One explanation for this delay is provided by the histologic data. Many osteoclasts were seen on the sur-

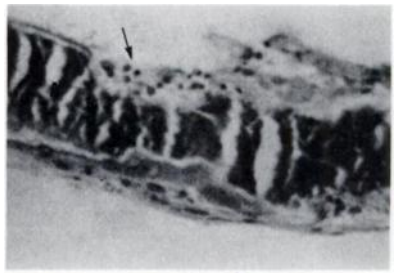

FIG. 4. Low-power view of 10-micron-thick undecalcified section from devitalized scapular implant (DV) treated with modified Goldner stain. This implant, removed 10 weeks after implantation, contains acellular nonvital calcified tissues (A), but no osteoid. Scalloped bone surfaces containing multinucleated osteoclasts (arrow), indicating an ongoing resorptive process, are seen along nonvital calcified tissues.

TABLE 1. TECHNETIUM CONCENTRATIONS IN BONE-FORMING (DM) AND RESORBING (DV) IMPLANTS AND NORMAL RAT BONE*

Rat	DM†	Control†	Difference	% Diff.	DV†	Control†	Difference	% Diff.
1	14,870	11,888	+2,982	+25.1	28,494	32,607	−4,113	−12.6
2	15,105	13,284	+1,821	+13.7	26,283	27,389	−1,106	−4.0
3	18,309	6,507	+11,802	+181.4	11,802	13,933	−2,131	−15.3
4	12,280	5,609	+6,671	+118.9	11,316	23,839	−12,523	−52.5
5	8,631	6,076	+2,555	+42.1	12,352	19,666	−7,314	−37.2
6	30,855	8,843	+22,012	+248.9	43,701	36,858	+6,843	+18.6
7	44,970	15,164	+29,806	+196.6	96,318	50,893	+45,425	+89.3
8	34,574	16,385	+18,189	+111.0	38,761	47,278	−8,517	−18.0
9	151,116	74,452	+76,664	+103.0	37,616	74,452	−36,836	−49.5
10	140,473	65,039	+75,434	+116.0	46,859	65,039	−18,180	−27.9
Mean‡			+24,794	+115.7			−3,845	−10.9
S.E.‡				24.2				13.0
t				4.77				0.84
p				<0.005				>0.40

* Concentrations are expressed as ^{99m}Tc counts per minute per gram of body weight.

† DM implants were prepared from tibias, femurs, and scapulae while DV implants were prepared from scapulae only. The control in each case was the normal host counterpart. Rats 9 and 10 received scapular DM as well as DV implants, and the control in both instances was the remaining normal scapula. The tabulated data were adjusted for differences between animals in the amount of Tc-PP injected by normalizing to 1 mCi of injected dose per kg of body weight. The data were also adjusted for differences in ^{99m}Tc decay.

‡ Analysis of the data by Student's t-test for paired experiments showed that the mean % difference between DM implants and their controls was significantly greater than zero ($p < 0.005$), while that between DV implants and their controls was not. S.E.: standard error of the mean % difference.

TABLE 2. TECHNETIUM UPTAKE BY CONTROL IMPLANTS: RATIOS OF IMPLANT TO NORMAL TIBIA*

Rat	Nonosteogenic matrix†	Polyethylene tubing
1	0.143	0.004
2	0.112	0.003
3	0.101	0.004
4	0.073	0.001
5	0.080	0.002
Mean	0.102	0.003

* The ratios were determined from well counter measurements of ^{99m}Tc cpm/gm wet weight at the time of harvest (6–10 weeks).

† The implants were prepared from rat tibias with 0.6 N HCl in 70% ethanol.

faces of devitalized scapular bone fragments by 1–2 weeks after implantation. At the time of death, numerous invaginations of the bony surfaces containing osteoclasts were evident (Fig. 4). Since the binding of bone-seekers in this system is by surface exchange, the interval between implantation and the initial detection of DV implants on images (4–5 weeks) probably represented the time required by the process of resorptive invagination to create a surface area large enough to concentrate sufficient Tc-PP to form an image.

Similarly, the 3-week delay in visualization of DM implants on images may be explained as follows. Bone-seekers have been shown to concentrate in areas of osteoid deposition (*2,3,12–14*) due perhaps to preferential binding of newly synthesized collagen (*15*). Our histologic data revealed the presence of osteoid seams (Fig. 3) in all DM implants at the time of their removal. It is likely, then, that the DM implants required a period of 3 weeks for the synthesis of sufficient host collagen to bind demonstrable amounts of the bone-seeker.

The data of Table 1 show that the specific ^{99m}Tc activities of DM implants were, on the average, twice those of normal bone. The concentrations of radionuclide per gram of resorbing bone were in most instances lower than those of normal bone. The data for the control implants (Table 2) show that the average uptake per gram of nonosteogenic matrix implants was only 10% of normal bone, while the activity of polyethylene tubing implants was negligibly low. The results of control implants suggest that most of the activity measured in the DV and DM implants, which was not removed by two saline rinses, undoubtedly represented the binding of Tc-PP by the processes under investigation.

Our data on Tc-PP uptake indicate that the process of bone formation produced greater regional concentrations of Tc-PP than normal bone. The concentrations by resorbing implants were at best equal to those of normal bone at a time when active osteolysis was demonstrable radiographically and histologically. It follows that bone-forming processes *alone* accumulate sufficient bone-seeker to produce positive images of osteolytic lesions, even though

both processes may be accelerated. Our results led us to believe that positive bone images of osteolytic lesions are entirely due to the incorporation of skeletal agents by compensatory bone-forming responses in adjacent tissues. This conclusion is an extrapolation of the results, but consistent with the current hypothesis.

It may be argued that the accretion of bone-seekers is directly proportional to blood flow irrespective of the particular process (i.e., osteogenesis or osteolysis). The published data are conflicting and insufficient to support this hypothesis (*16,17*). Although our histologic results did show evidence of vascularization in both types of implants, our results are not sufficient at present to warrant any conclusion in this regard.

Finally, note that in our implant system the initial detection of bone-forming implants on scintillation images and radiographs occurred at the same time. This contrasts with the common observation that a bone lesion is usually detected by imaging some time before it becomes radiographically apparent. The relatively early radiographic detection of implant calcification in this animal model is made possible by the location of implants in a muscular environment.

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