INVESTIGATIVE NUCLEAR MEDICINE

Localization of Tc-99m MDP in Epiphyseal Growth Plates of Rats

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The distribution and localization of Tc-99m methylene diphosphonate (Tc-MDP) in the epiphyseal growth plates of the rat were elucidated by contact and microautoradiography. The uptake of the tracer was found to be especially high in the calcified cartilage bars at the end of the vascular loops. In addition to areas of mineralization, increased uptake was found in the Howship's lacunae on the resorbing surfaces. This labeling corresponded with the fluorescence of tetracycline, which labeled both forming and resorbing surfaces, when given with short labeling interval. Distribution of Tc-MDP did not coincide with new production of collagen, as judged by H-3 proline labeling; nor was the uptake localized within cells with high alkaline phosphatase activity. The affinity of the tracer for the mineral phase was confirmed by decalcification of in vivo labeled sections with EDTA, which showed loss of radioactivity in contrast to sections incubated in water. By chromatography the activity in the decalcification medium could not be distinguished from that of Tc-MDP.

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The still improving resolution of imaging devices and the use of newer bone-seeking Tc-99m phosphorus compounds with higher lesion-to-background ratio and low radiation dose, have improved scintiscanning and extended its use. However, the physiological reaction of these agents with bone is still being discussed and various reaction mechanisms have been proposed.

Pyrophosphate, the diphosphonates, and their Tc-99m-labeled compounds are known to adsorb strongly to the surface of apatite crystals (*1–4*). By this mechanism the quantitative uptake is thought to be determined by either the local blood flow (*5–7*), or the rate of osteogenesis (*8–10*). Some evidence suggests an interaction with the fibrous collagen matrix, especially the immature collagen found in osteoblastic areas (*11–13*). Finally, phosphatase enzymes, particularly alkaline phosphatases, have been suggested as the target of the bone-seeking agents (*14,15*).

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In a macroautoradiographic study of the distribution of Tc-99m phosphorus compounds in osteoarthritic human hips, the bone-seeking agents were seen to accumulate in the osteophytes and in the denuded weight-bearing area—especially in the areas of endochondral ossification found both at the osteochondral junction of the osteophytes and in the walls of the pseudocysts (*16*).

Endochondral ossification is found primarily in the epiphyseal growth plate, with a good structural distinction between the different stages in the ossification process (*17*) (Fig. 1).

It is the intention of this study to elucidate the uptake of Tc-99m MDP in rat's epiphyseal growth plates using macro- and micro-autoradiography combined with various histologic-histochemical staining methods.

MATERIAL AND METHODS

Labeled compounds. Technetium-99m-labeled methylene diphosphonate (Tc-MDP) was prepared according to the manufacturer's instructions by adding Tc-99m as pertechnetate to a commercially available kit

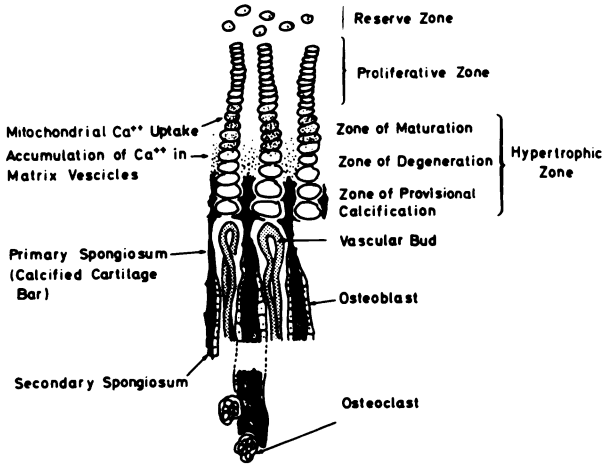

FIG. 1. Schematic illustration of different zones in epiphyseal growth plate and areas of calcification ■, ossification ■, and osteoblastic resorption in the enchondral ossification.

containing 4 mg of methylene diphosphonate and 0.25 mg of stannous chloride.

Tritiated proline (L-(^{3}H(G))-,NET-285)* was obtained with a specific activity of 3.7 and 5.0 Ci/millimol.

Tissue preparation and histological technique. Ten albino rats of uniform stock, 4- to 5-weeks-old, with an average weight of 63 g, were given 0.25–5 mCi Tc-MDP by tail vein 3 hr before killing. At necropsy one hind leg was immediately exarticulated at the hip and embedded in an aqueous solution of carboxymethyl cellulose on a microtome stage and frozen by immersion into hexane cooled by solid CO_2 (−75°C).

Five-micron sections of the knee region were cut in a heavy cryomicrotome at −20°C. Scotch tape was attached to the exposed surface of the frozen specimen before cutting, so that the section adhered to the tape.

Contact autoradiograms were made by Ullberg's technique (*18*), and the sections were placed on glass plates covered with G5 emulsion, 10 μ thick,† instead of on x-ray film.

These procedures were performed within the cryostat where the film was exposed for 18 hr. Each tape with its section was removed and the nuclear plates were developed with D19‡ for 4 min at 18°C and fixed for 8 min in sodium thiosulphate (10%), potassium metabisulphite (4%).

Sections for microautoradiography were fixed in Baker's formol calcium for 10 min at 4°C, then rinsed in distilled water and mounted on small metal frames. In the dark room these were dipped in melted G5 or K2 emulsion† using the technique described by Kopriwa and LeBlond (*19*). The emulsion was allowed to dry in the dark room and exposed for 18 hr. As a check for chemography, unlabeled sections were subjected to both autoradiographic procedures.

The following staining methods were used.

Poststaining. Mayers hematoxylin-eosin.

Prestaining. Alkaline phosphatase activity was demonstrated by Burstone's method (*20*) using α-naphtol-AS-BI-phosphate‖ as substrate and Fast Red Violet LB salt as coupling agent (pH 8.3).

Acid phosphatase activity was demonstrated by the method by Barka and Anderson (*21*) with α-naphtol-AS-BI-phosphate as substrate and hexazonium pararosanilin as coupling agent (pH 5.0). Microautoradiography was performed on sections stained for enzyme activity both with and without precoating of the sections with 0.5% celloidin.

Vital staining. Tetracycline labeling. In addition to administration of Tc-MDP, five rats were given 25 mg oxytetracycline 3 hr before killing.

The tetracycline-labeled sections were either photographed under incident UV-light§ before autoradiography was performed, or coated with 0.5% celloidin before autoradiography, thus reducing the fading of the fluorescence caused by the autoradiographical procedures.

All sections were dehydrated through graded alcohol and mounted in Euperal.¶

To compare the distribution of Tc-MDP with collagen production, H-3 proline (*22*) was given intravenously to two rats (4 μCi/g) 3 and 18 hr before killing, respectively. The hind legs were fixed in neutral buffered 4% formaldehyde, and one leg from each rat was decalcified in 4% EDTA (pH 7.2) at 4°C. The specimens were washed, dehydrated, double embedded in celloidin-paraffin, and sectioned at 6 μ. Microautoradiograms were made as described above, with exposure for 4 wk at 4°C in a closed box containing Drierite, and the sections were poststained with Mayer's hematoxylin-eosin.

The other hind leg from each rat was embedded in methylmethacrylate as described by Jowsey et al. (*23*) and sectioned on a milling machine to a thickness of 200 μ. The sections were ground and polished to a thickness of 90 μ before contact microradiograms were made using an x-ray tube** with a 10-min exposure at 12 kV and 12 mA. Spectroscopic plates type 649 Gh‡ were used, being developed for 5 min in D19 b.‡

After microradiography the same sections were placed on nuclear plates (G5) which were then exposed for 2 mo at 4°C. Concurrently, control specimens from a rat not given H-3 proline were subjected to the same procedures. The autoradiographic distribution of H-3 proline was compared with the histology, mineralization, and distribution of Tc-MDP found in the frozen sections.

Visualization of the regional blood flow. Regional blood flow in the bone was demonstrated autoradiographically using the fractional distribution of a small diffusible radioactive indicator given intravenously (*24*). The principle is based on the observation that the initial distribution of a tracer in most organs other than the brain is in proportion to the blood flow within the first

60 sec. With time, however, the various organs extract the tracer at different rates, and the relative distribution ceases to indicate blood flow.

Two etherized rats were given pertechnetate (50 and 5 mCi, respectively) by a tail vein and a string tourniquet was tied around the femur 15 sec and 5 min, respectively, after the injection. The animals were killed and exarticulated at the hip. The leg was immediately frozen and sectioned in a cryostat as described above. Nuclear plates with G5 emulsion were placed upon the sectioned surface of the frozen block containing approximately half of the specimens, and the contact autoradiograms obtained were compared with the latest cut sections stained with hematoxylin-eosin.

Decalcification experiments. In order to elucidate the affinity of Tc-MDP for bone, sections of bone labeled in vivo were decalcified, and the activity in the decalcification medium was examined in different chromatographic systems. Sections from the knee region of three rats given either 4 mg, 1 mg, or 0.1 mg of Tc-MDP were counted in a gamma spectrometer before and after 10 min of incubation in 10 ml of either distilled water or 4% EDTA at pH 7.2. The loss of activity was expressed as a percentage. In order to check the decalcification procedures, the sections were finally stained for calcium deposits by the method of von Kossa (*25*). Since the radioactive concentration after these procedures was not optimal for chromatographic studies, decalcification was performed on entire hind-leg bones labeled in vivo and cut into pieces. The following chromatographic systems were used on the EDTA solution after decalcification: (a) TCL-Plastic sheets Silica gel 60, 0.2 mm (No. 5748††) in n-butanol:ethanol:water (2:2:1), (b) ITLC SG, glass fiber sheets impregnated with silica gel‡‡ in acetone; (c) ITLC SG in saline; and (d) Whatman paper No. 1 in 1 *M* sodium acetate buffer, pH 4.75 (*26*).

The R_f values were compared with the R_f values of the following Tc-99m compounds in the same chromatographic systems: (a) Tc-MDP; (b) Tc-EDTA prepared from 1 ml $EDTANa_2$ in saline (10 mg/ml), 0.3 ml $SnCl_2 \cdot H_2O$ (1 mg/ml) in 0.1 *N* hydrochloric acid and 1 ml Tc-99m eluate (1 mCi/ml); (c) Tc-99m as pertechnetate; (d) Tc-99m Sn-colloid; and (e) Tc-99m albumin.

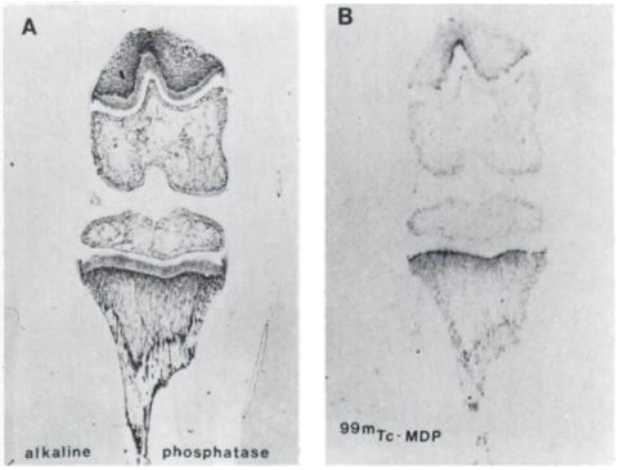

FIG. 2. Macroautoradiographic distribution of Tc-MDP in epiphyseal growth plate and its relation to alkaline phosphatase activity. (A) Section of knee region stained for alkaline phosphatase activity. (B) Corresponding contact autoradiogram. Particularly high uptake of Tc-MDP is seen at osteochondral junction. At low magnification, gross distribution of radionuclide roughly corresponds with alkaline phosphatase activity. However, no uptake is seen in the alkaline-phosphatase-active cells of the hyperthrophic zone of the epiphyseal cartilage, and in areas with more widespread activity, such as the periosteum, radioactive accumulation is seen in innermost mineralized layer and not in relation to area of enzyme activity (A and B ×3).

RESULTS

Contact autoradiography on nuclear plates showed the uptake of Tc-MDP to be particularly high in a narrow area at the osteochondral junction of the epiphyseal cartilage (Fig. 2). This area was found by microautoradiography to be the area of provisional calcification of the cartilage, and it was the calcified cartilage bars

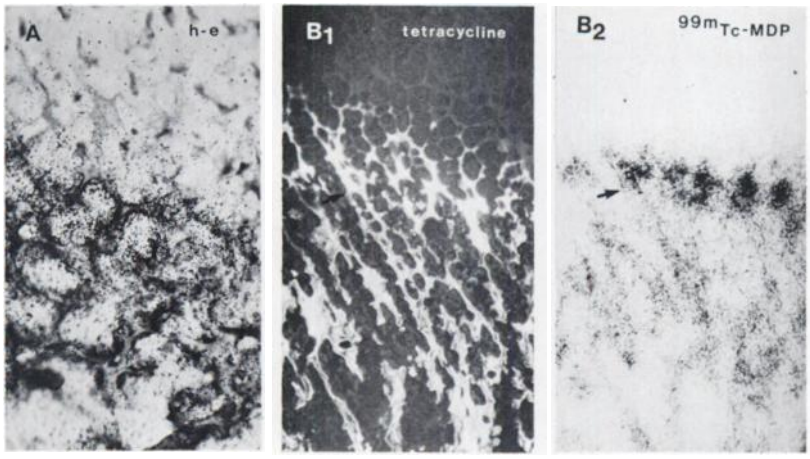

FIG. 3. Microautoradiographic localization of Tc-MDP in areas of mineralization. (A) Osteochondral junction, H-E staining (× 115). (B_1) Tetracycline fluorescence of osteochondral junction, with intense labeling of provisional calcification. (B_2) Corresponding light microphotograph showing distribution of Tc-MDP (×32). Highest uptake is seen in tetracycline-labeled calcified cartilage bars next to vascular buds.

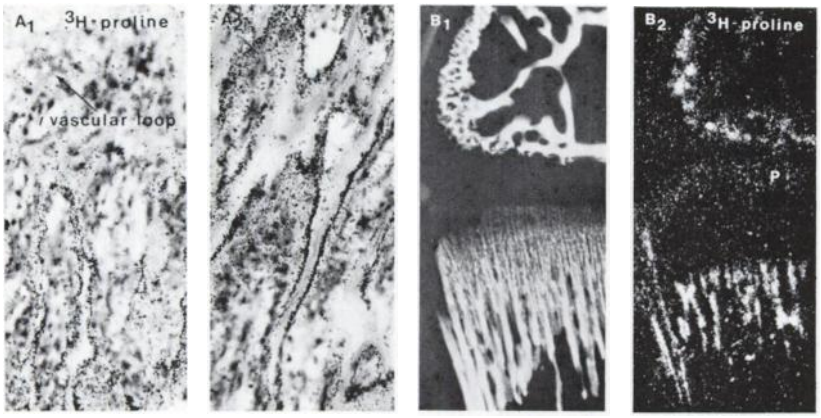

FIG. 4. H-3 proline localization at enchondral ossification as index of collagen formation. (A_1) Autoradiogram of the osteochondral junction (H & E staining). (A_2) Autoradiogram from deeper areas of metaphysis (A:X115). (B_1) Microradiogram of growth plate, showing degree of mineralization. (B_2) Corresponding contact autoradiogram (C:X19). H-3 proline is localized in ossification areas at surface of trabeculae. Osteoblastic activity starts beneath vascular loops and very little labeling is seen at provisional calcifications. Collagen formation of the cartilage is seen in zone of proliferation (P).

next to the vascular loops that were predominantly labeled with the tracer. However, in provisional calcifications of the articular cartilage, both the calcification surfaces facing the osteochondral junction and the superficial surfaces facing the joint space were labeled with Tc-MDP. The provisional calcifications showed an intense tetracycline fluorescence when a short labeling period was used (Fig 3).

The gross distribution of alkaline phosphatase activity in bone structures, as judged by the intensity of the color reaction, was similar to the distribution of Tc-MDP (Fig. 2). However, no uptake of the bone-seeking agent was seen in relation to the cells in the hypertrophic area of the epiphyseal cartilage, where a pronounced alkaline phosphatase activity was found. In periosteal areas with more widespread activity of the enzyme, it could be seen that the radioactivity was not localized to the area with enzyme activity, but to the underlying bone.

The distribution of H-3 proline, reflecting new production of collagen, did not correspond to the distribution of Tc-MDP, since labeling with H-3 proline was found to begin on the trabeculae beneath the vascular invasion of the cartilage (Fig. 4). The collagen production of the cartilage was seen mainly in the proliferative zone (Fig. 4B).

Increased uptake of Tc-MDP was found at resorbing surfaces as well as in areas of ossification and calcification. As the epiphyseal growth plate provides growth only in an area the size of the plate itself, a marked remodeling of bone is needed to maintain the shape of a tubular bone (Fig. 5A).

A pronounced resorption is seen particularly in the periosteal area under the flared metaphyseal bone, as was demonstrated by histochemical staining of osteoclasts for acid phosphatase activity (Fig. 5B). In this region, a spotty radionuclide uptake could be seen in relation to the osteoclasts within the Howship's lacunae (Fig. 6A). Free osteoclasts did not show any uptake. The autoradiographic resolution did not allow differentiation in the uptake between bone and osteoclasts within their lacunae. However, during frozen sectioning some osteoclasts were dislodged from their nests, and it could then be seen that some cells had been labeled, although not as much as the wall in the Howship's lacunae (Fig. 6B).

Tetracycline not only labeled areas of mineralization

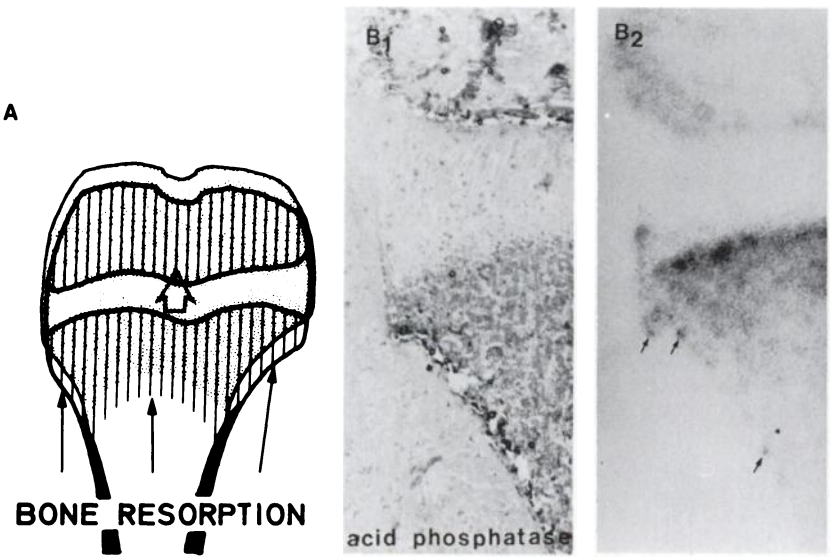

FIG. 5. Macroautoradiographic uptake of Tc-MDP in osteoclastic areas. (A) Schematic representation of remodeling during growth of proximal end of the tibia. (B_1) Acid phosphatase activity at growth plate illustrating localization of osteoclasts. (B_2) Corresponding contact autoradiogram (B:X19). Some spotty uptake is seen in osteoclastic area. Accumulation of Tc-MDP is not as high as in area of calcification of cartilage.

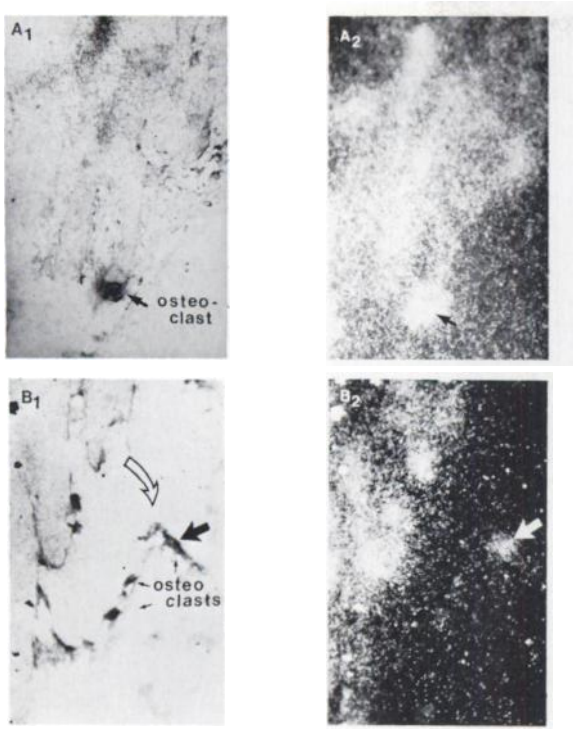

FIG. 6. Microautoradiographic uptake of Tc-MDP in periosteal osteoclastic area of metaphysis. (A_1) Acid phosphatase activity in section illustrating one major osteoclast within resorption lacuna at end of trabecula. (A_2) Corresponding dark-field micrograph illustrating silver grains (A:X50). (B_1) Acid phosphatase activity in osteoclasts dislodged from their lacunae during frozen sectioning. (B_2) Corresponding dark-field micrograph (B:X50). Radioactive uptake is seen in osteoclastic areas, first of all in walls of resorption lacunae, but also in some (probably active) osteoclasts.

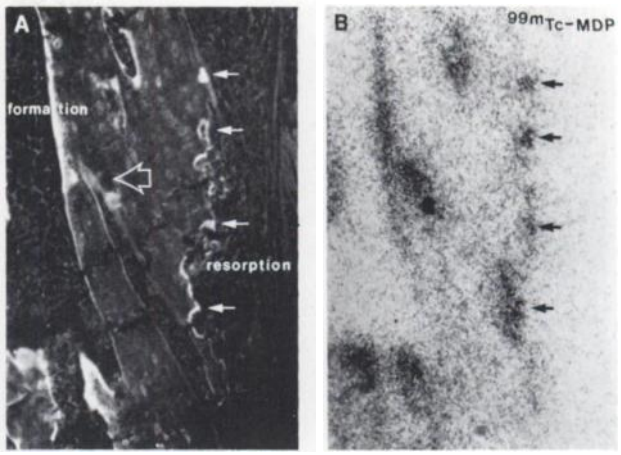

FIG. 7. Uptake of Tc-MDP in periosteal osteoclastic area compared with tetracycline labeling. (A) Tetracycline fluorescence micrograph. Bone trabecula is seen at center with periosteal area to its right and marrow to its left. (B) Autoradiogram showing corresponding uptake of Tc-MDP. Radionuclide accumulation coincides with tetracycline fluorescence with short labeling period, since both label areas of resorption as well as of formation (X70).

but, given a short labeling period, it also labeled the walls of the resorption lacunae. There seemed to be correspondence between the uptake of Tc-MDP and the fluorescence of tetracycline given simultaneously with the administration of the bone-seeking agent, both labeling forming as well as resorbing surfaces (Fig. 7).

The macroautoradiographic distribution of pertechnetate shows the entire metaphyseal bone to be highly vascularized (Fig. 8A), but with a low extraction rate (Fig. 8B) as compared with other tissues such as the skin.

Radioactivity counting on sections incubated in distilled water and 4% EDTA demonstrated that nearly all activity remained in the sections after water incubation (98.6%), but only 1.6% after EDTA decalcification. No difference was seen with different dosages of MDP. Von Kossa staining confirmed the EDTA-treated sections to be histologically decalcified (Fig. 9).

The radioactive compound in the decalcification medium was found to behave as Tc-MDP, since the following R_f values were obtained in the four chromatographic systems used: Tc compound in decalcification fluid: 0,0,0.9,0. Tc-MDP: 0,0,0.9,0. Tc-EDTA: 0.4,0-0.4,0.9,0.8. Pertechnetate: 0.7-0.8,0.9, 0.9-1.0,0.6-0.7. Tc-Sn-colloid: 0,0,0,0. Tc-albumin: 0,0,0-1.0,0-0.5.

DISCUSSION

Internal conversion electrons from the decay of Tc-99m, with energies of 119, 137, and 140 keV were responsible for the latent image produced in photographic emulsions. These energies are rather too high to provide good autoradiographic resolution. Since further dilution of the emulsion in order to produce a very thin emulsion layer gave stress-edge artifacts because of the irregular surface of a frozen section of bone, the autoradiograms of Tc-99m-labeled frozen sections could not be as good as those obtained from tritium-labeled paraffin sections. The surface irregularities could be eliminated by celloidin coating, but this increased the distance from the radioactive source to the emulsion. In a resolution study of Tc-99m with a dry autoradiographic technique on stripping film (AR-10),‡ Driessen et al. (*27*) found the resolution to be 22.6 μ, and they concluded that good histological localization was attainable, but reliable intracellular localization was not.

In our study, uptake of Tc-MDP was found in both mineralization and resorption surfaces, and the uptake coincided with the golden ultraviolet-induced fluorescence of tetracycline when this was given with a short labeling time, 3 hr before killing. In mineralization areas, particularly high accumulations of the bone-seeking agent were seen in the zone of provisional calcification. This zone was intensely labeled with tetracycline, but it was mainly the calcified cartilage bars next to the vascular loops that were tagged with the tracer. In the provisional calcification of the articular cartilage,

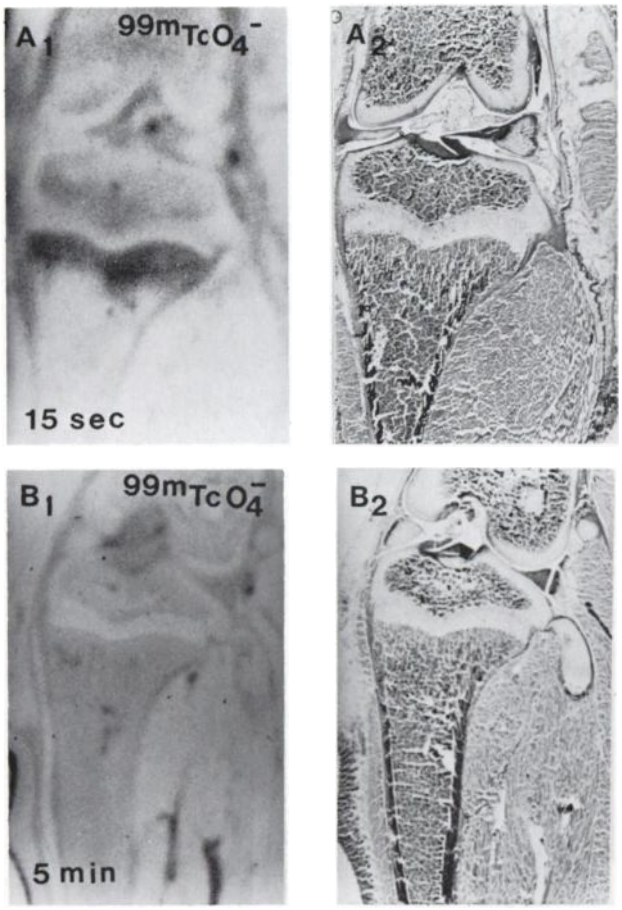

FIG. 8. Blood flow of knee region illustrated by the distribution of pertechnetate given intravenously 15 sec before arrest of flow. (A_1,B_1) Macroautoradiograms. (A_2,B_2) H & E staining of neighboring sections (A:X8, B:X6). Whole metaphyseal area appears highly vascularized, but with low extraction rates as compared with other tissues, such as skin (B: 5 min). Blood flow alone does not seem to account for high uptake of bone-seeking agents at provisional calcification (Fig. 2).

however, diffusion of Tc-MDP occurred from both the bone and from the synovial fluid, with radioactive labeling at both the osteochondral junction and the superficial surfaces of the calcifications facing the joint space. The uptake in calcified cartilage disagrees with the observation of Guillemart et al. (*28*), who found no accumulation of bone-seeking agents in the cartilage whether calcified or not.

For more than 20 years tetracycline has been used as a fluorescent probe specific for areas of mineralization. It is assumed that it is bound to Ca orthophosphate (*29*) or to the surface of the apatite crystal by chelation with calcium (*30,31*).

Resorbing surfaces, however, are also known to be visualized with tetracycline. Harris et al. (*32*) found that resorption cavities showed tetracycline fluorescence immediately after an intravenous dose, but this fluorescence was short-lived, and if 3 days had passed between the injection and sacrifice, this fluorescence was not then noted. In a study with triple fluorocrome labeling with intervals of 24 hr on young rat tibias (*33*), it was found that most of the Howship's lacunae were labeled with the last-given fluorocrome. The tetracycline is either loosely bound in these areas, or continuous osteoblastic activity perhaps causes dissolution of the tetracycline-labeled crystals.

The accumulation of Tc-MDP was not related to the production of collagen or to the alkaline phosphatase enzyme, although the overall distribution of enzyme activity in bone roughly paralleled the deposition of bone-seeking agents.

The vascularity of bone is not considered to be the only factor determining uptake. The entire metaphyseal bone was found to be well vascularized. Thus blood flow alone is not likely to explain the particularly high uptake in the calcified cartilage bars or the spotty uptake in resorption lacunae.

Loss of activity by decalcification with EDTA suggests that bone mineral is the target for the bone-seeking agents. The released activity in the decalcification fluid was compared with the five conceivable Tc-99m compounds, but it could not be distinguished from Tc-MDP. The fact that Tc-MDP remains as a unit in vivo contradicts the proposed theory of dissociation at the crystal surface (*34,35*).

The chemisorption of diphosphonates to the calcium phosphate mineral phase causes retardation of mineral ion efflux and influx from mineral surfaces as well as

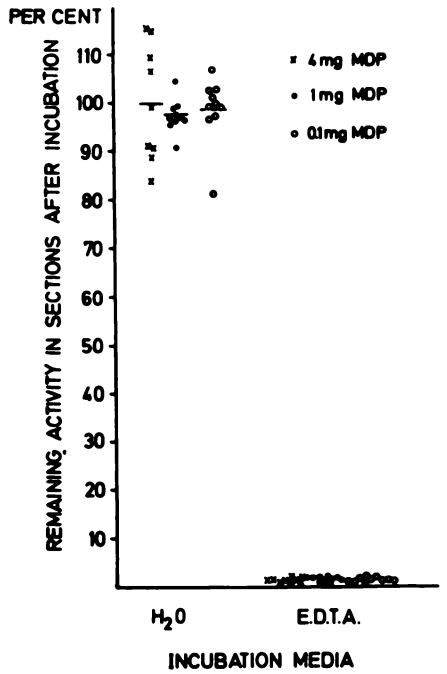

FIG. 9. Decalcification experiments on sections labeled in vivo with Tc-MDP and incubated in either water or 4% EDTA. Almost complete loss of activity from EDTA-incubated sections is seen, and released radioactive compound could not be distinguished from Tc-MDP by chromatography.

inhibition of precursor calcium phosphate to crystalline apatite (*2,3*). This effect of diphosphonates is used clinically in disorders of ectopic mineralization (*36*) and in disorders of excessive bone resorption such as in Paget's disease (*37*). This disease is characterized by an increased osteoclastic bone resorption and by disorganized formation of new bone, so that weak and immature woven bone replaces normal lamellar bone. While the osteoblastic activity of the disease is usually regarded as secondary to resorption, it nevertheless often dominates the scene (*38*). The selective affinity of Tc-MDP for mineralization and resorption surfaces found in this study gives histological support for the clinical effect of diphosphonates if the known inhibitory effect of these agents on crystal dissolution is borne in mind. The observed accumulation of labeled diphosphonates in resorption lacunae, as well as the histological observation that small but therapeutic dose levels of diphosphonate (HEDP) do not cause increased unmineralized osteoid in pagetic lesions (*39*), cast doubt on the suggestion (*40*) that a mineralization defect is a necessary prerequisite for diphosphonates to be effective.

As for the kinetics of calcium in bone, the findings of this study indicate that the uptake of Tc-99m phosphorus compounds is not necessarily correlated with the degree of apatite formation alone. Regarding the uptake in resorption surfaces, greater similarities are seen with the initial, short-term uptake of Ca-45 (*41*), where intense labeling is seen over resorption cavities and where highly mineralized, apparently nongrowing surfaces are labeled as well within the first day after administration of the radionuclide.

Not until several days after radionuclide administration is labeling only of poorly mineralized bone-forming surfaces seen. The initial uptake of calcium was supposed to depend on ion exchange and to occur on surfaces in contact with circulating fluid, but it seems possible that the distribution of exchangeable bone calcium could also depend on the available free surface area of bone mineral.

It is likely that the uptake of skeletal imaging agents, such as the Tc-99m phosphates and phosphonates, is determined by both the vascularity and the composition of the surface area of the apatite crystal, i.e., the larger the area available for adsorption, the higher the uptake. In apatite crystals just nucleated, the specific surface area (m^2/g) is high and there is a high ratio between the growing basal face perpendicular to the C-axis (001 face) and the other more inert prismal faces. It is possible that the Tc-99m compounds of phosphorus have a selective affinity for the 001 face, as proposed by Francis et al. (*42*).

In mature bone the available adsorption area may be further diminished because of crystal-collagen interference. In bone, apatite is found both as interfibrillar crystallites (*43*) and within the collagen fibrils occupying the gap between molecular ends of collagen, with adjacent molecules staggered to overlap the gap (*44,45*). The collagen molecules contain polar groups capable of hydrogen bonding with apatite surfaces. As suggested by Lee (*46*), the crystals are thought to fix the collagen molecules via etch pits in the basal plane (001 face) where cross links between molecular ends are embedded, thereby stiffening the bone.

Most important regarding the uptake of Tc-99m compounds of phosphorus is the fact that collagen occupies chemical groups on the apatite crystal surface, decreasing the available surface area. In a measurement of specific surface area using the method of Brunauer, Emmett, and Teller (B.E.T. method) (*47*) of low gas adsorption, Holmes et al. (*48*) showed that the B.E.T. technique gave the same specific surface values as those measured by the x-ray scattering method only when the mineral was freed from collagen by anhydrous ethylenediamine treatment. Thus, at least in the dry state, even the smaller nitrogen molecule used in the B.E.T. technique, could not penetrate the collagen screen. This blocking of the adsorption surfaces by collagen could explain the difference in uptake found in areas of mineralization and mature bone, as well as the uptake seen in connection with active osteoclasts, which dissolve both the matrix and the mineral component of bone. In these resorption areas, free crystals are located between the frayed surface of the bone and the ruffled border of the osteoclasts, between the cytoplasmatic processes of the ruffled border (*49–51*), and within intracellular vacuoles of the osteoclasts (*52*). These crystals are assumed to be the target of bone-seeking agents in resorption areas.

FOOTNOTES

* New England Nuclear Co, Boston, Mass.
† Ilford
‡ Kodak
‖ Sigma 22508
§ Leitz Ploem Opak fluorescence microscope, type G filter.
¶ GBI labs. Ltd. Manchester, England.
** Macklett OEG-50.
†† Merck
‡‡ Gelman No. 61885

ACKNOWLEDGMENT

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MIDEASTERN CHAPTER
SOCIETY OF NUCLEAR MEDICINE
11th ANNUAL MEETING

April 9-11, 1981 **Uniformed Services University of Health Sciences** **Bethesda, Maryland**

The 11th Annual Meeting of the Mideastern Chapter will be held on the campus of the Uniformed Services University of Health Sciences, adjacent to the Naval Hospital in Bethesda. The program will include invited speakers, teaching sessions, submitted papers, and exhibits. The theme is Functional Imaging, but papers will be presented on other topics. AMA Category 1 credit available.

A limited number of rooms have been reserved at the Marriott, Bethesda, 2 Pooks Hill Road, Bethesda, MD 20014; (301) 897-9400. Contact the Marriott for reservations.

For further information, write or phone the Program Chairman listed below or E.U. Buddemeyer, Sc.D. (301)528-6890.

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EASTERN GREAT LAKES CHAPTER
SOCIETY OF NUCLEAR MEDICINE
SECOND ANNUAL MEETING

May 15, 1981 **The Prince of Wales Hotel** **Niagara-on-the-Lake, Ontario**

The Eastern Great Lakes Chapter of SNM announces its Second Annual Meeting to be held May 15, 1981 at The Prince of Wales Hotel, Niagara-on-the-Lake, Ontario, Canada.

The program will include continuing education courses to be given by: Dr. Michael Loberg on "Hepatobiliary Radiopharmaceuticals and Imaging"; Dr. Terry Mandel on "Krypton-81m Gas Generator and Imaging"; and Dr. Günes Egē on "Lymph Node Imaging."

The technologist program will include in-depth lectures on "Hepatitis Testing and Infection Control" by Mr. Tom Dias and "Processor Quality Control" by John Blanowicz.

There will also be a presentation of contributed papers. Abstracts, typed single-spaced, not to exceed 300 words including title, author(s), and address, should be mailed to:

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Deadline for submission of abstracts is March 6, 1981.