^{99m}Tc-POLYPHOSPHATE: HISTOLOGICAL LOCALIZATION IN HUMAN FEMURS BY AUTORADIOGRAPHY

Robert L. Tilden, John Jackson, Jr., William F. Enneking, Frank H. DeLand, and James T. McVey

Veterans Administration Hospital and University of Florida, Gainesville, Florida

Extranuclear electrons of metastable technetium make it possible to obtain autoradiographic resolution that extends to the limits of light microscopy. Autoradiographs show the direct correlation of this scanning agent and the substructures of bone tissue. Experiments are described that are part of an effort to learn of the biological organization and fixation mechanisms for ^{99m}TcSnPP in bone tissue. Microautoradiography may also be used to make more precise dosimetry calculations at the cellular level. Knowledge of the quantitative and qualitative deposition of ^{99m}Tc-polyphosphate will be of primary importance in defining the precision, sensitivity, and specificity of bone scanning with this agent.

Metastable pertechnetate complexed to polyphosphate with stannous chloride (^{99m}TcSnPP) is currently receiving much attention by clinicians for its excellent properties as a bone scanning agent. Technetium has a short half-life of 6 hr and produces essentially monoenergic gamma rays of 140 keV. The degree to which ^{99m}TcSnPP localizes in bone is related to factors of medical importance such as skeletal metastasis, pyogenic osteomyelitis, and bone repair (*1*). However, the chemical and physiological mechanisms by which this radiopharmaceutical is localized and fixed to bone are not known. The technique described in this paper provides a useful approach for defining the fixation process more clearly.

High-resolution autoradiography of some gamma-emitting isotopes is possible by using extranuclear beta particles of low penetrating power (*2–5*). From the decay of metastable technetium, there is an abundance of non-nuclear radiation contributed by internal conversion and Auger electrons. The internal conversion electrons are emitted with the major line spectra at 1.4 keV and with an abundance approximately equal to that of the 140-keV gamma photons. The Auger spectrum is below 0.5 keV and equally as abundant (*6*). The excellent autoradiographic resolution obtained with radioactive technetium was less than 0.4 micron. Three factors contribute to the resolution: (A) the low penetrating power of the energies; (B) the extremely thin sections (1 micron) of undecalcified bone; and (C) a monolayer of photographic emulsion that provides a geometry that reduces the lateral scatter of electrons. The extranuclear x-rays and nuclear gamma rays do not adversely affect the photographic results.

Technetium-99m-polyphosphate bone scans were performed before surgery on patients scheduled for insertion of a total-hip prosthesis because of severe degenerative hip disease. Rectilinear scans showed abnormal increased concentration of radioactivity in the involved hip. Three hours before the resection of the femoral head, ^{99m}Tc-polyphosphate was again administered.

METHOD

Macroautoradiography and microautoradiography were carried out on each specimen. Macroautoradiography refers to mapping radioactivity of an entire cross section of the femoral head, and microautoradiography refers to the distribution at the cellular level. After receiving the specimen at surgery, 2-mm-thick coronal sections were cut with a water-cooled rotary saw.

Macroautoradiography of ^{99m}TcSnPP in bone. Macroautoradiographs of 2-mm-thick coronal sections of the femoral head were obtained by direct contact with 16-hr exposure on x-ray spot film (DuPont SF-2). By this method the overall relationship

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For reprints contact: Frank H. DeLand, Nuclear Medicine Service, VA Hospital, Gainesville, Fla. 32601.

FIG. 1. Bone from subchondral area stained with Richardson's methylene blue azure II. Silver granules representing radioactivity due to ^{99m}Tc-label on polyphosphate defines area of bone that borders marrow space that appears more immature than surrounding bone by histologic staining. (X 450)

between sites of radioactivity and gross-bone structure were obtained.

Microautoradiography of ^{99m}TcSnPP in bone. Because of the low-energy emissions producing the autoradiographs, intimate and uniform contact between the specimen and the photosensitive material was important and best achieved with microautoradiography using a nuclear tracking emulsion. Smaller sections (2 × 2 × 3 mm) were cut from the subchondral and cancellous portions of the coronal sections and then fixed, postfixed, dehydrated, and embedded according to Luft's method (7), with one exception: the time allowed for embedding was reduced from 24 to 10 hr to minimize loss of radiation due to decay. Just before cutting the thin sections, radioactivity was confirmed in the embedded specimen by counting in a deep well scintillation counter. One-micron-thick sections were cut with a glass knife on the Porter-Blum MT2 ultramicrotome. After the sections were fixed on glass slides, they were dipped in Ilford-K5 nuclear tracking emulsion at 14 hr (2.3 technetium half-lives from the time of surgery). The exposure time for microautoradiography was 8 hr. After the emulsion was photographically processed with Microdol-X and Kodak rapid fix, histologic stains were applied through the emulsion using Richardson's methylene blue and azure II to accent bone detail and density. Separate slides were stained with hematoxylin and eosin to emphasize nuclear material. Photomicrographs were obtained with Panatomic-X and Kodachrome II, 35-mm films.

RESULTS

The silver grains of the emulsion that reflect the locale of the radioactivity are arranged in precise patterns that show: (A) relationships to different stages of bone maturity; (B) proximity to bone marrow; and (C) relationships to osteocytes.

The photomicrographs (Fig. 1–4) were selected

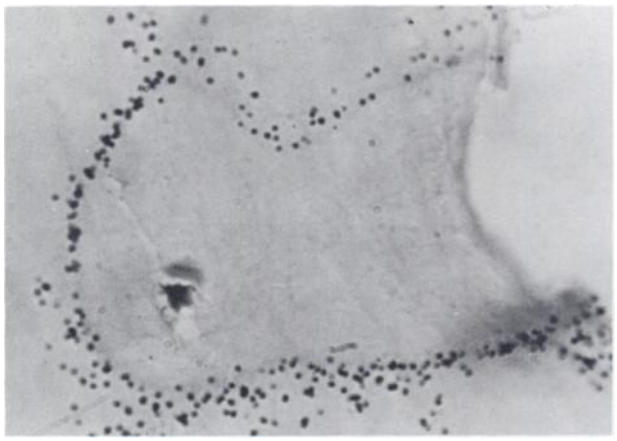

FIG. 2. Bone from subchondral area stained with H & E to emphasize nuclear material. Again lacuna is seen surrounded by localized radioactivity that extends to surface of marrow space. (X 950)

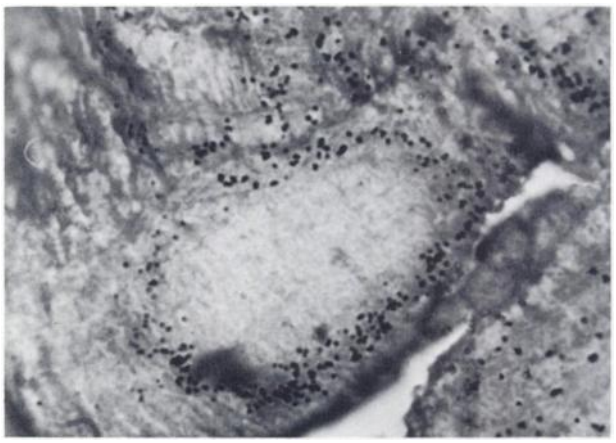

FIG. 3. Bony trabecula in cross section taken from subchondral area and stained with Richardson's methylene blue. Paler, more densely mineralized core is surrounded by less densely mineralized bone over which silver granules indicate concentration of radioactivity. H & E stains of adjacent sections show presence of osteocytes, indicating that this is living bone. (X 950)

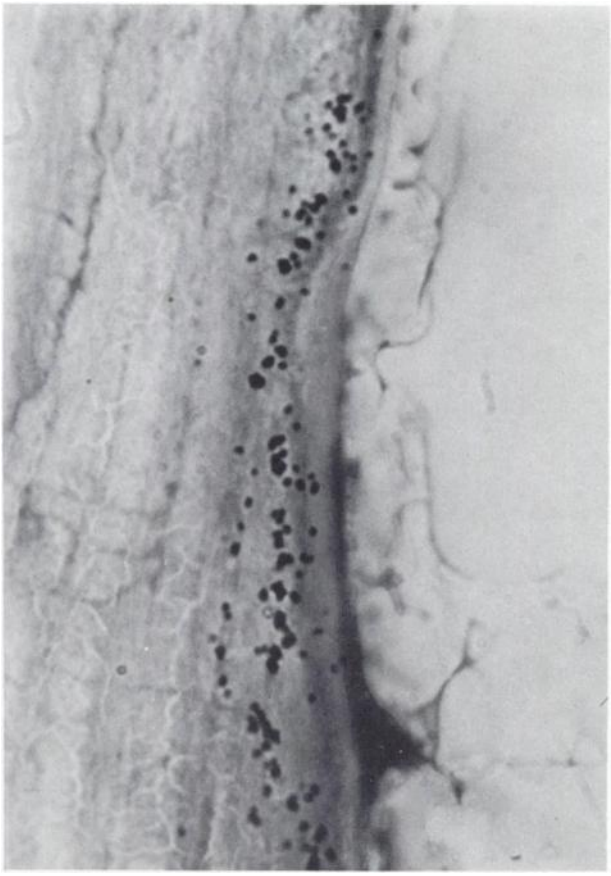

FIG. 4. Section of cancellous bone stained with Richardson's methylene blue. Fatty marrow lies to right. On left is trabecula of bone. Band of osteoid lies between bone and marrow. As indicated by overlying silver granules, radioactivity seems to be just beneath osteoid. H & E stains on adjacent sections verified that this was living bone. (X 950)

from regions of viable bone as indicated by the presence of osteocytes, identified on the histologic preparations stained with hematoxylin and eosin.

The ^{99m}Tc-polyphosphate was deposited predominately in linear patterns at the junction of bone of different maturities. Figures 2, 3, and 4 were photographed from 1-micron sections stained with Richardson's methylene blue and azure II, which stain bone maturity differentially. In Fig. 3 a small trabecula of bone has been cut in cross section. The histologic stains indicated that the core, surrounded by a discrete line of radioactivity, was more mature than the peripheral bone. There are scattered grains of activity in the less mineralized and lesser mature bone.

In a thin section of cancellous bone (Fig. 4) the line of radioactivity lies at the interface of osteoid next to the marrow cavity and the mineralized bone.

Localization of ^{99m}Tc-polyphosphate has been compared with results reported in the literature (*8*) using ^{85}Sr and ^{45}Ca and with results obtained at this institution with ^{45}Ca (*9*). At this time we have not been able to draw any firm correlations between our findings and those with other radionuclides.

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