Gallium-67 Citrate Localization in Osteoclast Nuclei of Paget's Disease of Bone

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Gallium-67 citrate scintigraphy has been used to indicate the extent of bone involvement in patients with Paget's disease of bone and is an excellent marker in monitoring the effects of specific therapy. Since gallium uptake is dependent on cellular function, autoradiographic techniques can be applied to cells of Paget's lesions to understand better the mechanism of [^{67}Ga]citrate uptake. Bone biopsies were obtained from sites of increased uptake using [^{67}Ga]citrate scintigraphy in two patients with Paget's disease. In both patients electron microscopic autoradiographs demonstrated a high concentration of silver grains over the nuclei of osteoclasts. The cellular mechanism is unknown but may be related to the known inhibitory effect of calcitonin on osteoclast activity. The association of [^{67}Ga]citrate with the nucleus of the osteoclasts is unique and different from tumor cells in which there is a high association of [^{67}Ga]citrate with the lysosome fraction within the cytoplasm.

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In 1969, Edwards and Hayes demonstrated the clinical value of gallium-67 (^{67}Ga) citrate scintigraphy in the detection of soft-tissue tumors and infections (*1*). Although numerous publications have since appeared demonstrating the effectiveness of gallium scans in localizing a wide spectrum of tumors and infectious processes, no definite mechanism of [^{67}Ga]citrate uptake has been proven. Several studies have demonstrated radioactive gallium citrate localization in the cytoplasm of tumor cells, mainly in the lysosome fraction (*2–11*). Autoradiographs of [^{67}Ga]citrate labeled tumor cells examined by light microscopy have demonstrated cytoplasmic localization (*9*). Autoradiographic studies utilizing the electron microscope have localized the isotope to electron dense, single membrane bound subcellular organelles which are typical of lysosomes (*2*). Manfredi et al. in 1978, demonstrated that [^{67}Ga]citrate was localized as an amorphous electron dense body in single membrane-bound lysosome-like cytoplasmic organelles and to a lesser extent in the nuclei and cytoplasm (*11*). Recent work attributes the transport of injected gallium to transferrin (*12–17*). Localization is then believed to occur within lysosomes and endoplasmic reticulum (*18*).

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Gallium-67 citrate scintigraphy in Paget's disease of bone was discussed by Waxman and colleagues (*19*). This group determined that patients with Paget's disease of bone demonstrated intense gallium citrate activity at the site of the pagetic lesion similar to the abnormalities noted on technetium-99m diphosphonate bone scans in patients with Paget's disease. However, unlike the bone scan the gallium citrate scan accurately reflected changes in Paget's disease as influenced by treatment with calcitonin, which is known to decrease osteoclast numbers and inhibit osteoclast activity (*20*). This group postulated that gallium uptake is a more appropriate measure of activity of Paget's disease than a noncellular marker such as a technetium-99m diphosphonate containing bone scan agent. At that time, however, it was not clear which cellular population in the pagetic lesion was responsible for the increased gallium concentration. In addition therapeutic amounts of gallium are known to inhibit bone resorption and can be used to treat hypercalcemia (*21*).

The purpose of the current study was to determine whether autoradiography performed on a specimen from the bone lesion of patients with known Paget's disease of bone could help explain the excellent correlation of the [^{67}Ga]citrate scan with the degree of success or failure of therapy in patients with Paget's disease.

The current study describes electron microscopic autoradiographs obtained from two patients with Paget's

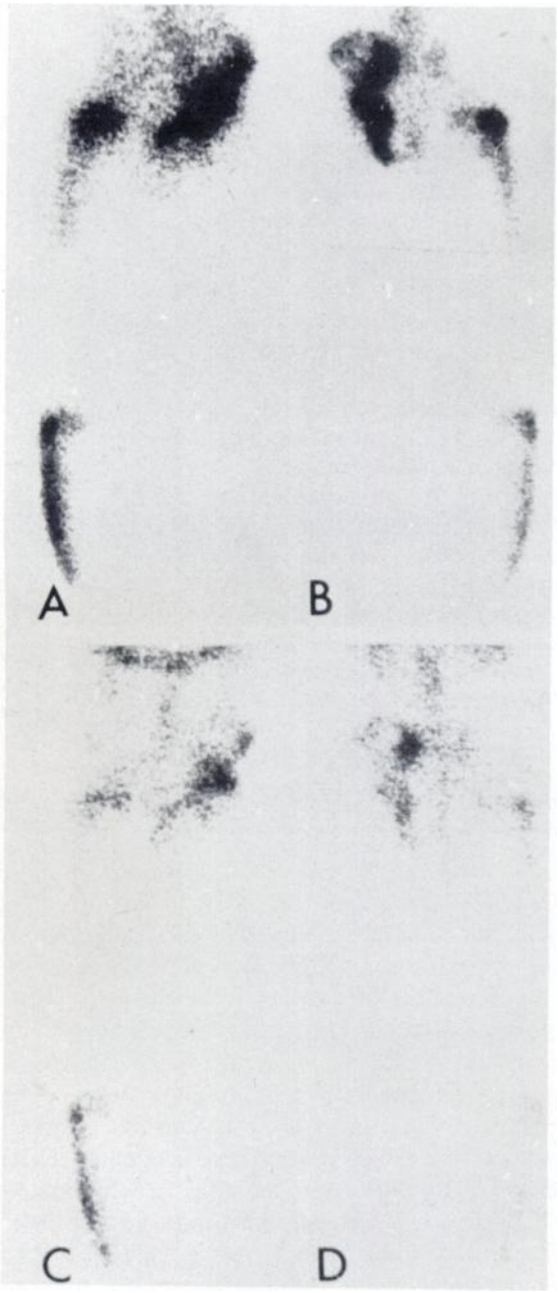

FIGURE 1
Gallium scans of patient with Paget's disease of bone in the head of the right femur, ilium and tibia. A is anterior view, B is posterior view prior to treatment. C and D are anterior and posterior gallium scans of same lesions five months following daily treatment with 0.5 mg human calcitonin per day (× 7,500).

disease in whom silver grains marking [^{67}Ga]citrate were localized in the nuclei of osteoclasts to a markedly greater concentration of grains than in the cytoplasm, or in other cells in the Paget's bone lesion. Thus, these findings correlate well with the changes in osteoclast numbers and function noted after calcitonin treatment (*20*).

METHODS AND MATERIALS

Two patients with classic radiographic features of Paget's disease of bone were studied by electron microscopic autoradiography. Confirmation of the diagnosis was made by bone biopsy. The patients underwent serial total body bone scans with a dual-headed rectilinear scanner and Anger camera after intravenous injection of 5–7 mCi of [^{67}Ga]citrate. The gallium scans were performed 48 hr after injection. Five months following the initial scan after calcitonin treatment a diagnostic [^{67}Ga]citrate scan was again performed.

Iliac crest biopsies were performed 48 hr postinjection immediately following the scan using a Meunier trephine. The bone was fixed in 5% cold glutaraldehyde in phosphate buffer for 2 hours without demineralization or post-fixation in osmium tetroxide. Dehydration and embedment in Spurr plastic (Polysciences, Warrington, PA) followed by polymerization was accomplished at 70° C within 24 hr. Sections were cut on an ultratome and carefully mounted on one hole grids held in place by the formvar (Ted Pella, Inc., Tustin, CA) film deposited on glass slides (*22*). After staining the bone sections on the grids with uranyl acetate and lead citrate the photographic emulsion was applied by dipping the slides holding the sections

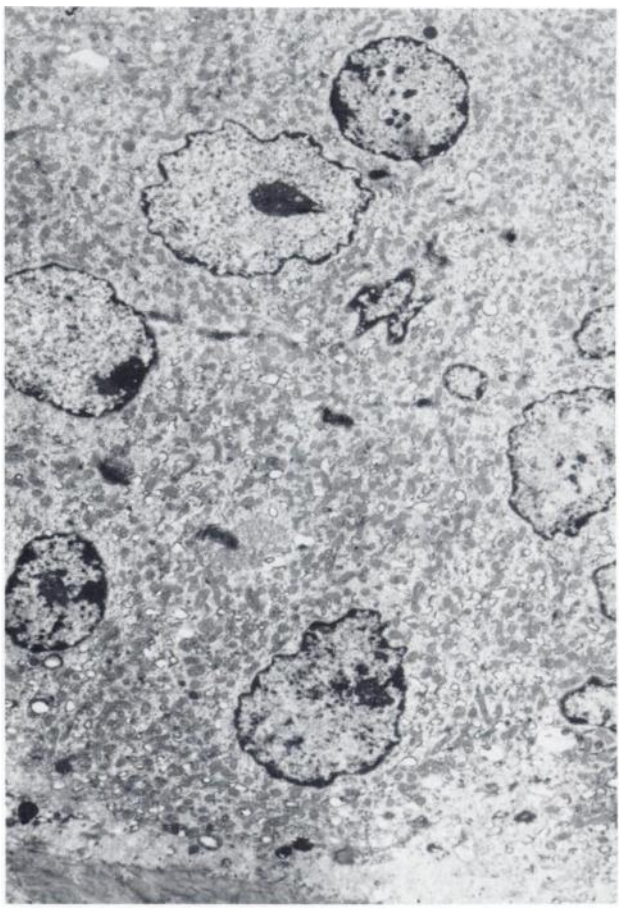

FIGURE 2
Negative control. Electron autoradiograph of Paget's osteoclasts adjacent to bone (lower left) after gallium scan and prior to treatment. Section has been demineralized removing ^{67}Ga. Several nuclei can be seen illustrating lack of silver grains (× 6,500).

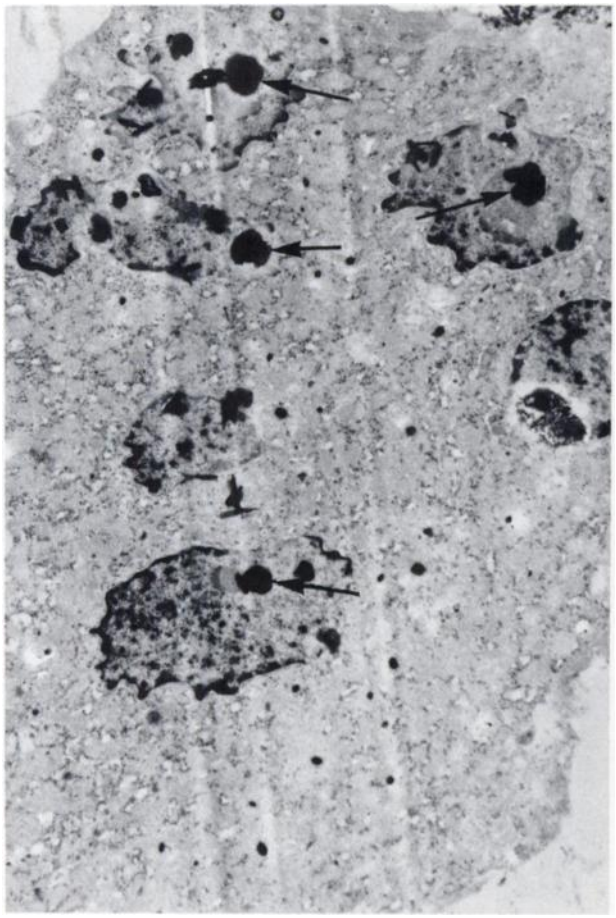

FIGURE 3
Electron autoradiograph of Paget's osteoclast after gallium scan from same specimen as in Figure 2. Note large accumulations of silver grains over nuclei (arrows) marking location of [^{67}Ga]citrate deposition. Undermineralized (× 8,000).

mounted on grids in diluted Kodak NTB-2 nuclear track emulsion in the dark. The sections on grids adherent to glass slides were air dried and allowed to develop for 3–5 days in the cold. The autoradiographs were developed with D-19 and fixed with F-10. The one hole grids were removed from the glass slides and the bone sections examined by electron microscopy performed in a Ziess EM-9S or an AEI Corinth 500 electron microscope at 60 kV. Radioactive specimens were compared to sections of the same biopsy from which the [^{67}Ga]citrate had been removed by EDTA prior to electron microscopic examination as a negative control.

RESULTS

The [^{67}Ga]citrate scan for one patient is shown in Figure 1, illustrating the skeletal abnormality due to Paget's disease detected with [^{67}Ga]citrate, prior to treatment (A and B). Following treatment a reduced uptake was seen, (Fig. 1, C and D) illustrating the marked decrease in uptake after calcitonin therapy. Electron microscopic autoradiographs taken from bone sections at the time of the [^{67}Ga]citrate scan prior to treatment of each patient appear in Figures 2–5. Figure 2 shows a typical Paget's osteoclast from which the gallium has been removed by EDTA prior to autoradiography illustrating a lack of electron dense particles (negative control). Figure 3 is an electron microscopic autoradiogram made following a ^{67}Ga scan that identifies ^{67}Ga induced deposition of silver grains (arrows) predominantly localized as amorphous electron dense bodies over the nuclei of large multinucleated giant cells (osteoclasts) typical of Paget's disease. The densities were not exclusively associated with nuclear membranes or any recognizable component such as the nucleoli (Fig. 4). A few densities were found on lysosomes but the cytoplasm of the osteoclasts was sparsely populated with silver grains (Fig. 5). Other cells such as macrophages, lymphocytes, and polymorphonuclear leukocytes were also labeled occasionally. However, the concentration of particles on osteoclast nuclei in both patients was striking (Compare Fig. 3 with Fig. 5.)

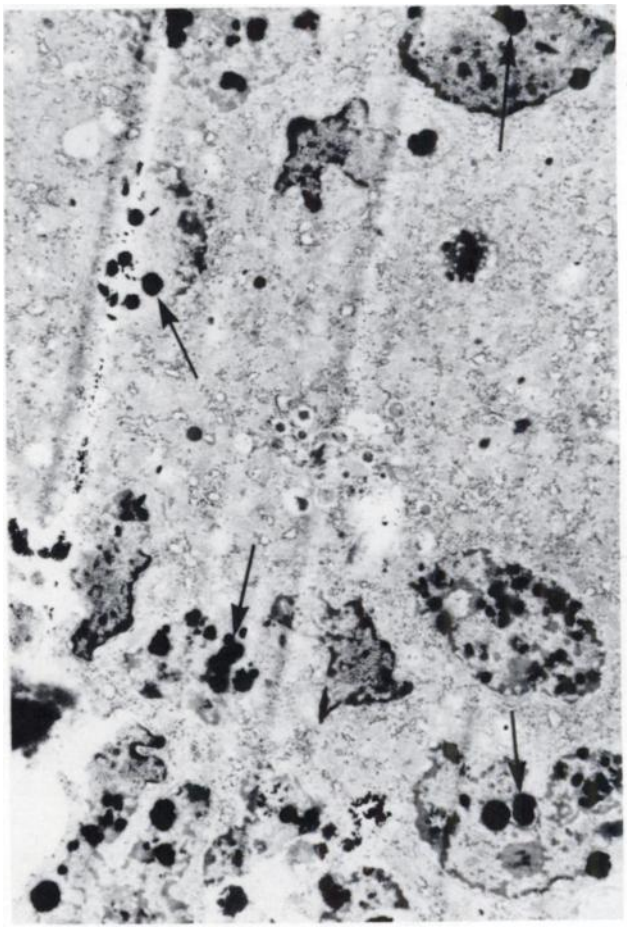

FIGURE 4
Electron autoradiograph of another Paget's osteoclast illustrating localization of ^{67}Ga over nuclei. The Paget's osteoclast also illustrates concentration of ^{67}Ga over nuclei but not over osteoclast cytoplasm despite knife marks on the section, thus eliminating artifact as the source of the deposition of silver grains marking radioactivity (× 7,500).

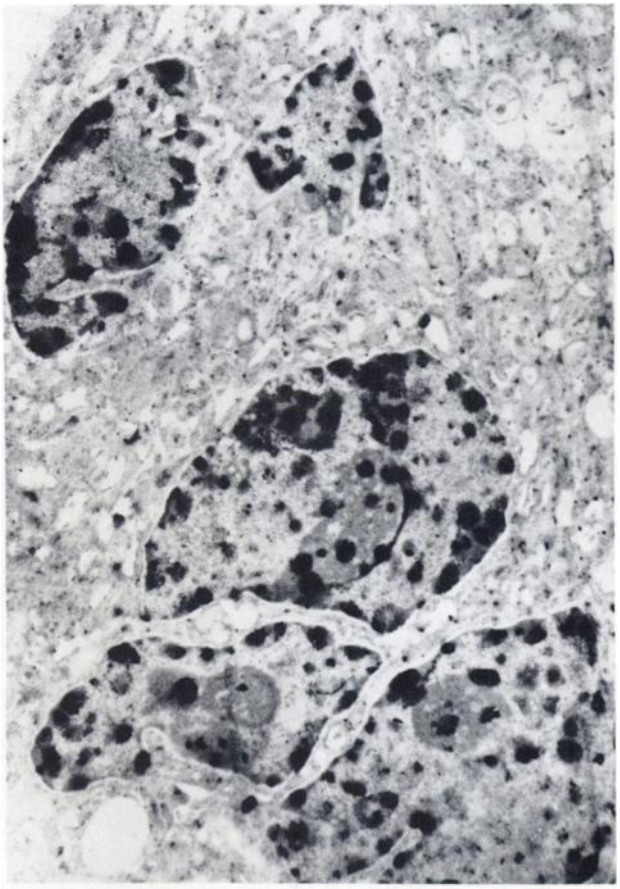

FIGURE 5
Second Paget's patient—section not demineralized. Note concentration of ^{67}Ga over nuclei as in lesion of first patient. The higher magnification shows that the cytoplasm is again almost free of silver grains (× 10,400).

DISCUSSION

The localization of [^{67}Ga]citrate over the nuclei of osteoclasts in patients with Paget's disease of bone is well demonstrated using autoradiographic techniques. The mechanism of uptake may be related to specific physiologic, pathologic and therapeutic observations. Osteoclasts are known to take an active part in the growth and remodeling of bone occurring in the metaphysis of young animals (*23*). The fact that gallium is effective in treating the hypercalcemia of neoplasia as reported by several investigators (*21,24*) correlates well with the possible inhibition of osteoclast function by gallium (*21*). Increased uptake of [^{67}Ga]citrate in the skeleton is reported in hyperparathyroidism and renal bone disease (*24*). Both of these diseases are associated with increased numbers and activity of osteoclasts. Calcitonin is known to decrease the number and activity of osteoclasts in Paget's disease (*20*). Treatment of pagetic patients with calcitonin who were sequentially followed using ^{67}Ga scintigraphy demonstrated a decrease of ^{67}Ga on bone scans. This finding correlated well with biochemical parameters used to measure response to calcitonin therapy (*19*).

The precise spatial and functional relationship of ^{67}Ga activity to the nucleus of the osteoclast is not clear. The cytoplasmic increase found in tumor cells may be secondary to a mechanism of accumulation which is entirely different than that seen in the osteoclasts of Paget's disease. The exact binding site within the nucleus is unclear, but may represent a co-factor with a high affinity for iron or iron-like elements.

Nuclear accumulation of [^{67}Ga]citrate by the osteoclast in patients with Paget's disease appears to be the single most important factor in determining the extent of gallium citrate accumulation on scans of patients undergoing gallium studies. Reduction in osteoclast concentration in patients undergoing calcitonin therapy appears to explain the reduction of [^{67}Ga]citrate activity seen on the gallium scan following calcitonin therapy.

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