# Calcification and Uptake of Tc-99m Diphosphonates in Neuroblastomas: Concise Communication

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**Sixty-six percent of 54 patients with neuroblastoma demonstrated uptake of bone-seeking radioagents by the primary tumor. This is a higher incidence than previously reported. Uptake was slightly more common in abdominal than thoracic tumors. There was a significant correlation between the size of the tumor and tracer uptake. Calcification was demonstrated in the primary tumor in almost 90% of the 54 patients. This is a much higher incidence of calcification than previously reported. Microscopy shows that the calcification is not always due to tumor necrosis; it also occurs in areas of viable tumor cells. Tracer uptake is believed to be related to calcium metabolism. The rate of metabolic activity rather than the total amount of calcium present within the tumor may be the most important factor in determining the amount of uptake. No significant relationship was found between tracer uptake and tumor stage or homovanillic acid and vanillylmandelic acid metabolic activity.**

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Calcification in primary neuroblastoma masses is well recognized and is helpful diagnostically in distinguishing this tumor from Wilms' tumor. This calcification has been reported in up to 50% of patients with neuroblastoma (*1*) and was believed due to necrosis in parts of the tumor mass. This study demonstrates that calcification may be found in almost every neuroblastoma and that it occurs in sites of living tumor cells as well as in areas of necrosis.

There have been reports of uptake of bone-seeking radiotracers (Tc-99m diphosphonates) by neuroblastoma masses (*2,3*). We find a higher incidence of such uptake. This study seeks a possible explanation for this, and also discusses the mechanism by which the uptake occurs.

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## MATERIALS AND METHODS

During the period 1973–1981, 54 patients with neuroblastoma had diphosphonate bone images made as part of their initial evaluation. In all cases the diagnosis of neuroblastoma was confirmed by microscopy of either the primary tumor or biopsy tissue obtained from bone marrow, lymph node, liver, or pleural fluid.

The scintigrams were done with Tc-99m-tagged hydroxyethylidene diphosphonate (HEDP) or methylene diphosphonate (MDP). In all cases the region of the tumor was imaged as well as the skeleton. The dose of radiotracer was calculated according to a formula offered by Webster et al. (*4*). Each patient was imaged 2–3 hr after injection of tracer. Fifty-four patients had abdominal radiographs, and 35 of the children had transmission computerized tomography (TCT) of the primary tumor mass. In all cases 1- or 1.5-cm contiguous TCT slices were displayed through the area of the tumor.

Calcification of the primary tumor was evaluated on the plain abdominal radiographs, the TCT, and when

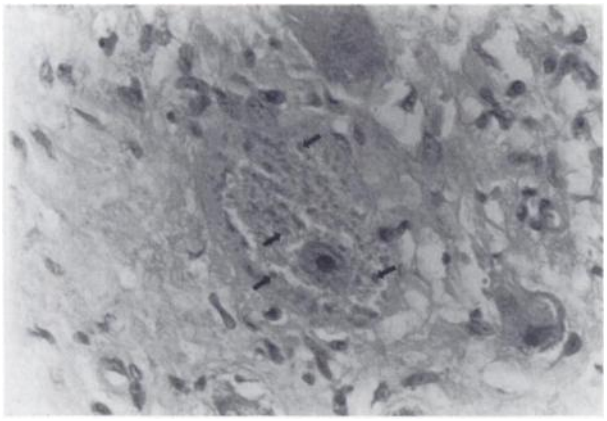

FIG. 1. Microscopic calcification (arrows) within neuroblastoma cell (X750).

available, by microscopy of the primary tumor mass. Lymph-node and marrow biopsies were not evaluated for calcification.

Twelve- and 24-hr urinary excretions of vanillylmandelic acid (VMA) and homovanillic acid (HVA) were determined in 44 cases. VMA-to-HVA ratios were available for 27 of these patients. VMA was measured by the method of Pisano-Crout-Abraham, and HVA by the method of Goldenberg and Friedland. Normals are <20 μg HVA per milligram creatinine, and <12 μg VMA/mg creatinine.

It is difficult to calculate tumor volume accurately. In order to permit comparisons, an index was calculated for tumor size. It is the product of the greatest height, width, and anteroposterior dimension). These were obtained from TCT, or from plain film or pathological specimen when TCT was unavailable. This tumor index could be determined for 46 cases.

## RESULTS

There were 28 females and 26 males in the study. The mean age at tumor diagnosis was 20 mo. Primary tumor tissue was available for microscopy in 34 patients. Of these, 16 had total excision of the primary tumor and 18 had biopsies. In 16 other patients diagnosis was made by biopsy of bone marrow, lymph nodes, liver, or pleural fluid.

**Calcification of the primary tumor mass.** Calcification was found in 45 of the 54 patients. Microscopy was the most sensitive method for identifying calcium, with TCT being better than plain radiography. There was no correlation between the size or site of the tumor and the presence or absence of calcium. Microscopic review showed that the calcification was not always related to necrotic or dystrophic tissue. Only one patient had evidence of tissue necrosis in the area of calcification. Microscopic calcification (Fig. 1) occurred in areas of viable tumor cells. There was no correlation between the presence of microscopic calcification and the maturity of the tumor.

**Diphosphonate uptake by the tumor.** In 33 of the 50 patients, tracer uptake was found in the primary tumor mass. There was no significant correlation between this uptake and VMA and HVA metabolism (Table 1), nor between tumor stage and tracer uptake. Sixty-five percent of the tumors were Stage 4 (those having distant metastases) on initial presentation. The incidence of uptake in this group was 66%. This is the same as the incidence of tracer uptake for the whole tumor population.

There was a statistically significant correlation between the primary tumor mass and the uptake of tracer. Tumors showing uptake were much larger than those in which none could be seen ($p < 0.032$).

Tracer uptake was related to tumor site. There were two pelvic, one neck, 42 abdominal, and nine mediastinal tumors. Uptake seemed more frequent in abdominal primary tumors (69%) than in mediastinal (44%), but the difference was not statistically significant.

There was no statistically firm correlation—by imaging, microscopy, or radiography between the presence of calcium and the uptake of tracer. Anatomically, however, foci of high uptake tended to agree with radiographic or TCT densities (Figs. 2 and 3).

## DISCUSSION

Reports of the incidence of calcification in neuroblastomas range from 25–50% (*1*). In our series calcification has been identified radiographically in 62% of

**TABLE 1. CORRELATION BETWEEN TUMOR METABOLIC ACTIVITY AND Tc-99m DIPHOSPHONATE UPTAKE**

| | VMA (μg/mg creatinine) | | HVA (μg/mg creatinine) | | VMA:HVA | |
|---|---|---|---|---|---|---|
| | uptake + | uptake − | uptake + | uptake − | uptake + | uptake − |
| Mean | 105 | 77 | 87 | 33 | 1.47 | 2.51 |
| Standard deviation | 116 | 70 | 151 | 26 | 1.07 | 3.01 |
| Number of cases | 27 | 15 | 24 | 10 | 18 | 9 |
| p value: | >0.05 | | 0.05 | | 0.05 | |

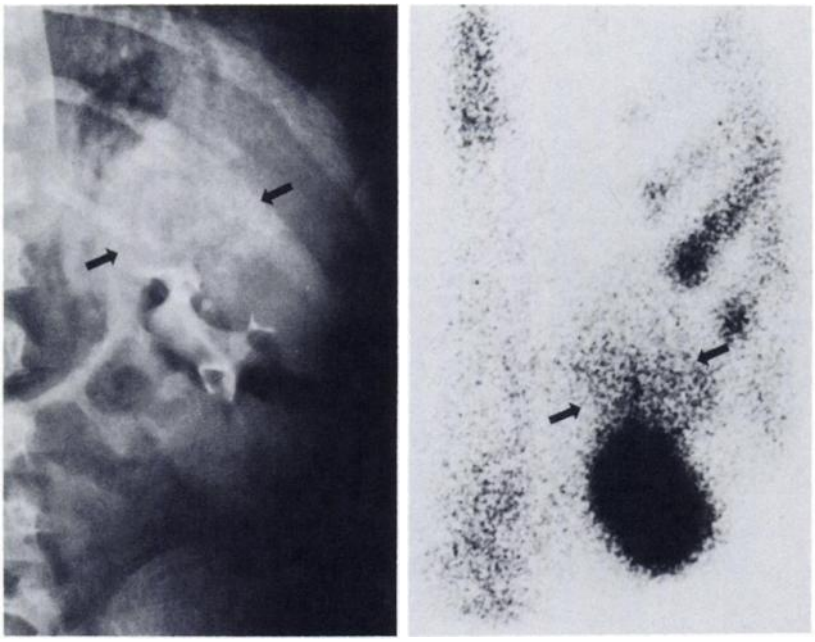

FIG. 2. Tracer uptake in tumor mass (arrows) corresponds to site of calcification seen on plain film (arrows).

patients. This higher incidence may be due in part to our fairly routine use of TCT. When microscopic evidence of calcification is included, our incidence approaches 90%, and the true incidence may be even higher. Firstly, four of the patients in whom no calcium could be demonstrated on TCT or plain radiograph did not provide any tissue from the primary tumor for microscopy. Some of these may indeed have had calcification. In addition, in several cases where calcification was definitely seen on TCT or plain film, it was spotty rather than diffuse, and could easily be missed in a biopsy. Patients with Stage 4 tumors had only biopsy of the tumor, not total resection.

It has been suggested that the mechanism by which the calcification occurs in neuroblastomas is related to tumor necrosis. Our histological studies dispute this. They lead us to believe that calcification is related to some characteristic metabolism in the neuroblastoma cell that results in calcium deposition. This may be independent of other metabolic parameters such as VMA and HVA activity. If the deposition is part of an active metabolic process then one might postulate an ion-exhange mechanism for the tracer uptake. This could occur only in nonischemic tissues, not in necrotic areas, and might explain why tumors such as neuroblastoma take up the tracer whereas calcified lymph nodes (e.g., in histoplasmosis) do not.

Sixty-six percent of the patients in this study showed uptake of Tc-99m HEDP or MDP by the primary neuroblastoma mass. Our figure agrees with the previous report of Young and L'Heureux, who found a 62% incidence of MDP uptake in a series of 16 patients (*2*) but is higher than that of Howman-Giles who reported a 35% incidence of uptake in 63 patients (*3*). The reason for our high incidence is unclear, but it may in part be related to the imaging technique. We routinely did high count and intensity images over the region of the tumor, and this may identify more patients with only a moderate degree

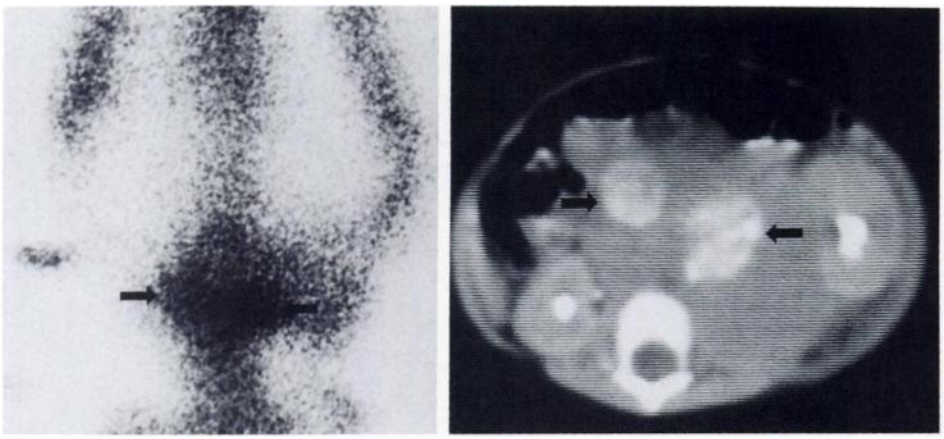

FIG. 3. Tracer uptake in tumor mass (arrows) is concentrated in area of calcification demonstrated by TCT (arrows). TCT Section.

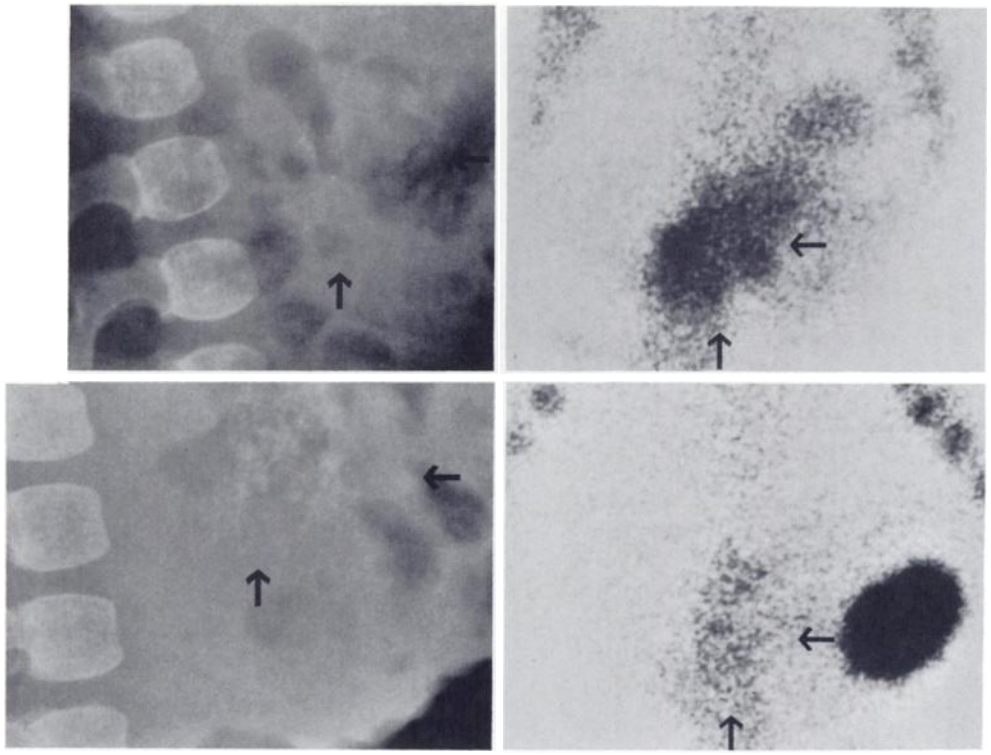

**FIG. 4.** Stage 4 neuroblastoma shows increasing calcification by radiograph, but decreasing uptake of Tc-99m diphosphonate. Initial radiograph and bone image (upper); radiograph and bone image at 5 m of therapy. (Arrows indicate tumor area).

of tracer uptake in the primary tumor. This is suggested because of the correlation between uptake with tumor size. Small tumors can be missed. The low incidence of uptake in the thorax relative to the abdomen may also be explained in terms of technical problems. The ribs may provide a partial shield, masking small areas of uptake in intrathoracic tumors. The abdominal uptake may be higher as well. Without special views (e.g., obliques), small-tumor activity may be difficult to distinguish from renal activity.

Several hypotheses for the mechanism of radiouptake in neuroblastomas and other soft tumors have been proposed. One theory, which correlates with the uptake in bone in certain pathologic states, holds that it is secondary to increased blood flow (*2*). At our institution, however, we routinely do flow studies with injections into a dorsal pedal vein for all abdominal tumors in children (*5*). Blood flow to a neuroblastoma is no more than to other abdominal tumors such as the Wilms's. Also against the hyperemia hypothesis is the fact that while tumor uptake may be seen early, it is usually better on the delayed film. Leakage of tracer through abnormal capillary walls has also been proposed as a mechanism of the uptake (*6*). Hypervascularity has been reported, however, in about half of neuroblastomas (*7*), with some patients showing arterial encasement (*8*). In a single case reviewed by McCartney and Nusynowitz, electron microscopy did not reveal any abnormality of the capillary wall (*6*). Another hypothesis relates uptake of Tc-99m phosphate agents to phosphate enzyme systems that bind the tracer. This has been evaluated in some breast tumors that led to increased acid phosphatase (*9*), but never in neuroblastoma.

The final mechanism proposed for the tracer uptake involves ion exchange at the crystal surface in areas of calcification (*2*). The technetium phosphate agents are felt to initiate calcium, so that areas of calcification are made visible by the tracer on their surfaces. This is felt to be an important mechanism in the early stages of bone uptake.

Although we have not demonstrated a statistically significant correlation between uptake and areas of demonstrable calcium, we believe calcium metabolism at the cellular level to be important. The evidence supporting this is as follows:

A. Calcification is far commoner in neuroblastoma than in any other pediatric tumor.

B. Pulmonary metastases from osteogenic sarcoma may frequently calcify and demonstrate sequestration of bone-seeking nuclides (*10*).

C. Frequently with time, radiographic areas of calcification will increase; they never diminish. Tracer uptake by a neuroblastoma may become less in follow-up bone images (Fig. 4).

Thus, one may hypothesize that the uptake may very well be related to calcium metabolism in the tumor. Future studies are planned to evaluate the role of bone-seeker uptake in the follow-up of patients with neuro-

blastoma, to look for tumor recurrence or metastases. We also propose to see whether there is any correlation between tumor uptake and prognosis.

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