# The Effect of Methylene Diphosphonate on the Tissue Distribution of Gallium-67

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Nonradiolabeled methylene diphosphonate (MDP) was administered intravenously to CFW Swiss Outbred female mice 2 hr prior to i.v. injection of [$^{67}$Ga]citrate. The dose of MDP ranged from that usually administered for bone scanning (17.9 μg/kg) to 1,000 times the usual bone scan dose (17.9 mg/kg). The animals were killed 24 hr after administration of [$^{67}$Ga]citrate and organ distribution determined as compared to control animals who received no MDP. At MDP doses one to ten times usual bone scan dose, the only organ showing significantly different $^{67}$Ga uptake was the lung and this difference in pulmonary uptake was accounted for by incidental pulmonary infection. At MDP doses 100 to 1,000 times usual bone scan dose, significantly altered $^{67}$Ga uptake was noted in lung, spleen, kidney, and gastrointestinal tract. The only consistent pattern of association of MDP administered dose and alteration in $^{67}$Ga uptake, however, was noted in the spleen where uptake was augmented with increased dose, and the bone where increased MDP dose depressed $^{67}$Ga uptake. Even this effect, however, was modest. It is concluded that at usual MDP dose levels used in bone imaging, no significant alterations occur in $^{67}$Ga distribution in normal mouse tissue. It is inferred that $^{67}$Ga scans performed following bone scans can be interpreted as if both radiopharmaceuticals had been administered separately.

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Previous studies have shown that phosphorus containing compounds facilitate the in vitro transfer of Gallium-67 ($^{67}$Ga) from transferrin to ferritin (*1*) and from lactoferrin to ferritin (*2*). Some of these compounds, such as pyrophosphate, may actually bind significant amounts of $^{67}$Ga (*1,2*). Because methylene diphosphonate (MDP) as well as other phosphates, complexed to technetium-99m ($^{99m}$Tc), are often used clinically in conjunction with $^{67}$Ga (*3–5*), especially in evaluation of suspected osteomyelitis it is important to know if these phosphorous containing compounds alter the normal distribution of gallium and thus the interpretation of a patient's gallium scan. Therefore, the goal of our study was to determine the effect of MDP on the uptake and distribution of $^{67}$Ga in vivo.

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## MATERIALS AND METHODS

We used CFW Swiss Outbred female mice* at least 65 days old and over 24 g in weight. They were housed in an exposed environment and carried normal bacterial contaminants.

These animals were injected initially with a sterile saline solution of MDP administered through the tail vein. The MDP used was from an Osteolite $^{99m}$Tc Medronate kit†. It was reconstituted according to the manufacturer's instructions with the exception that no $^{99m}$Tc was added. The dose of MDP injected into the mice was based on the usual dose used for bone scanning in humans (17.9 μg MDP per kg body weight). The animals were randomly divided into four test groups and one control group. Test groups received the following doses: 0.448 μg MDP (17.9 μg/kg) for the usual concentration group, 4.48 μg MDP (179.0 μg/kg) for the 10× usual concentration group, 44.8 μg MDP (1.79 mg/kg) for the 100× usual group and 448 μg MDP (17.9 mg/kg) for the 1,000× usual group. All doses of MDP were injected in 0.15 ml of sterile saline. The control group was injected with 0.15 ml of sterile saline containing no MDP.

In order to simulate the sequence in which [$^{99m}$Tc]MDP

**TABLE 1**
**24-hr Organ Uptake of $^{67}$Ga as Function of Quantity of MDP Injected 2 hr Prior to $^{67}$Ga Administration**

| Organ | Dose MDP | Group size | % Inj. dose per g tissue | s.e.m. | Organ | Dose MDP | Group size | % Inj. dose per g tissue | s.e.m. |
|---|---|---|---|---|---|---|---|---|---|
| Blood | Control | 18 | 1.87 | 0.11 | Spleen | Control | 18 | 2.63 | 0.18 |
| | 17.9 μg/kg* | 10 | 1.75 | 0.23 | | 17.9 μg/kg | 10 | 2.14 | 0.23 |
| | 179 μg/kg† | 9 | 1.92 | 0.13 | | 179 μg/kg | 9 | 2.63 | 0.18 |
| | 1.79 mg/kg‡ | 6 | 1.93 | 0.11 | | 1.79 mg/kg | 6 | 3.31 | 0.08¶ |
| | 17.9 mg/kg§ | 10 | 2.03 | 0.15 | | 17.9 mg/kg | 10 | 4.62 | 0.24** |
| Heart | Control | 18 | 0.88 | 0.04 | Kidney | Control | 18 | 5.67 | 0.25 |
| | 17.9 μg/kg | 10 | 0.82 | 0.08 | | 17.9 μg/kg | 10 | 5.13 | 0.40 |
| | 179 μg/kg | 9 | 0.95 | 0.07 | | 179 μg/kg | 9 | 5.62 | 0.22 |
| | 1.79 mg/kg | 6 | 0.93 | 0.07 | | 1.79 mg/kg | 6 | 3.87 | 0.20** |
| | 17.9 mg/kg | 10 | 0.99 | 0.10 | | 17.9 mg/kg | 10 | 7.66 | 0.50¶ |
| Lung | Control | 18 | 7.36 | 1.60 | GI | Control | 18 | 4.84 | 0.28 |
| | 17.9 μg/kg | 10 | 2.37 | 0.24¶ | | 17.9 μg/kg | 10 | 3.94 | 0.34 |
| | 179 μg/kg | 9 | 3.60 | 0.76 | | 179 μg/kg | 9 | 5.59 | 0.34 |
| | 1.79 mg/kg | 6 | 1.66 | 0.16¶ | | 1.79 mg/kg | 6 | 3.21 | 0.22** |
| | 17.9 mg/kg | 10 | 4.53 | 1.01 | | 17.9 mg/kg | 10 | 10.12 | 1.32** |
| Liver | Control | 18 | 5.26 | 0.33 | Muscle | Control | 18 | 0.37 | 0.02 |
| | 17.9 μg/kg | 10 | 4.64 | 0.44 | | 17.9 μg/kg | 10 | 0.35 | 0.03 |
| | 179 μg/kg | 9 | 5.35 | 0.32 | | 179 μg/kg | 9 | 0.41 | 0.03 |
| | 1.79 mg/kg | 6 | 4.47 | 0.34 | | 1.79 mg/kg | 6 | 0.47 | 0.04 |
| | 17.9 mg/kg | 10 | 5.86 | 0.30 | | 17.9 mg/kg | 10 | 0.33 | 0.04 |
| | | | | | Bone | Control | 18 | 12.14 | 0.84 |
| | | | | | | 17.9 μg/kg | 10 | 12.44 | 0.89 |
| | | | | | | 179 μg/kg | 9 | 11.46 | 0.98 |
| | | | | | | 1.79 mg/kg | 6 | 9.82 | 0.27 |
| | | | | | | 17.9 mg/kg | 10 | 9.49 | 0.68 |

* 1X usual bone scan dose.
† 10X usual bone scan dose.
‡ 100X usual bone scan dose.
§ 1,000X usual bone scan dose.
¶ $p < .0125$.
** $p < .0025$

bone and $^{67}$Ga scans might be performed clinically, the test animals were injected through the tail vein with ~5 μCi of [$^{67}$Ga]citrate† at 2 hr following administration of the MDP. The [$^{67}$Ga]citrate was diluted in sterile saline to a concentration of 33.3 μCi per ml. All gallium dose calibrations were performed with a dose calibrator‡. 0.15 ml of this stock solution was then drawn into a 1 cc syringe for injection. The weight of the syringe and needle were measured before and after injection to determine the volume of $^{67}$Ga solution injected.

In addition, injection standards were made from the stock solution of $^{67}$Ga. These standards were prepared by diluting approximately 0.15 ml of the $^{67}$Ga solution (exact volume determined by weighing the syringe before and after injection) with 50 ml distilled water. 1 ml aliquots of this solution were counted at the same time and under similar conditions as the tissue samples.

Twenty-four hours following the radiogallium injection, the mice were anesthetized with ether. Approximately 0.5 ml of blood was drawn through cardiac puncture and placed in a pre-weighed specimen tube. The mice were then sacrificed using cervical dislocation and dissection was performed. Samples of the following organs were removed from each mouse rinsed in saline, blotted dry and placed in a pre-weighed specimen tube: heart, lungs, liver (sections of the right and left lobes), kidney, spleen, section of the large intestine without feces, muscle (sections of the quadriceps and gastrocnemius), bone (femur and tibia) and the complete tail. All samples were weighed prior to counting.

The filled specimen tubes, along with the tubes containing the 1 ml aliquots of standard solution, were counted in an automatic gamma counter§ within one hour after dissection. A single energy window of 50-500 keV was used and counts were all obtained over a 1-min period.

The total injected dose of $^{67}$Ga per animal was calculated as follows. The activity, in terms of cpm, of the 1 ml aliquot of the injection standard was multiplied by 50 to account for dilution. This number was divided by the weight of $^{67}$Ga stock solution added to the 50-ml flask; thus activity (cpm) per mg of injection solution was obtained. The activity (cpm) per mg was multiplied by the mg of solution injected into each mouse. The activity which remained in the tail and the activity on the pad which covered the injection site for ~1 min after the syringe was withdrawn was subtracted from the total injected activity. This final effective administered dose of $^{67}$Ga (in cpm) was used to derive tissue activity in terms of percent

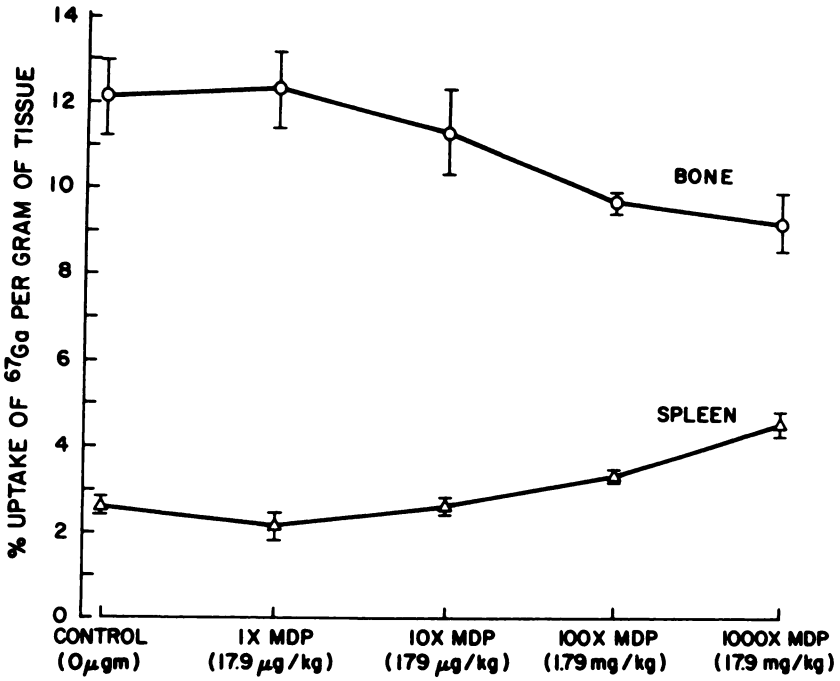


**FIGURE 1**
**Relation between 2 hr loading dose of MDP and $^{67}$Ga uptake at 24 hr**

injected dose per gram.

The mean and standard error of the mean for the percent injected dose per gm in each MDP concentration group and in the control group were calculated for each tissue. The mean percent injected dose per gram tissue was compared between each test group and the control group. The Behrens-Fisher t-test with Bonferroni adjustment for multiple comparisons was used to determine significant difference between the test and control groups.

## RESULTS

Table 1 summarizes the results of this biodistribution study. At usual concentration of MDP (17.9 μg/kg), only the lungs showed significantly depressed uptake of $^{67}$Ga ($p < 0.05$). At 10X usual concentration of MDP no organ showed significantly altered $^{67}$Ga uptake compared to control values. At 100X and 1,000X usual concentrations, MDP did alter the uptake of $^{67}$Ga in the following organs: lung, spleen, kidney and gastrointestinal (GI) tract. Only the spleen showed a discrete pattern of change in uptake with increasing MDP concentration. Spleen uptake increased with increasing MDP dose (Fig. 1). The uptake of $^{67}$Ga in the kidney and GI tract at 100X and 1,000X MDP, although significantly different from their respective control groups, did not follow any pattern. Bone activity tended to decrease as a function of increasing MDP dose but the difference was not statistically significant when corrected for multiple comparisons.

## DISCUSSION

When [$^{67}$Ga]citrate is injected intravenously it binds rapidly to transferrin (*6,7*). Its localization in tumors and inflammatory lesions, as well as normal tissues, is dependent on its relative affinity for transferrin as well as its in vitro transfer to other iron binding molecules, such as lactoferrin and ferritin (*8,9*). Previous studies in our laboratory have demonstrated that certain phosphate containing compounds influence in vitro transfer of $^{67}$Ga between ferritin and lactoferrin and transferrin, respectively (*1,2*). Also, pyrophosphate competes with transferrin, lactoferrin and ferritin for binding of $^{67}$Ga (*1,2*). Methylene diphosphonate is closely related structurally to pyrophosphate, the difference being the presence of a bridging oxygen atom between the two phosphate groups in pyrophosphate compared to a bridging carbon atom in MDP. Thus, it seems possible that MDP might influence the in vivo distribution of $^{67}$Ga. This would create a major potential clinical problem since [$^{99m}$Tc]MDP is commonly used in conjunction with $^{67}$Ga in the diagnosis of osteomyelitis. It is important to know if MDP, injected i.v. 2-4 hr prior to i.v. administration of [$^{67}$Ga]citrate, influences the in vivo distribution of $^{67}$Ga.

Our study indicates that, at doses of MDP up to ten times that used in the clinical setting, the MDP has little influence on $^{67}$Ga distribution in normal tissues. The lung was the only organ in which a significant change was noted. However, gross inspection of the lungs of many of the animals revealed inflammatory changes; subsequent dissection revealed small vascular emboli in four of ten animals in the "usual" concentration of MDP (0.448 μgm) group. We strongly suspect that incidental pulmonary infection, a common laboratory problem with CFW Swiss mice, was responsible

for the observed changes in pulmonary uptake. Moreover, there was no consistent relation between MDP dose and pulmonary uptake.

At higher concentrations of MDP, lung, kidney, GI tract and spleen showed significant differences in $^{67}$Ga uptake. However, the only organ that showed a constant relationship between MDP dose and $^{67}$Ga uptake was the spleen. The spleen demonstrated increasing uptake with increasing dose of MDP. However, this change was modest and unlikely to be of clinical significance. The bone showed a trend toward decreased uptake as a function of increased dose of MDP (p value at 1,000X's usual dose less than 0.05 compared to control). We have no explanation for the change in splenic uptake. However, in the case of bone, MDP is known to localize in this organ and, specifically, to chemabsorb on hydroxyapetite crystal (*10,11*) as well as competitively inhibit phosphatase (*12*). It is possible that this "coating" of hydroxyapetite or the phosphatase inhibition could be responsible for the observed trend toward decreasing $^{67}$Ga uptake as a function of MDP concentration. This change was so small, however, that even in bone lesions producing markedly increase MDP uptake, significant inhibition of $^{67}$Ga localization would not be expected.

## CONCLUSION

On the basis of our data, MDP does not significantly affect the uptake and distribution of $^{67}$Ga in vivo in mice when administered at doses equivalent to those usually used for bone imaging (17.9 $\mu$g/kg). Only when MDP is administered at 100 and 1,000 times this dose do significant changes in $^{67}$Ga distribution occur. Clinically, our study infers that $^{67}$Ga scans which are preceded by [$^{99m}$Tc]MDP scans can be interpreted as if both radiopharmaceuticals had been administered separately.

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## FOOTNOTES

* Charles River Kingston Breedery, Wilmington, MA.
† DuPont NEN Medical Products, North Billerica, MA.
‡ Capintec CRC-5R, Ramsey, NJ.
§ Beckman Instruments, Model Gamma 8000, Irvine, CA.

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