Technetium-99m Stannous Phytate as an Imaging Agent for Lymph Nodes

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This report describes preliminary results using technetium-99m stannous phytate as a lymph-node imaging agent in animals. After the subcutaneous administration there is good visualization of the draining nodes, best obtained 2–4 hr from the time of injection. There is also visualization of the liver, spleen, kidneys, and bladder. This agent appears suitable for lymph-node imaging in areas where the extranodal concentration does not interfere.

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The early detection of lymph-node metastases in malignant disorders is of the utmost importance in the management of the patients. Unfortunately there are certain shortcomings in current roentgenographic and radiotracer techniques available for this purpose (*1–3*). The gamma imaging of the lymph nodes has been restricted thus far by the size of the particles introduced and the radiation dose delivered to the site of injection.

We describe here our preliminary results, obtained in animals, with technetium-99m stannous phytate ("Tc-phytate") as an imaging agent for lymph nodes. It combines the excellent physical characteristics of Tc-99m with the small particle size of calcium phytate, thus providing a unique radiotracer for lymph-node imaging.

MATERIALS AND METHODS

For this study sodium phytate (inositol hexaphosphate) with stannous ion was used and each kit vial contained 10 mg sodium phytate and 1 mg $SnCl_2$. Four ml of [^{99m}Tc] pertechnetate (4 mCi/ml), were added to the contents of a vial. To study each group of lymph nodes, 0.1 ml of this preparation was injected subcutaneously into the dorsum of the paw. Sixteen rats, five rabbits, and four dogs were used for this study, and 64 sites in all were injected. Only the lower extremities were injected in rats. The im-

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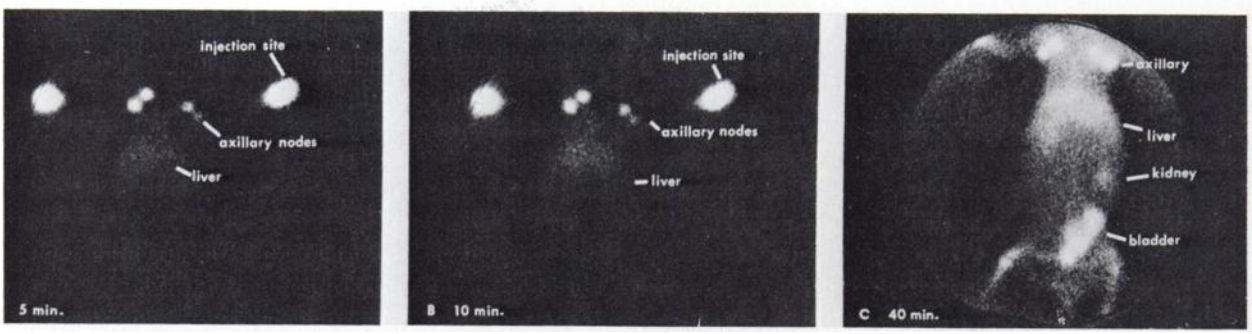

FIG. 1. Scintiphotos of site of injection and upper part of body of a rabbit at 5, 10, and 40 min after subcutaneous injection show good concentration of Tc-phytate in axillary nodes and early accumulation of activity in liver, kidney, and bladder. There is also some bone-marrow activity.

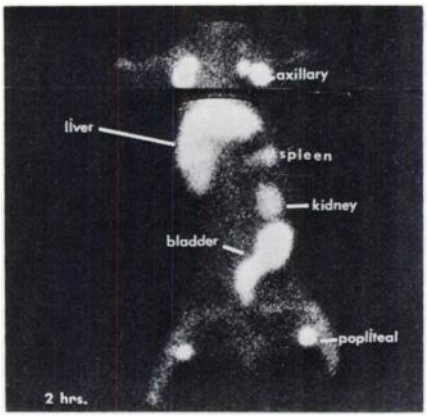

FIG. 2. Scintiphoto obtained 2 hr after subcutaneous injection of Tc-phytate in a rabbit demonstrates significant activity in axillary and popliteal lymph nodes. There is minimal background activity at this time. Note significant concentration of activity in liver, spleen, kidneys, and bladder. Some bone-marrow uptake can be seen.

ages of the draining nodes and the rest of the body were obtained at frequent intervals (5 min–8 hr).

In nine rats and three rabbits, images were obtained with colloidal Au-198 for comparison with images obtained with the Tc-phytate. Colloidal Au-198 was injected subcutaneously into the lower extremities at the completion of the Tc-phytate images. In these animals the images were obtained 24 hr after the injection of colloidal gold.

The images were obtained with a scintillation camera. Low-energy collimators were used for Tc-phytate scintiphotos, and a medium-energy collimator for colloidal Au-198.

The rate at which Tc-phytate was cleared from the site of administration was determined in two dogs. In these animals all four extremities were injected. The dogs were allowed to move freely after injection. The sites of injection were monitored by a scintillation probe attached to a scaler, and counts obtained every hour for 6 hr were used to calculate the clearance rates.

In two rabbits a urine sample was taken at 2 hr postinjection and subjected to chromatography. This was done to determine whether the radioactive contents in the bladder resemble Tc-phytate or $^{99m}TcO_4^-$. An eluting solution of 70% methanol and Whatman No. 1 paper were used for chromatography. These chromatograms were compared with those of Tc-phytate and $^{99m}TcO_4^-$ solutions as freshly prepared or as mixtures incubated for 30 min with samples of nonradioactive rabbit urine, with or without added calcium ion.

RESULTS

Within minutes after subcutaneous administration of Tc-phytate, there was evidence of transfer of activity to the rest of the body. The draining lymph nodes began to appear on the scan several minutes after injection (Fig. 1). Early visualization of the liver, spleen, kidney, and bladder occurred in rats and rabbits and, to a lesser degree, in dogs. The activity in the kidney and bladder appeared more intense when the agent was injected into the lower extremities than into the upper. The liver was visualized within minutes after administration of the agent. During the first hour there was some background activity, which diminished significantly as the study progressed.

The lymph nodes were clearly visualized in the first hour. The optimum scanning time was 2–4 hr after administration (Figs. 2 and 3), but scans of good quality could be obtained at 6–8 hr (Fig. 4).

There was excellent correlation between the results of Tc-phytate and colloidal Au-198 images when body studies were performed in the same animal. Because of significant bladder activity, it was impossible to identify para-aortic nodes clearly with the Tc-phytate, but with colloidal Au-198 there was no bladder activity (Fig. 5). Also, Tc-phytate images demonstrated the presence of more activity in the liver in the early hours than did the 24-hr colloidal Au-198 scintigrams (Fig. 3). When Tc-phytate was injected intravenously, there was very little kidney and bladder activity, moderate activity in the skeleton, and intense activity in liver and spleen (Fig. 6).

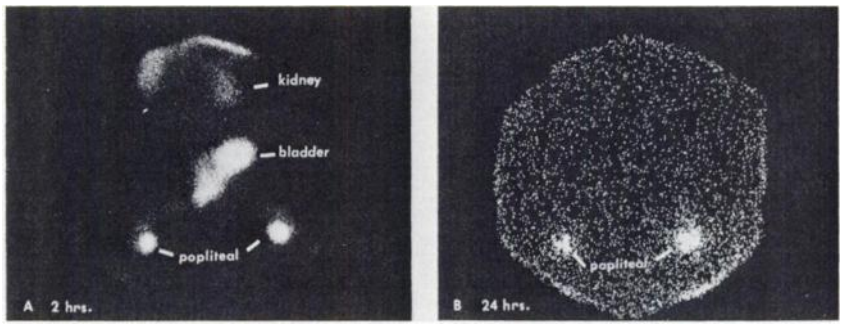

FIG. 3. A) Images obtained 2 hr after subcutaneous injection of Tc-phytate into a rabbit demonstrate good concentrations in lymph nodes, kidney, bladder, and liver. B) After 24 hr, colloidal Au-198 activity was observed only in popliteal area. No liver activity was seen.

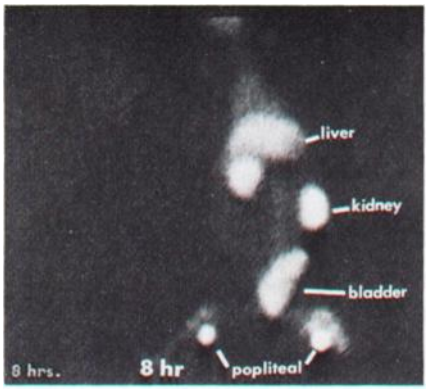

FIG. 4. Images made 8 hr after subcutaneous injection of Tc-phytate demonstrate good lymph-node concentrations.

The rate of clearance of Tc-phytate from the site of injection in two dogs is shown in Fig. 7. From the regression analysis, the average standard deviation of slopes was 31% and 9.2% for the two dogs. The average effective half-lives were 3.2 ± 20% and 3.54 ± 22% hr, but the difference is not statistically significant. From these data, the calculated biologic half-time for the clearance of Tc-phytate is 7.85 hr.

The radiation dose delivered by a 1-mCi injection of Tc-phytate can be estimated from the dog data. The highest tissue dose occurs at the site of injection. Assuming that all of the activity there is uniformly distributed over a 5-g mass (1.7 cm^3), and that the biologic clearance is as measured in dogs (7.85 hr), the calculated dose at the site of injection is 56 rads/mCi administered. For comparison, using the same assumptions, the dose at the point of injection of colloidal Au-198 is 1440 rad/mCi administered or about 25 times as much.

For purposes of dosimetry calculation, the activity, A, in the lymphoid tissue and liver is given by

$$A = A_0 [1 - \exp(-.693\, t/T_b)] \cdot [\exp(-.693\, t/T_p)], \quad (1)$$

where A_o is the administered activity, T_b is the half-time for the biologic clearance from the site of injection, and T_p is the physical half-life of Tc-99m. By integration, the average time for the activity in the organs is 2.3 hr. The calculated dose to the liver from the Tc-phytate, assuming that all of the activity eventually enters the liver, is approximately 0.1 rad/mCi injected.

The dose to the lymphatic system was calculated by assuming that the mass of lymphatic tissue on one side of the body is 350 g. It is assumed for purposes of dosimetry that the lymph system can be represented by an ellipsoid having axes in the proportion of 1:3:8. The estimated absorbed fraction for a 140-KeV photon in such an ellipsoid is approximately 0.09. The calculated dose to the lymph-node chain is then about 0.4 rads/mCi administered. The corresponding Au-198 dose to the liver and lymph nodes is approximately 100 times as great.

In ascending chromatography, the R_f values for Tc-phytate and $^{99m}TcO_4^-$ solutions, whether freshly prepared or as mixtures incubated for 30 min with samples of nonradioactive rabbit urine, were 0 and 0.67, respectively. With the urine from the experimentally injected rabbits, 84% of the activity ranged from $R_f = 0$ to 0.5, with peaks at 0, 0.25, and 0.44; not more than 2% was at $R_f = 0.67$, where $^{99m}TcO_4^-$ would be found, and another small peak (2–3%) was detected at $R_f = 0.94$. The addition of calcium did not change the above-described pattern.

We conclude from these data that it is not unaltered Tc-phytate or $^{99m}TcO_4^-$ that has entered the bladder.

DISCUSSION

Since its introduction as a lymph-node imaging agent, colloidal Au-198 has been used to demonstrate the anatomy of the lymphatic system (*3,4*). This tracer, however, has two major disadvantages: (a) a high radiation dose delivered to the site of injection and to the liver, and (b) an unfavorably high gamma energy, which requires a high-energy collimator and a thick crystal. This agent has been found useful in outlining the lymph system in malignant disorders. It has also been used for the evaluation of the dynamics of lymph-node drainage in different disorders (*5*). The particle size of colloidal

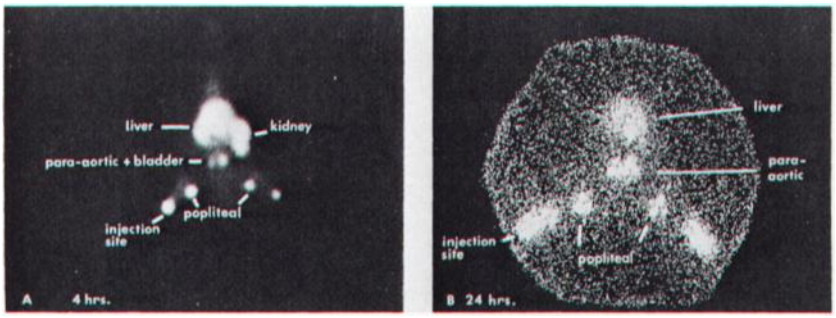

FIG. 5. Scintigram obtained from a rat 4 hr after subcutaneous injection of Tc-phytate (A) demonstrates activity in injection sites, popliteal and possibly para-aortic lymph nodes, bladder, kidney, and liver. Image (B) was obtained 24 hr after injection of colloidal Au-198 in same animal. There is good correlation between the two studies, except that colloidal gold did not concentrate in the bladder.

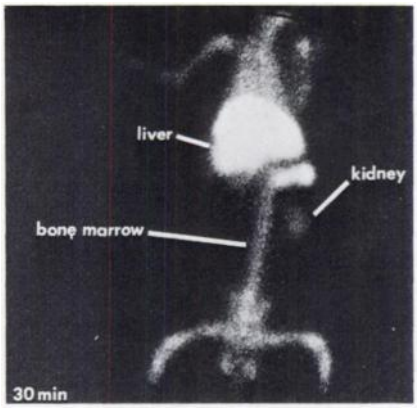

FIG. 6. Intravenous injection of Tc-phytate into dog demonstrates significant activity in liver, spleen, and bone marrow. There is minimal visualization of kidney, and no demonstrable bladder activity.

Au-198 is several millimicrons (*3–7*). The optimum scanning time is 6–8 hr after the injection (*5*), but most investigators prefer to wait for 24 hr.

Technetium-99m sulfur colloid has been used as a lymph-node imaging agent with some success (*6,7*). Since the particles in this agent are considerably larger (0.5–2 microns) than in colloidal Au-198, simultaneous administration of hyaluronidase is recommended with the former to facilitate the transport of the particles. Despite this precaution, the particles appear to move very slowly from the site of injection, and incomplete delineation of the lymph-node system is a possibility, even if lymphatic drainage occurs at a normal rate. Moreover, hyaluronidase is an allergenic substance, and the routine use of this enzyme could cause complications.

A Tc-antimony colloid has been used for lymph-node imaging (*8*). Its particle size is smaller than that of Tc-99m sulfur colloid (4–12 mμ against 500–2,000 mμ). With the use of this agent, excellent images of the internal mammary nodes have been obtained. Since 1–35% of the radiocolloid is transported from the site of interstitial injection over the first 24 hr, the clearance is somewhat variable.

Because of its favorable physical characteristics In-111 colloid has been proposed as a good lymph-node imaging agent (*9*). Its investigators indicate that the particle size in this preparation is comparable to that of colloidal Au-198, but particles prepared by the same technique using In-113m, and reported elsewhere by the same authors (*10*), appear to be significantly larger than Au-198 particles. Furthermore, indium-111 attached to this colloid is more expensive than [^{99m}Tc] pertechnetate.

In this report we have described our preliminary results using Tc-phytate as a lymph-node imaging agent. This phytate was introduced by Subramanian to outline the reticuloendothelial activity in the liver and bone marrow (*11*). The agent prepared by the addition of pertechnetate to a lyophilized kit containing Sn-phytate is a clear noncolloidal solution. After i.v. injection, the soluble compound becomes a microcolloid by reacting with serum calcium to form insoluble calcium phytate, which is phagocytized by the reticuloendothelial cells in the liver, spleen, and marrow. The biologic distribution in the reticuloendothelial system may be controlled by adjusting the ratio of phytate to the stannous ion concentration and the total quantity injected (*11*). Because of the presence of calcium ion in the lymph (*12*), insoluble calcium phytate particles are formed during passage through the lymph vessel to the lymph node after

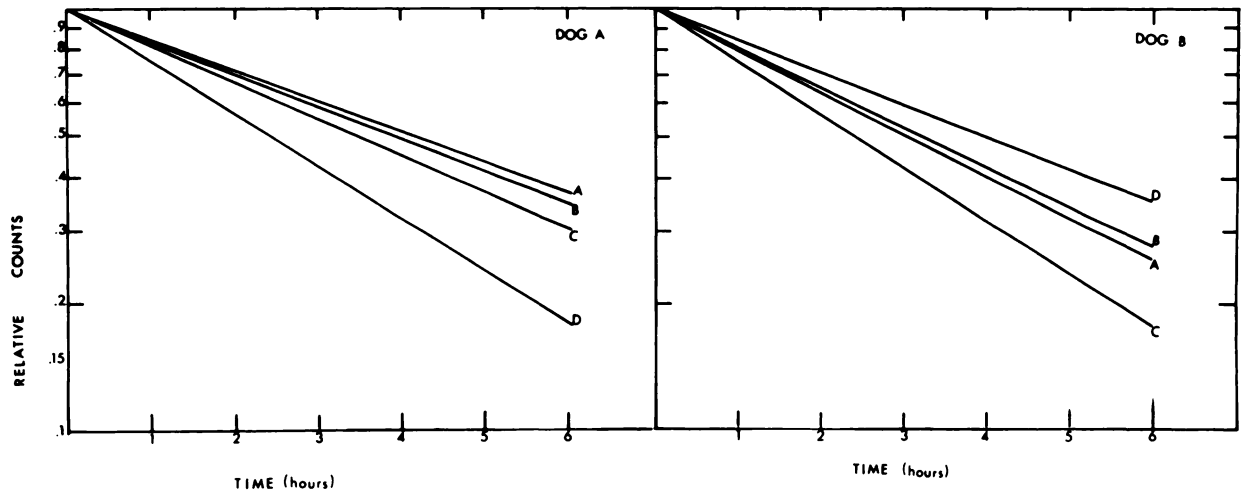

FIG. 7. Graphs show rate of clearance of Tc-phytate from site of injection in two dogs. Plots are semilog, and represent eight injection sites as follows: (A) left front paw; (B) left rear paw; (C) right front paw; (D) right rear paw.

injection of Tc-phytate. These particles are caught by phagocytic cells in the lymph nodes, which they outline. The activity in the liver and bone marrow results from drainage of particles into the systemic circulation through the thoracic duct, followed by active phagocytosis in the reticuloendothelial system of the liver and bone marrow. We noticed visualization of the kidneys and bladder, especially in the rabbits, but have no explanation for this finding. The urine samples examined by chromatography revealed a pattern different from those observed with either Tc-phytate or $^{99m}TcO_4^-$ solutions.

In an earlier study, good visualization of the popliteal and axillary lymph nodes was obtained after subcutaneous injection of Ga-67 phytate and Tc-phytate in rabbits (*13*). Images were obtained 6, 24, 48, and 72 hr after injection. In our study we noticed lymph-node concentration of Tc-phytate several minutes after administration. The lymph nodes were more intensely outlined as the study progressed. The optimum time for scanning of the nodes was 2–4 hr after injection. Although we have no human data available, the radiation dose calculated based on our animal data indicates that the radiation dose is definitely far below that delivered by colloidal Au-198 and is within the acceptable range for a diagnostic test.

CONCLUSION

In conclusion, we have reported our results with the experimental use of Tc-phytate as a lymph-node imaging agent. This tracer appears to clear rapidly from the site of injection and to localize in the draining lymph nodes. Good images are obtained within 2–4 hr after administration, and the radiation dose is low. Therefore, if clinically indicated, the study can safely be repeated at short intervals. Tc-phytate may prove useful in the evaluation of lymph-node disease, especially in the staging of malignant disorders.

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