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**COPPER-67 LABELED PORPHYRIN LOCALIZATION IN INFLAMED
TISSUES.**

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Abstract

A series of experiments compared the uptake of 5,10,15,20-tetrakis(4-carboxyphenyl) porphinato [^{67}Cu] copper (II), $^{67}\text{CuTCPP}$, by the lymph nodes of inflamed and two sets of control rats. The results demonstrate that $^{67}\text{CuTCPP}$ localizes in greater concentration in inflamed lymph nodes than in noninflamed control lymph nodes. This enhanced uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes was 3.6 times greater than was the uptake by control lymph nodes. A time course study demonstrated that the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes reached the maximum level by 24 hours post-injection of $^{67}\text{CuTCPP}$ and remained constant throughout the 96 hours examined. It was also found that the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes was not exclusively dependent upon an increase in the weight of inflamed lymph nodes. These studies show that $^{67}\text{CuTCPP}$ has potential as a lymphoscintigraphy agent.

Key words: lymphoscintigraphy, lymph nodes, copper-67, porphyrin, radionuclide imaging

INTRODUCTION

The ability to image lymph nodes has become important in the diagnosis and treatment of lymphatic disorders and in malignant disease. The use of radiocontrast lymphangiography allows the anatomical visualization of selected lymph nodes; however, this procedure has several shortcomings (Thornton and Pickering, 1985). The main problems of lymphangiography include: (1) tedious cannulation of lymph vessels, (2) induced pulmonary microemboli, (3) hypersensitivity reaction to the contrast media, (4) obstruction of small or abnormal lymphatic channels, and (5) difficulty in repeat studies (Thornton and Pickering, 1985; Steckel et al., 1975; Henze et al., 1982; Dworkin, 1981). As a result of these limitations, a variety of radiolabeled agents have been examined for their ability to localize lymph nodes. The first radiopharmaceutical clinically used for imaging lymph nodes was ^{198}Au colloid. Because of the unacceptably high radiation dose at the site of injection, this agent was abandoned for colloids labeled with $^{99\text{m}}\text{Tc}$ (Haubold, 1976). A colloid that has exhibited good qualities for lymphatic imaging is $^{99\text{m}}\text{Tc}$ labeled antimony sulfide. This agent has been used in studies to evaluate lymph nodes in patients with breast cancer, truncus melanoma, and pelvic lymph node metastases (Ege and Cummings, 1980; Ege, 1983; Jones et al., 1985). More recently, $^{99\text{m}}\text{Tc}$ nanocolloidal albumin (Mazzeo et al., 1986), and $^{99\text{m}}\text{Tc}$ -rhenium colloid (Matsubara et al., 1986) have been used to image lymph nodes. Because these colloidal agents are administered subcutaneously, they localize in only those lymph nodes draining the injection area. This localized uptake limits the number of lymph nodes that can be imaged by these agents (Vaum et al., 1982). Radiolabeled antitumor monoclonal antibodies have also been used to delineate metastatic deposits in regional lymph nodes (Nelp et al., 1987; Weinstein et al., 1984; Bunn et al., 1984); however, the use of monoclonal antibodies is not devoid of problems. First, monoclonal antibodies must be specific for the cancer present in the lymph node, second, the

localization of monoclonal antibodies in lymph nodes is dependent upon the presence of cancerous cells in the node.

During the 1940's it was observed that many porphyrins have an affinity for lymphatic and neoplastic tissue (Figge *et al.*, 1948; Rasmussen-Taxdal *et al.*, 1955). However, in these early studies the use of porphyrins was of limited diagnostic utility because the imaging technique depended solely upon differential fluorescence for detection in the target tissue. More recently, the use of radiolabeled metalloporphyrins has significantly improved the clinical usefulness of these compounds (Nunn, 1979; Vaum *et al.*, 1982; Robinson, Jr., *et al.*, 1986). Even though there have been reports of the potential use of radiolabeled porphyrins for lymph node imaging (Vaum *et al.*, 1982; Robinson Jr., *et al.*, 1986), as well as lymphatic ablation (Fawwaz *et al.*, 1969; Fawwaz, *et al.*, 1971; Fawwaz, *et al.*, 1974; Chihiro, *et al.*, 1985), there have been no reports on routine *in vivo* diagnostic use of porphyrins in clinical medicine. Therefore, neither monoclonal antibodies nor many of the common lymph node imaging agents can physiologically distinguish inflamed lymph nodes from noninflamed lymph nodes.

In this study, we demonstrate that 5,10,15,20-tetrakis(4-carboxyphenyl) porphinato [^{67}Cu] copper (II) will localize in greater amounts in inflamed lymph nodes than in noninflamed lymph nodes. The porphyrin serves to carry the radionuclide to the lymph nodes, whereas the ^{67}Cu has suitable decay properties (62 hour half-life and gamma photon emission) for use in gamma camera imaging.

MATERIALS AND METHODS

Copper-67

High specific activity ^{67}Cu (mean specific activity 8,000 Ci/g) was

produced by the proton bombardment of ZnO targets at Los Alamos Meson Physics Facility. Copper-67 was purified using an electrochemical procedure previously described by Bentley *et al.*, 1984; Taylor and Bentley, 1984; and Mercer-Smith *et al.*, 1987.

Porphyrin Radiolabeling

The synthesis of the porphyrin activated for metalation, N-benzyl-5,10,15,20-tetrakis(4-carboxyphenyl) porphine (N-bzHTCPP, has been described previously (Mercer-Smith *et al.*, 1987). The synthesis and metalation mechanism of N-bzHTCPP to form 5,10,15,20-tetrakis(4-carboxyphenyl) porphinato [^{67}Cu] copper (II), (henceforth denoted as $^{67}\text{CuTCPP}$; Figure 1) have been described elsewhere (Mercer-Smith *et al.*, 1987 and Lavalley, 1987). The $^{67}\text{CuTCPP}$ in aqueous phosphate buffer was sterilized by passage through a Millex GS 0.2 filter (Millipore, Bedford, MA) into a sterile crip-sealed vial.

Animals

Inbred male Fischer (F-344) rats (Charles River Laboratories, Wilmington, MA) weighing 180-200 g were used in all experiments. The animals were housed in plastic cages in a temperature controlled room, bedded with sawdust, and maintained on a 12-hour light cycle with water and rat chow provided *ad libitum*.

Inflammation Procedure

Inflamed Animals: Animals to be inflamed were lightly anesthetized with ether during the administration of the inflaming agents. Each animal was subcutaneously injected with 0.01 ml (0.005 mg) of a 1:1 solution of complete Freund's adjuvant (Sigma Chemical Co., St. Louis, MO) and sterile saline into each foot pad using a tuberculin syringe with a 25 G needle. Four days after the

administration of Freund's adjuvant, each animal was injected intraperitoneally with 0.2 ml (0.2 mg) bacterial endotoxin (*Salmonella abortus equi*).

Control Animals: Control animals (age and sex matched) were treated in a similar manner as were the inflamed animals. Sterile saline was administered in place of Freund's adjuvant and bacterial endotoxin. Each control animal was injected with saline at the same time and with the same volume as were the inflamed animals. In addition to the saline injected control animals, a second group of non-injected control animals was used. This group of animals was not injected with saline or any inflaming agent.

Administration of $^{67}\text{CuTCPP}$

Three days following the injection of bacterial endotoxin (7 days after Freund's adjuvant injection), both inflamed and non-inflamed control animals were injected with $^{67}\text{CuTCPP}$. Prior to the administration of $^{67}\text{CuTCPP}$, animals were anesthetized with 0.4 ml (20 mg) Nembutal (pentobarbital sodium, Abbott Laboratories, North Chicago, Il). The tails were shaved with a surgical razor and scrubbed with 70% ethanol. A small rubber tourniquet was applied to the tail to facilitate the intravenous injection. Sterile $^{67}\text{CuTCPP}$ [1.2×10^{-9} moles (1.0×10^{-6} gm)] was then intravenously injected into the tail vein of each rat. The injection site was wiped with 70% ethanol, and the animals were returned their cages. Because the specific activity of the ^{67}Cu varied with each experiment, a specific mass of $^{67}\text{CuTCPP}$ (1.0×10^{-6} gm) was injected in all experiments. This eliminated any dose effects.

Dissection and Evaluation of Lymph Nodes

Lymph nodes were dissected from inflamed and control animals at either 6, 12, 24, 48, 72, or 96 hours post-injection of $^{67}\text{CuTCPP}$. A total of 13 lymph nodes were removed from each animal. Lymph nodes from all regions of the

body were removed. Figure (2) graphically illustrates the location of the dissected lymph nodes.

At each time period inflamed and control animals were sacrificed, and the lymph nodes were surgically removed. Care was taken to remove the extraneous tissue surrounding the lymph nodes. Dissected lymph nodes were then placed into 20 ml plastic counting vials containing formalin and counted in a NaI autogamma counter (Packard, Packard Instruments, Downers Grove, IL). A calibration standard of $^{67}\text{CuTCPP}$, which was calibrated on a GeLi gamma counter (EG&G Ortec, Oak Ridge, TN) coupled to a multichannel analyzer (Canberra series 35 plus, Meriden, Ct) was counted with the lymph node samples. When the $^{67}\text{CuTCPP}$ had decayed to background levels, the lymph nodes were weighed.

Data Processing

Radioactive measurements (counts) from the $^{67}\text{CuTCPP}$ lymph nodes were entered into an IBM AT personal computer (International Business Machines, Boca Raton, FL) and analyzed using the software program Lotus (Lotus Development Corporation, Cambridge, MA). Radioactivity measurements from the lymph nodes of an individual animal were decay corrected to the injection time of the animal (time zero) (This decay correction allowed lymph nodes from an individual animal and from different animals to be compared). In all experiments, 8 animals (4 inflamed and 4 control) were examined at each time point. The overall uptake of $^{67}\text{CuTCPP}$ by the lymph nodes from the 4 inflamed animals was averaged and compared to the average uptake by lymph nodes from the 4 control animals. A two-tailed T-test was used to determine significance between the uptake of $^{67}\text{CuTCPP}$ by lymph nodes from control and inflamed animals.

RESULTS

Uptake of $^{67}\text{CuTCPP}$ by Individual Lymph Nodes

In order to determine whether inflamed lymph nodes localized greater amounts of $^{67}\text{CuTCPP}$ than did control lymph nodes, a comparison was made between the uptake of $^{67}\text{CuTCPP}$ by inflamed and control lymph nodes. Table (1) illustrates the uptake of $^{67}\text{CuTCPP}$ by various lymph nodes from 4 inflamed, 4 saline control, and 4 non-injected animals at 48 hours post-injection of $^{67}\text{CuTCPP}$. In all lymph nodes examined, the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes was greater than was the uptake by lymph nodes from either control group. This was especially evident in the inflamed popliteal lymph nodes, in which the uptake was 7 times greater than was the uptake by saline control popliteal lymph nodes. When individual lymph nodes from inflamed

and saline control animals were combined (averaged), the overall uptake of lymph nodes from inflamed animals was significantly greater ($p < .05$) than was the uptake by lymph nodes from saline control animals (Table 1). Lymph nodes from inflamed animals localized approximately two times more $^{67}\text{CuTCPP}$ than did lymph nodes from either control group. When the uptake of $^{67}\text{CuTCPP}$ by lymph nodes from the two control groups was compared, the localization of $^{67}\text{CuTCPP}$ by lymph nodes from the saline injected animals was slightly greater than was the uptake by lymph nodes from the non-injected animals.

Changes in Individual Lymph Node Weights

A comparison of changes in weight of the various lymph nodes from inflamed, saline control, and non-injected control animals illustrates that lymph nodes from inflamed animals were heavier than were lymph nodes from control animals at 48 hours post-injection of $^{67}\text{CuTCPP}$ (Table 2). The inflamed popliteal lymph nodes had the greatest weight increase of all the lymph nodes examined. The weight of inflamed popliteal lymph nodes was approximately 4 times greater than was the weight of saline control popliteal lymph nodes. This increase in weight of inflamed popliteal lymph nodes may have contributed to the enhanced uptake of $^{67}\text{CuTCPP}$ by inflamed popliteal lymph nodes as in seen in Table (1). When the weights from the various lymph nodes of inflamed and control lymph nodes were averaged, lymph nodes from inflamed animals were significantly ($p < .05$) heavier than were lymph nodes from either control group (Table 2). The average weight of lymph nodes from saline control animals was slightly heavier than was the weight of lymph nodes from non-injected control animals.

Time Course: Uptake of $^{67}\text{CuTCPP}$

Figure (3) illustrates the uptake of $^{67}\text{CuTCPP}$ by lymph nodes from inflamed and control animals over a 6 to 96 hour time period (post-injection of

the $^{67}\text{CuTCPP}$). At each time period 4 inflamed, 4 saline control and 4 non-injected control animals were examined. The uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes during the first 12 hours following the injection of $^{67}\text{CuTCPP}$ was not significantly different ($p>.05$) from the uptake by saline control animals; however, by 24 hours post-injection the uptake of $^{67}\text{CuTCPP}$ had increased significantly ($p<.05$) over that of saline control lymph nodes. At this time point (24 hours), inflamed lymph nodes localized approximately twice as much $^{67}\text{CuTCPP}$ as did the saline control lymph nodes. This degree of uptake remained constant over the remaining time periods (48 to 96 hours). These results suggest that the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes had reached the maximum concentration by 24 hours and that the level of uptake did not change during the 24 to 96 hour time period. Because time periods longer than 96 hours were not examined, it could not be determined at what time period the $^{67}\text{CuTCPP}$ began to clear from the lymph nodes.

An examination of the time response for the saline control group demonstrates that the uptake of $^{67}\text{CuTCPP}$ during the first 12 hours following the injection of $^{67}\text{CuTCPP}$ was low; however, the uptake of $^{67}\text{CuTCPP}$ during the remaining time periods (24 to 96 hours) was relatively constant. The uptake of $^{67}\text{CuTCPP}$ by non-injected control animals over the 6 to 96 hour time period was similar to the saline control group. The overall uptake of $^{67}\text{CuTCPP}$ by the saline control group was slightly greater than was the uptake by the non-injected control group. At 3 time points (24, 48 and 96 hours) this increased uptake of $^{67}\text{CuTCPP}$ by lymph nodes from saline injected animal was somewhat enhanced but not highly significant.

Time Course: Changes in Weight

The weight of inflamed lymph nodes during the 96 hour time period examined increased during the first 48 hours and then remained constant over the remaining time periods (Figure 4). Because the inflaming agents were

administered 7 days prior to the injection of $^{67}\text{CuTCPP}$, it was expected that (1) maximum inflammation would have occurred by the time the $^{67}\text{CuTCPP}$ was injected and (2) that the lymph node weights would have reached their maximum level. However, the short time interval between bacterial endotoxin and $^{67}\text{CuTCPP}$ injection (4 days) may have induced an inflammation response that resulted in an increase in lymph node weight during the first 48 hours following the injection of $^{67}\text{CuTCPP}$. The weight of saline control lymph nodes remained relatively constant during the time periods examined; however, a slight increase in weight was observed during the 48 to 96 hour time periods. A comparison between the weights of inflamed lymph nodes to the weight of saline control lymph nodes (Figure 4) illustrates that by 24 hours the weights of inflamed lymph nodes were significantly ($p < .05$) heavier than were the weights of saline control lymph nodes. During this time period (24 hours) inflamed lymph nodes localized greater amounts of $^{67}\text{CuTCPP}$ than did the control lymph nodes (Figure 3). These results suggest that changes in lymph node weight may have a role in the uptake of $^{67}\text{CuTCPP}$.

Relationship Between The Uptake of $^{67}\text{CuTCPP}$ and Weight Increases

The final study examined how changes in inflamed lymph node weight affected the uptake of $^{67}\text{CuTCPP}$. In Figure (5), changes in the uptake of $^{67}\text{CuTCPP}$ between inflamed and saline control lymph nodes are compared to the changes in weight between inflamed and saline control lymph nodes at various time periods (6 to 96 hours). For all time periods studied, (except for 6 hours in which the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes was less than in the saline control groups), inflamed lymph nodes localized greater than 3 times more $^{67}\text{CuTCPP}$ than did saline control lymph nodes (solid bars). (A value of 1 indicates equal change by inflamed and control lymph nodes). The maximum change in uptake of $^{67}\text{CuTCPP}$ between inflamed and saline control lymph nodes occurred between 48 and 96 hours. During this time period, inflamed lymph nodes localized 3.6 times more $^{67}\text{CuTCPP}$ than did saline control lymph nodes (Figure 5). In contrast to the change in the $^{67}\text{CuTCPP}$ localization between inflamed and saline control lymph nodes, the change in weight between inflamed and saline control lymph nodes (hatched bars) was relatively constant over the time period examined (Figure 5). At all time periods (6-96 hours) inflamed lymph nodes were 1.8 times heavier than were saline control lymph nodes. Therefore, the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes, compared with saline control nodes, was twofold greater than was the increase in weight. This suggests that the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes is not completely dependent upon the increase in lymph node weight and may involve other physiological factors.

DISCUSSION

Because of the important role the lymphatics play in disease, including cancer, and the problems associated with lymphangiography, there is a need for alternative lymph node imaging procedures. Ideally, these alternative procedures should be simple to perform and not traumatic to the patient. The results we are presenting demonstrate that $^{67}\text{CuTCPP}$ will localize in greater amounts in inflamed lymph nodes than in noninflamed lymph nodes. Therefore, $^{67}\text{CuTCPP}$ may prove to be useful as a diagnostic radiopharmaceutical for imaging diseased lymph nodes.

Unlike other lymphoscintigraphy agents, which distinguish diseased lymph nodes on a morphological basis (increase in size or lack of flow to lymph nodes), $^{67}\text{CuTCPP}$ was shown to localize in greater amounts in inflamed lymph nodes than in noninflamed lymph nodes. Our data demonstrate that $^{67}\text{CuTCPP}$ uptake was not exclusively dependent upon changes in lymph node size (weight), as was shown in Figure (5). Inflamed lymph nodes were shown to localize approximately 3.6 times more $^{67}\text{CuTCPP}$ than did the control lymph nodes, while the weight of inflamed lymph nodes increased only 1.8 times that of control nodes. Therefore, during the period of maximum uptake (48 to 96 hours), there was an approximate twofold increase in the uptake of $^{67}\text{CuTCPP}$ that was not caused by the increase in lymph node weight. If the uptake of $^{67}\text{CuTCPP}$ was solely dependent upon factors that affect lymph node size or weight, the change in uptake of $^{67}\text{CuTCPP}$ induced by the inflammatory process would have been comparable to the change in weight by the inflamed lymph nodes. For example, in Figure (5), the change in the uptake of $^{67}\text{CuTCPP}$ by inflamed nodes when compared to control nodes would have been similar to the change in weight of inflamed lymph nodes when compared to control lymph nodes. The mechanism for this enhanced uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes is not understood at this time. Several possible explanations will

be discussed in the following paragraphs.

First, it is possible that a fraction of the uptake is dependent upon normal inflammatory processes that increase lymph node weight, such as increased cell proliferation and increases in fluid and blood volumes. However, the majority of the uptake is dependent upon factors that do not affect changes in lymph node weight. These factors may include cell membrane permeability changes, which are activated during the inflammatory process and which allow $^{67}\text{CuTCPP}$ to penetrate the lymph node cells. In a similar manner, binding sites may be activated that allow $^{67}\text{CuTCPP}$ to be bound to individual cells.

Second, macrophages, which are known to phagocytize most foreign materials, may become activated during the inflammatory process and ingest $^{67}\text{CuTCPP}$. This action would explain the effects seen in Figure (5). Under normal noninflamed conditions, non-stimulated macrophages may phagocytize $^{67}\text{CuTCPP}$ at a low rate. In the inflamed lymph nodes macrophages become activated (stimulated) and may phagocytize greater amounts of $^{67}\text{CuTCPP}$. Therefore, in the inflamed lymph nodes, the same number of macrophages as in the non-stimulated lymph nodes could theoretically ingest larger amounts of $^{67}\text{CuTCPP}$. Therefore, a small change in lymph node size or macrophage number in the inflamed lymph node could result in a large uptake of $^{67}\text{CuTCPP}$.

The third possible explanation is related to the mechanism proposed for entry of carbon into the lymph nodes. Blau (1978) demonstrated that colloidal carbon was concentrated in the noninflamed lymph nodes in the region of the high endothelial venules (HEV). Because of the unique permeability of these venules, which allows functional lymph node venous communication, a number of investigators have proposed that porphyrins may act similarly to colloidal carbon and localize in these areas of high endothelial cells (Vaum et al., 1982, and Robinson, Jr., et al., 1986). Because HEV's increase in density during the inflammatory response, the uptake of $^{67}\text{CuTCPP}$ would also be dependent upon changes in lymph node weight. Further studies will be required to determine the

mechanism by which $^{67}\text{CuTCPP}$ localizes in inflamed lymph nodes.

Even though the mechanism of $^{67}\text{CuTCPP}$ localization is not understood, the potential of $^{67}\text{CuTCPP}$ as an imaging agent is apparent. Because the maximum uptake of $^{67}\text{CuTCPP}$ is reached by 24 hours post-injection, clinical studies could be started within a reasonable time following the administration of $^{67}\text{CuTCPP}$. In addition, the maximum uptake of $^{67}\text{CuTCPP}$ remains constant for at least 96 hours. This would allow clinical studies to be performed at later time periods if necessary. The 62 hour half-life of ^{67}Cu would also allow studies to be performed at these longer time periods. Copper-67 has three major gamma photons (91, 93, and 185 keV), which comprise 71% of the total photon yield (Ramam and Pinajian, 1969). These photon energies are well within the detection capabilities of current nuclear gamma cameras. The strong chelate formed between ^{67}Cu and the porphyrin allows for a very stable complex. This complex has been shown to very stable in human and rat sera (Roberts, et al., 1987); thus, the background levels of uncomplexed $^{67}\text{Cu}^{2+}$ should be very low.

Because the uptake of $^{67}\text{CuTCPP}$ is dependent upon physiological factors that are not exclusively related to the increase in lymph node size caused by inflammation, the potential exist for a very sensitive lymph node imaging agent. Due to the physiological process involved in the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes, small changes in lymph node size occurring during the early stages of an inflammatory response may result in a large uptake of $^{67}\text{CuTCPP}$. Under optimal conditions, $^{67}\text{CuTCPP}$ may be able to identify lymph nodes that are in the early stages of an inflammatory response and which do not have detectable changes in size.

The route of administering a lymph node imaging agent can also have an effect on which lymph nodes can be visualized. Vaum and coworkers (1982) pointed out that oil lymphangiography, ^{99m}Tc antimony trisulfide colloid, and other colloidal imaging agents that are injected subcutaneously, have the inherent limitation of imaging only those nodal groups that drain the injection

area. The intravenous route of administration has several advantages as discussed by Robinson Jr. and coworkers (1986). The intravenous administration of a lymph node imaging radiopharmaceutical offers the advantage of a noninvasive procedure for evaluating mediastinal, mesenteric, cervical, and other deep seated lymph nodes, which are difficult to image using subcutaneous injection. Therefore intravenous (I.V.) lymphoscintigraphy affords the ability to image lymph nodes which cannot be imaged by more conventional techniques. For example, the following procedure could be used as a potential application to evaluate lymph nodes. A single I.V. injection of a radiopharmaceutical, such as $^{67}\text{CuTCPP}$, could be used to evaluate lymph nodes in all regions of the body. Regions of the body in which lymph nodes are identified as positive using the I.V. technique could be more clearly reevaluated using a subcutaneous injected radiopharmaceutical. If $^{67}\text{CuTCPP}$ proves to be a successful lymph node imaging agent, it could be used in the initial lymph node screening procedure.

SUMMARY

Our results clearly demonstrate that lymph nodes from inflamed animals localize greater amounts of $^{67}\text{CuTCPP}$ than do lymph nodes of control animals. The uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes reached the maximum level by 24 hours following the injection of $^{67}\text{CuTCPP}$ and remained constant throughout the 96 hour time period examined. During this interval inflamed lymph nodes localized approximately 3.6 times more $^{67}\text{CuTCPP}$ than did control lymph nodes. The mechanism involved in this process is unknown; however, it may involve changes in lymph node weight as well as some intrinsic physiological process that does not contribute to weight changes. The fact that some physiological factor is involved in the uptake of $^{67}\text{CuTCPP}$ by inflamed lymph nodes is encouraging in terms of developing a nuclear medicine lymph

node imaging agent.

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Table 1

Percent Uptake of $^{67}\text{CuTCCP}$ By
Individual Lymph Nodes

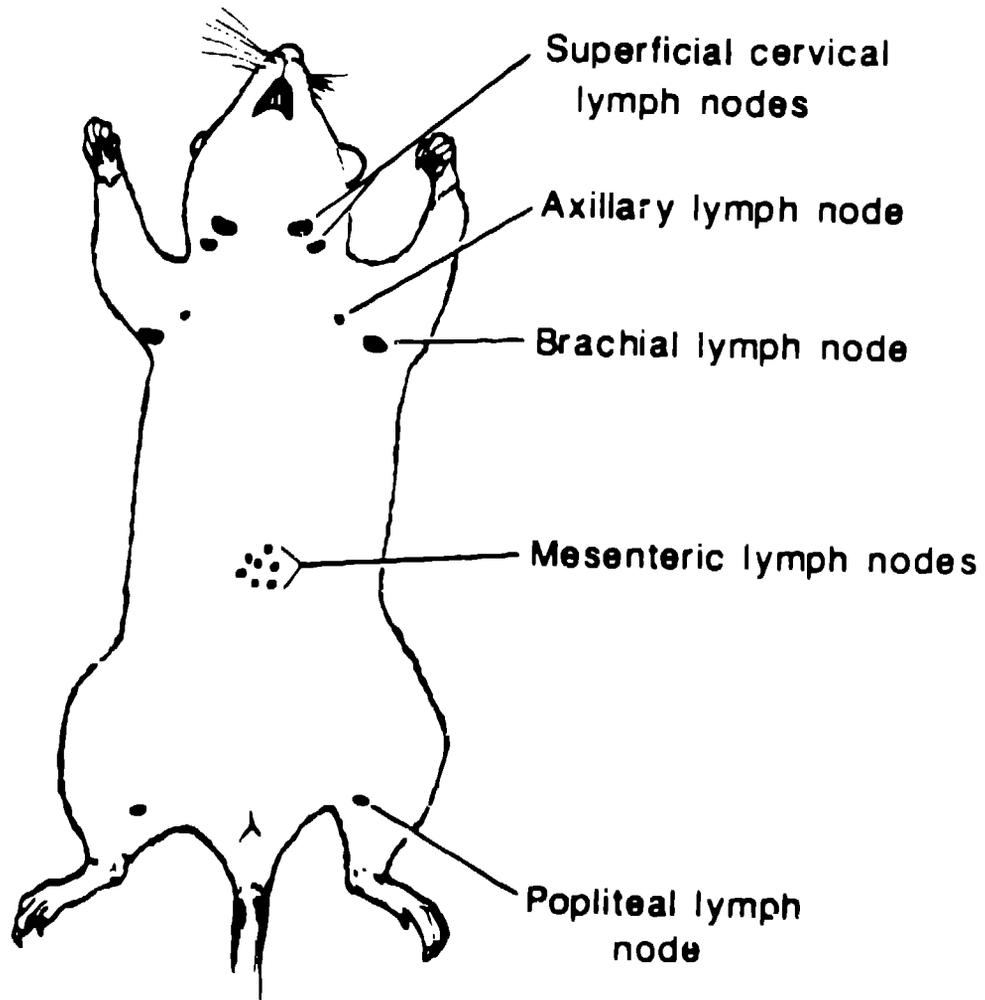
LYMPH NODE	INFLAMED	SALINE CONTROL	NON-INJECTED CONTROL
Cervical (R) ¹	0.030 ± 0.016 ²	0.017 ± 0.005	0.011 ± 0.001
Cervical (L)	0.023 ± 0.012	0.015 ± 0.003	0.011 ± 0.003
Axillary (R)	0.021 ± 0.003	0.017 ± 0.008	0.003 ± 0.001
Axillary (L)	0.023 ± 0.010	0.007 ± 0.002	0.005 ± 0.001
Brachial (R)	0.021 ± 0.001	0.014 ± 0.003	0.009 ± 0.006
Brachial (L)	0.019 ± 0.004	0.006 ± 0.002	0.004 ± 0.001
Popliteal (R)	0.022 ± 0.011	0.004 ± 0.001	0.002 ± 0.001
Popliteal (L)	0.033 ± 0.008	0.003 ± 0.001	0.003 ± 0.001
Mesentery	0.038 ± 0.010	0.024 ± 0.012	0.022 ± 0.003
MEAN³	0.026 ± 0.002	0.012 ± 0.002	0.008 ± 0.002

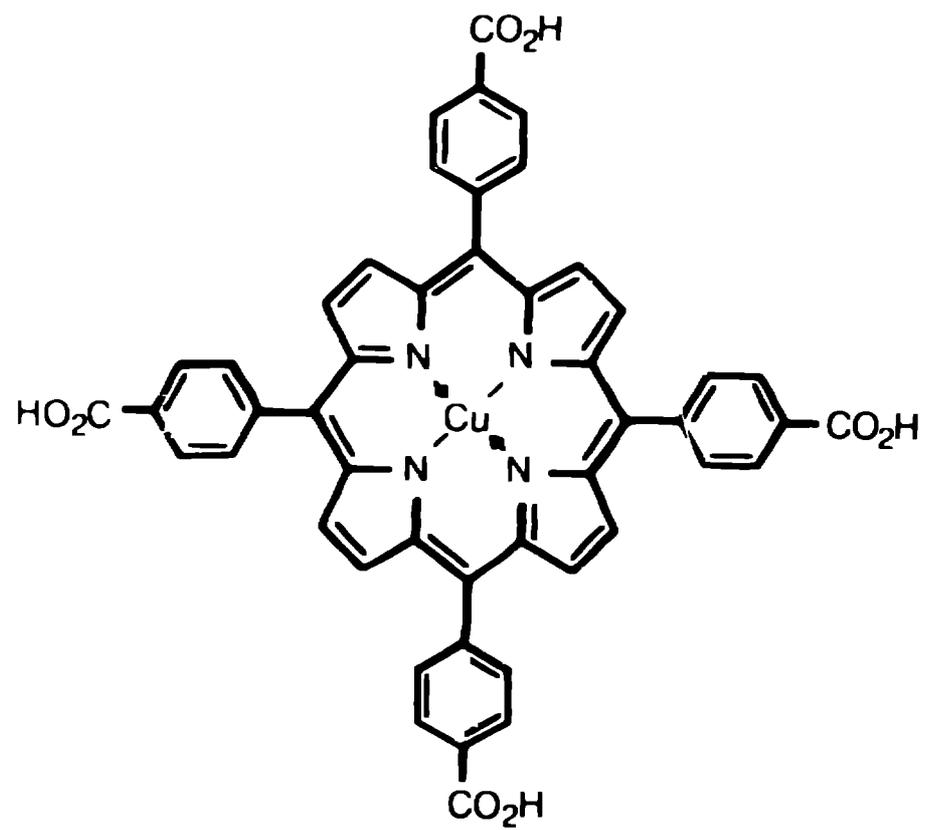
¹ Individual lymph nodes from either inflamed, saline control or non-injected control animals. (R=right and L=left lymph nodes).

² Each value represents the mean percent uptake of injected dose of $^{67}\text{CuTCCP}$ and the standard deviation of 4 individual lymph nodes.

³ The mean and standard error of the mean of the percent uptake of injected dose of all lymph nodes from each group (inflamed, saline control and non-injected control).

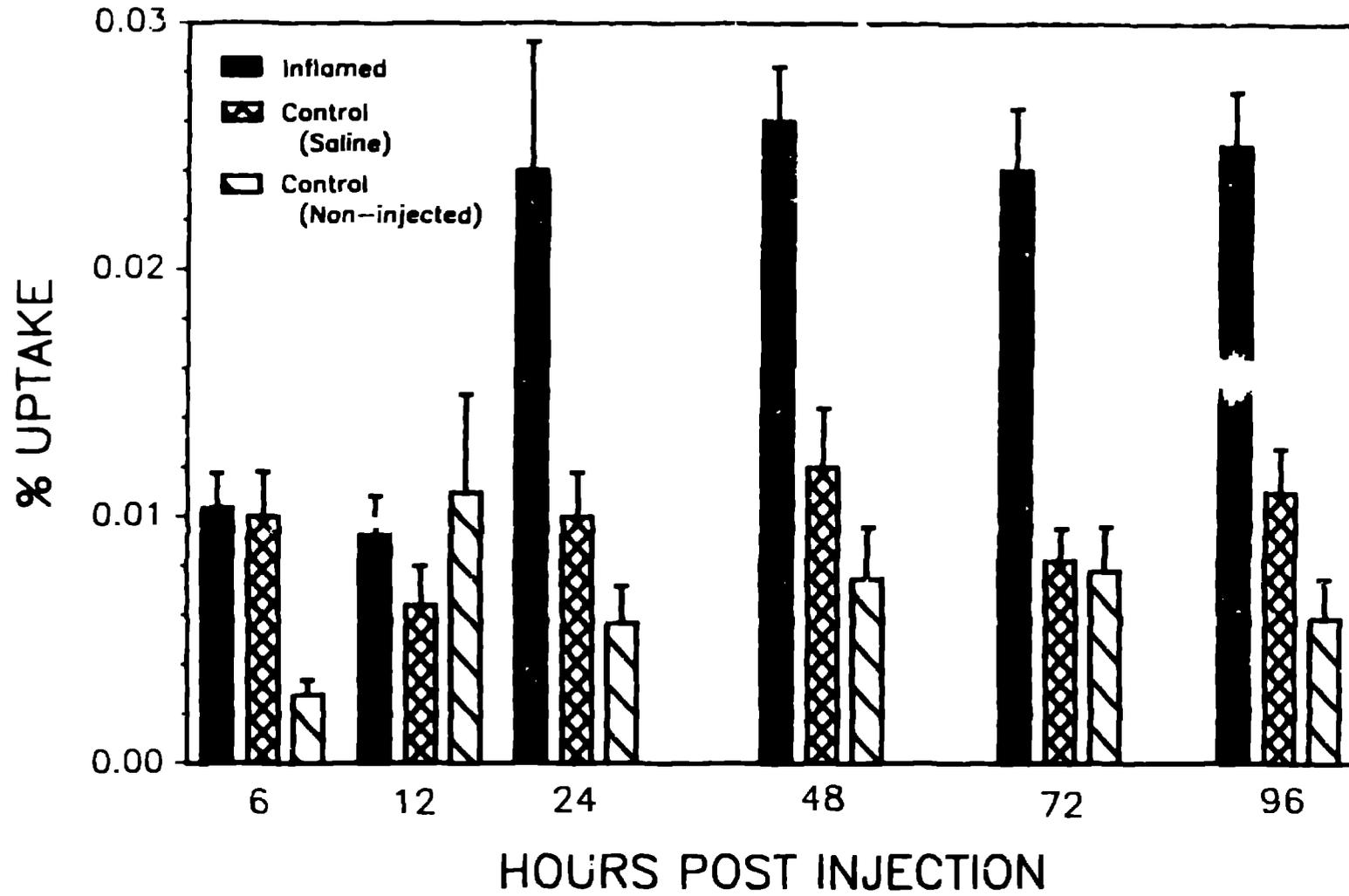
LOCATION OF LYMPH NODES IN THE RAT





% UPTAKE OF INJECTED DOSE

ALL LYMPH NODES



LYMPH NODE WEIGHTS

ALL LYMPH NODES

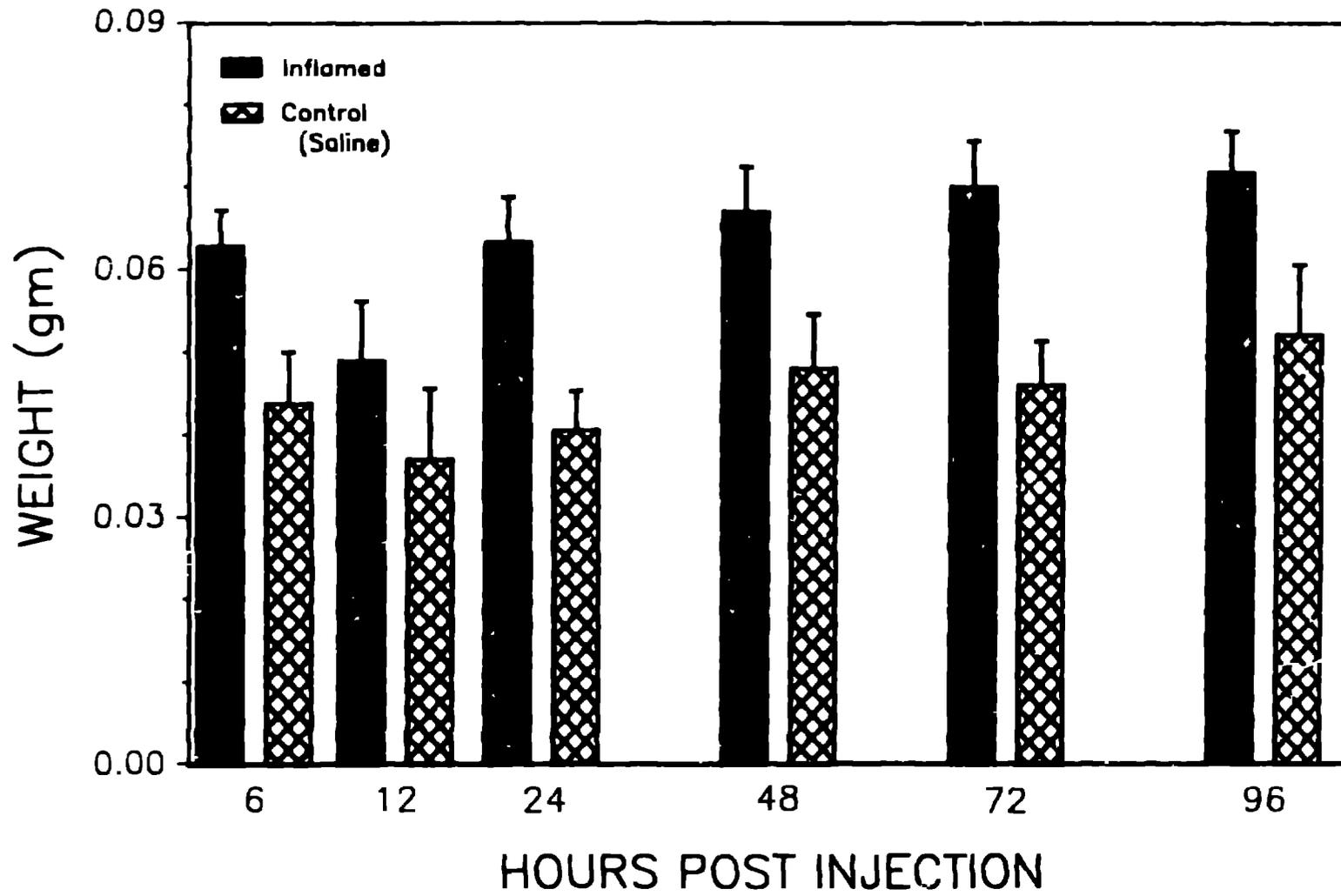


Figure 1

RATIO OF INFLAMED TO CONTROL LYMPH NODES FOR % UPTAKE AND WEIGHT

