# Trapping of Calibrated Microspheres in Rat Lymph Nodes

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The diagnostic and therapeutic usefulness of intralymphatic microspheres depends on the site and duration of lodgement of the injected particles within the lymph nodes. The size of the spheres is probably the most important factor that determines where and for how long the particles will remain trapped.

This study was carried out to test, under controlled conditions, the filter capacity of lymph nodes for spheres of various calibers.

## *Materials and methods*

*Microspheres.* – Sterile, nonradioactive, complete spheric, homogeneous ceramic microspheres (3 M Co., St. Paul) were used. The size of the microspheres had been designated as 5 to 10 μ, 15 ± 5 μ, 25 ± 5 μ, 35 ± 5 μ, and 50 ± 10 μ. The spheres were kept in a 10% dextran solution.

*Animals.* – A total of 229 male, albino Sprague-Dawley rats, weighing 250 to 350 gm were used. They were housed under standard conditions at a temperature of 72 F, with free access to a standard laboratory pellet diet *(Rockland)* and water. For injections of microspheres, the rats were anesthetized with a solution of pentobarbital (Pentobrocanal) administered intraperitoneally in a dose of 4 mg/100 gm of body weight. They were killed with ether in groups of 6 to 12 animals at time intervals of 4 hours and 1, 2, or 7 days. A complete autopsy was done on each animal, and all regional nodes were removed for histologic study (Fig. 1).

*Injection procedure.* Anatomic Considerations. – A typical example of the draining lymphatic vessels and lymph nodes after ink injection is shown in Fig. 1. Anatomic variations have been discussed in a previous paper (1).

Technique of Injection. – The microspheres were injected into the superficial lymphatic channels at the hilus of the left testis or the adjacent epididymal fat tissue. Details of the technique have been previously described (1). For this study, a sterilized 27-gauge needle fitted to an 18-inch plastic tube was used with a 2-ml glass syringe, and a Luer-Lok tip. The suspension of microspheres in dextran was diluted 3 to 4 times with a

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balanced salt solution that was faintly stained with Evans blue to demonstrate the injected lymphatic channels. The injected volume ranged from 0.03 to 0.08 ml. All containers, syringes, tubes, and needles were siliconized and sterile. In spite of this precaution, micropheres tended to cling to the walls, so that hemocytometer counts prior to the injection indicated little about the number of microspheres that actually reached the lymph nodes. Because of their size, the spheres of sizes 35 and 50 μ were very difficult to inject. In only nine animals were we successful in injecting particles of these sizes.

*Histologic techniques.* – Fixation, embedding, and staining procedures have been described previously (1). Sections were cut at a thickness corresponding to the size of the injected microspheres, that is, for lymph nodes with 25 μ sections were cut.

Histologic measurements of microspheres are only valid if the size of the particles is not affected by the heat and the chemical substances used during the embedding procedure. According to the company specifications, the microspheres should be completely resistant to the chemicals and temperatures used. To further test the effect of the embedding procedure, lymph nodes with microspheres were divided into two halves. Fresh frozen sections or touch preparations were prepared from on half and paraffin sections were prepared from the other. A total of 100 microspheres were then individually measured in each of the frozen and paraffin sections and in the touch preparations, and the medium values and the variations in size were compared.

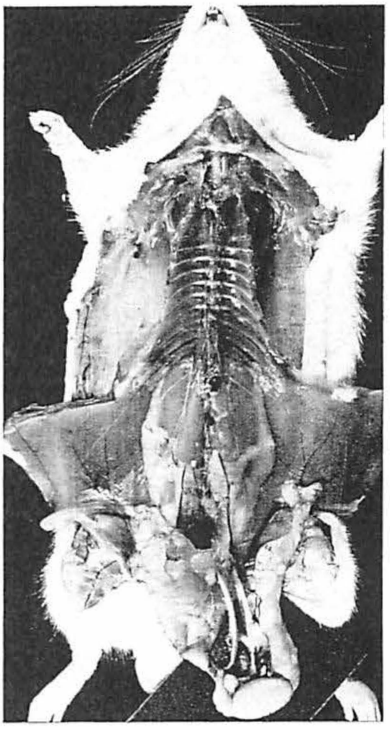

Fig. 1 Retroperitoneal lymphatic system of rat after ink injection into left epididymal lymphatic channels. Main drainage leads to para-aortic (pararenal and celiac) and left upper mediastinal lymph nodes which are stained black. Few small lymphatic channels lead to lymph nodes near the bifurcation of the aorta.

Histologic measurements were carried out with a calibrated measuring ocular (Zeiss; Kpl 12.5 × W Pol, and calibrator slide, 5 + 100/100 mm).

## *Results*

The injection procedure was successful in 47% of the rats. A total of 108 rats showed microspheres in at least one of the sections of the regional lymph nodes. Table 1 shows the number of rats with positive lymph nodes in the 12 experimental groups, and the total number of microspheres counted in the histological sections. Table 2 shows the size-related distribution of the microspheres in the different lymph-node compartments after death in rats sacrificed at varied intervals. The distribution is expressed as a percentage of the total numbers of microspheres shown in Table 1.

Table 1 Number of Microspheres in 12 Groups* of Rats with Positive Lymph Nodes.

| Time after injection | Sphere size | | | | | |
|---|---|---|---|---|---|---|
| | 10 μ | | 15 μ | | 25 μ | |
| | No. of rats | Total number of microspheres | No. of rats | Total number of microspheres | No. of rats | Total number of microspheres |
| 4 hours | 8 | 4,206 | 8 | 929 | 6 | 46 |
| 1 day | 8 | 4,186 | 8 | 360 | 10 | 431 |
| 2 days | 12 | 6,900 | 8 | 509 | 8 | 534 |
| 7 days | 9 | 8,011 | 6 | 767 | 8 | 885 |

* Nine rats in whom spheres of 35 and 50 μ were injected are excluded.

Table 2 Size Related Distribution of Microspheres in Different Lymph-Node Compartments and Time Elapsed After Intralymphatic Injection.*

| Size of spheres in microns | Marginal sinus | | | Intermediary sinus | | | Cortical pulp | | | Medullary sinus | | | Medullary cord | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 15 | 25 | 10 | 15 | 25 | 10 | 15 | 25 | 10 | 15 | 25 | 10 | 15 | 25 |
| Time after injection | | | | | | | | | | | | | | | |
| 4 hours | 71 | 99 | 100 | 18 | 1 | 0 | 3 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 |
| 1 day | 34 | 92 | 99 | 22 | 1 | 0 | 22 | 6 | 1 | 19 | 1 | 0 | 3 | 0 | 0 |
| 2 days | 21 | 80 | 91 | 15 | 1 | 1 | 50 | 18 | 7 | 8 | 1 | 1 | 6 | 0 | 0 |
| 7 days | 6 | 78 | 90 | 21 | 5 | 0 | 49 | 16 | 10 | 16 | 1 | 0 | 8 | 0 | 0 |

* Distribution is expressed in % of total number of spheres counted in all positive lymph nodes of all rats, that is, all animals that received microspheres of the same size and that were sacrificed after the same time interval.

The size of the microspheres was not significantly affected by the embedding procedure. Averages of comparative measurements of the diameters of the particles in the 5 to 10 μ and 25 μ groups varied by about 10%. Although most of the spheres were within the size range indicated by the company literature, a few spheres in the 25 μ group were as small as 15 or as large as 42 μ (Fig. 2 A). Extreme sizes in the 15 μ group were 12 and 24 μ, and in the 5 to 10 μ group, the smallest spheres were 2 μ, while none was larger than 10 μ.

Fig. 2 Microspheres. A. In subcapsular and intermediary sinuses of para-aortic (renal) lymph node. Rat sacrificed 2 days after injection. The spheres that progressed farthest toward hilus were 15 to 18 μ, the ones in peripheral portion of intermediary sinuses were 18 to 30 μ, and the largest spheres in subcapsular sinus were 42 μ. (Hematoxylin and eosin; ×120.) B. In subcapsular sinus of para-aortic (renal) lymph node. Rat sacrificed 2 days after injection. This sphere was 62 μ. (Hematoxylin and eosin; ×360.) C. In medullary sinus of para-aortic (renal) lymph node. Rat sacrificed 2 days after injection. Spheres in this field were between 2 and 8.5 μ and were phagocytized by transformed sinus histiocytes. (Hematoxylin and eosin; ×880.) D. In intermediary sinus of para-aortic (renal) lymph node. Rat sacrificed 4 hours after injection. Spheres in this field were 9 to 20 μ. (Hematoxylin and eosin; ×440.)

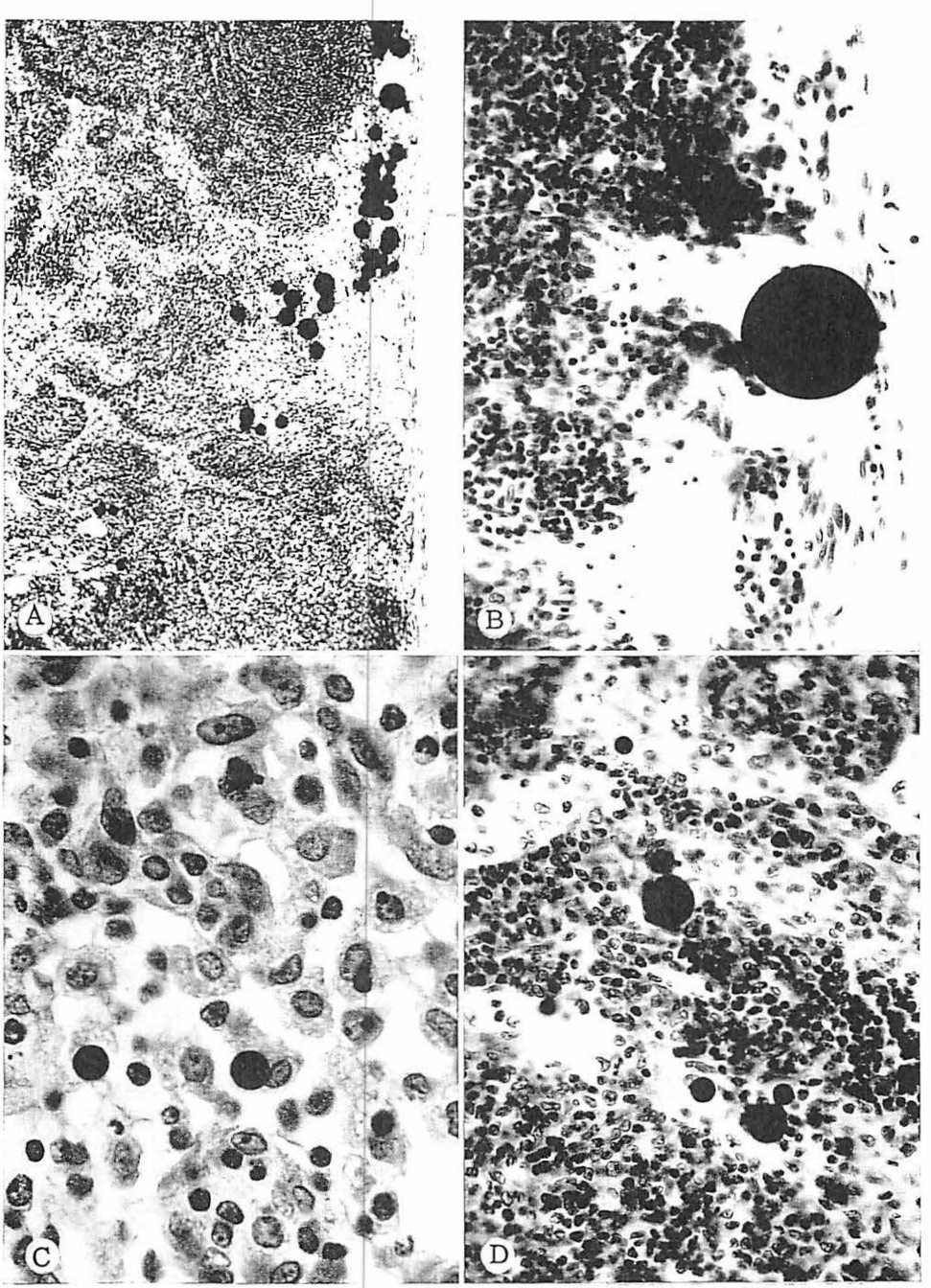


Fig. 2

Dislocation or breakage of spheres during the cutting procedure was rarely observed, and this was seen only with spheres larger than 30 μ. Usually in these instances, well-circumscribed spaces in the tissue indicated the original position of the spheres.

The position of the microspheres was easy to ascertain in most but not all cases. For instance, the subcapsular sinuses were not always identifiable; on occasion, sinuses were difficult to delineate from the pulp, or the plane of sectioning was not exactly through the hilus. The medullary cords tended to show plasmacellular transformation, which permitted them to be delineated from the cortical pulp. Histiocytosis was found more often in the intermediary sinuses than in the medullary sinuses, but the sinuses were never sharply separated. Clustering of microspheres was another occasional phenomenon that made counting difficult.

Although not shown in Table 2, the smaller spheres usually were found closer to the hilus; that is, in the same lymph nodes, they seemed to travel faster than the slightly larger spheres (Fig. 2 A).

Microspheres larger than 30 μ seemed to remain in the subcapsular sinuses and did not enter any of the other lymph-node compartments (Fig. 2 B). Spheres that were 25 μ and larger occasionally clustered and caused tissue necrosis or, rarely, microabscesses. In such a situation, spheres that were 35 or 50 μ could reach an intermediary sinus or the cortical pulp. Sinus histiocytosis developed in most instances, often accompanied by much phagocytosis of spheres that were 10 μ and smaller (Fig. 2 C).

Table 2 shows that the intermediary sinuses were reached within 4 hours after injection by 18% of the spheres in the 5 to 10 μ group and 1% of the spheres in the 15 μ group (Fig. 2 D). At this time, the cortical pulp was infiltrated by only 3% of the 5 to 10 μ spheres and less than 1% of the larger particles. After 1 day, a small percentage of spheres in the 15 μ group reached the cortical pulp and the medullary sinuses, but 2 days were needed for small numbers of spheres in the 25 μ group to reach the medullary sinuses. Most spheres in the 25 μ group which seemed to have passed the limiting membrane of the marginal sinus were somewhat smaller than 25 μ by actual measurement and, in most cases, seemed to remain just beyond the limiting membrane. In the medullary cords, spheres 10 μ or smaller were found, regardless of the time interval after injection.

### *Discussion*

Radioactive microspheres have been used to study the blood supply (2) or radiation treatment of experimental tumors (3, 4). Radioactive microspheres were also used for the treatment of a limited number of patients with advanced malignant tumors (5–8). In these cases, spheres that were 15 μ or 40 to 60 μ were either injected into blood vessels or applied locally.

In very few cases, intralymphatic radiotherapy with microspheres-$^{90}$Y has been attempted (9, 10). Spheres that were 1 to 4 μ were used. Of all available techniques (11), this type of intralymphatic therapy seemed best suited because there is no spillover of radioactivity into the general circulation or diffusion into the surrounding tissues and

because the carrier is nontoxic. Unfortunately, the injected material localized in the normal parts of the lymph nodes. Because of the short penetration of β rays, radioactive microspheres of this type can be expected to affect only microemboli. Therefore, intralymphatic radiotherapy seemed to serve best for the prophylactic treatment of lymph nodes, that is, for the treatment of expected microtumor emboli (11). More studies are necessary to establish how maximal intranodal tumor radiation can be achieved. Rat lymph nodes may serve as a model because most of their structural elements seem to resemble those of the larger human lymph nodes.

We may assume that the spaces in the limiting membranes of rat lymph nodes are not large enough or cannot be stretched enough to let particles larger than 30 μ pass. This confirms previous observations (12).

The distribution pattern of the smaller microspheres showed striking similarities to the distribution of Walker carcinosarcoma cells in the phase of the premetastatic intranodal spread (1). Both tumor cells and microspheres tended to spread around the entrance of the afferent lymphatics in a disk-like fashion through the subcapsular sinuses and then to infiltrate only the adjacent segment of the node through spaces in the limiting membrane of the marginal sinus. Another common feature was the tendency of tumor cells and microspheres to cluster in the peripheral cortical pulp, usually close to the limiting membrane, and to penetrate through secondary follicles just as easily as through the remainder of the cortical pulp. Neither tumor cells nor microspheres were ever seen to collect in greater numbers in the medullary sinuses.

In spite of the previously mentioned shortcomings of the method, the similar distribution pattern of intralymphatically injected tumor cells and microspheres in the 10 to 15 μ range indicated that lymph flow and other mechanical factors affected tumor cells and spheres in almost the same manner.

Although spheres that were 1 to 4 μ localized in human inguinal and iliac lymph nodes (9), the present study indicated that microspheres of about 15 μ also are easy to inject and would be optimal for intralymphatic radiotherapy because of their slower passage through the lymph nodes.

## *Summary*

Intralymphatically injected, calibrated microspheres up to a size of 30 μ penetrated the limiting membrane of the marginal sinuses of rat lymph nodes. Larger spheres were difficult to inject and seemed to remain in the marginal sinuses.

Within 4 hours after injection, the medullary sinuses were reached by a large number of spheres that were 10 μ or smaller. Two days were needed by spheres that were 25 μ to reach, in small numbers, the medullary sinuses. The spheres in the 15 μ range showed a distribution that was between the two groups. In the medullary cords, only spheres smaller than 10 μ were found, even at the end of the 7-day study period.

The lymph-node distribution pattern of spheres that were 10 to 15 μ resembled that of intralymphatically injected Walker carcinosarcoma cells.

The location of microspheres within the lymph nodes depended on particle size and time elapsed after injection. Microspheres in the 15 μ range appear most promising for intralymphatic radiotherapy of tumors.

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# ABSTRACTS

## Basic Science

STERNS, E. E., E. R. VAUGHAN (Surg. Res. Lab. Queen's Univ., Kingston, Ontario, Canada): **The Lymphatics of the Dog Colon.** Cancer 26 (1970), 218–231

A study of the lymph drainage patterns of the dog colon has been done by indirect lymphography. The technique was carried out under normal and various physiologically abnormal conditions which included acute sterile inflammation, mechanical obstruction, and thoracic duct obstruction. Ethiodol was injected into the subserosa of the bowel through a laparotomy incision. Drainage patterns, size and number of nodes, and intramural spread of the contrast medium were all noted and documented.

This paper appears to be an interesting reference work for surgeons, anatomists, physiologists, and lymphologists interested in the lymph drainage of the gastrointestinal tract. B. J. LEE

SATO, C. (Dept. of Radiat. Res., Tohoku Univ. School of Med.): **Earlier Recovery of Lymphopoiesis and Immunological Response by Bone Marrow Shielding after Leukemogenic Irradiation in Mice.** Gann 60 (1969), 393–400

This well documented article demonstrates that thigh shielding, during total body radiation in mice, promotes more rapid recovery of lymphoid cells in the thymus, more rapid regeneration of lymph nodes and earlier recovery of lymphocyte counts in the peripheral blood. There was a concomitant striking reduction in thymoma incidence in shielded mice when compared to a large series of controls.

This paper is provocative when one considers the current emphasis on ever-increasing tumor doses and port sizes which are being recommended by many radiotherapists for the treatment of various types of cancer. The author relates the high rate of protection against oncogenesis in these shielded animals to the earlier and more complete recovery of the stem cell population, and documents his reasons for this conclusion. B. J. LEE

WIEDERHIELM, C. A.: **Dynamics of Transcapillary Fluid Exchange.** Proc. Symp. N.Y. Heart Ass. (1968), 29–63

An analog computer program is described that stimulates fluid balance at the capillary level and takes into account fluid and protein fluxes into and out of the interstitial space. The model assumes the identity of lymph and interstitial fluid. Solutions are obtained for tissue hydrostatic pressure, tissue fluid osmotic pressure, interstitial space volume and lymph