The Technique and Applications of Lymphography

J. W. Davidson, A. L. Fletch, G. McIlmoyle and W. Roeck*

ABSTRACT

The technique of radiographic examination by lymphography of normal lymphatic structures regional to the hind limbs is described in the rabbit, dog and rhesus monkey. Radiographic appearances of normal lymph nodes and possible applications to veterinary science are indicated.

Normal lymph node microstructure is illustrated using in vivo intralymphatic injection of a brightly colored radiopaque silicone rubber. Angiogenesis in active germinal follicles during the period of an immune response is displayed. Further applications of the casting technique are suggested.

RÉSUMÉ

Les auteurs décrivent l'application de la technique de l'examen radiographique des structures lymphatiques normales drainant les membres postérieurs, au moyen de la lymphographie, chez le lapin, le chien et le singe rhesus. Ils commentent l'apparence radiographique des ganglions lymphatiques normaux, ainsi que ses applications éventuelles à la science vétérinaire.

L'illustration de la microstructure d'un ganglion lymphatique normal s'effectue in vivo par l'injection intralymphatique d'un silicone opaque aux radiations et de couleur vive. On met en évidence l'angiogénèse dans les follicules germinatifs fonctionnels, au cours d'une réaction immunologique. On suggère d'autres applications de cette technique de moulage.

*Radiological Research Laboratories (Davidson, McIlmoyle and Roeck) and Division of Laboratory Animal Science (Fletch), Faculty of Medicine, University of Toronto, Toronto 181, Ontario.

Submitted May 16, 1972.

INTRODUCTION

The advantages of a safe *in vivo* method of displaying pelvic and retroperitoneal glands in humans include early detection of disease and correct choice of optimal sites for biopsy. During the past two decades, study on radiographs of lymphatic structures regional to the limbs, by cannulating lymph vessels and introducing iodinated contrast media, has become a well-established technique in man (14, 15, 22). Because pelvic and retroperitoneal lymph glands are impalpable, lymphography has found its principal application in evaluating the extent of malignant lymphomas and the spread of neoplasms via lymphatic structures (10-16).

In animals, lymph vessels were first discovered by Gaspar Aselius (1581-1626) of Milan who recognized lacteals in the mesentery of the dog during vivisection. The topography and anatomy of lymphatic structures in man was appreciated by William Hunter (1784), but it was not until 1929 that radiographic demonstration of lymph trunks was possible following injection of iodinated media (11). Since then, lymphatic cannulation has been frequently performed in animals to study the physiology of lymph flow (6, 7). Studies of the radiographic appearances of metastatic disease from the V2x tumour in the popliteal nodes of the rabbit (21) led to more widespread application of lymphography using Ultra Fluid Lipiodol, an oily contrast medium, which displayed both lymph vessels and nodes (17).

To date undue emphasis on difficulties in the technique of lymphography has precluded its extensive use in veterinary practice. In this laboratory, interest in examining lymph nodes of rabbits, dogs and monkeys during immune reactions arose from a lack of understanding of radiographic appearances in man (4). This communication describes the technique of

Fig. 1A. Rabbit Hindleg — Medial aspect — Dye in Lymphatics.

— Blue stained lymph trunks. ↓

— Occluding suture. ←

— Loose tie suture. ↘

lymphography and the resulting radiographic appearances in animals. In addition, a further application of *in vivo* intralymphatic injection using a brightly coloured radiopaque silicone rubber is illustrated.

MATERIALS AND METHODS

LYMPHOGRAPHY

Lymphatic Cannulation — For sedation, intra-muscular Innovar Vet[1], 0.25 ml per kg body weight in the New Zealand white rabbit, and 0.10 ml per kg in the domestic dog or rhesus monkey *(Macacca mulatta)* was used because of the minor nature of the procedure. This possibly avoided the pulmonary complications of more prolonged and deep barbiturate anaesthesia. However, many other forms of sedation or light anaesthesia are equally suitable and disadvantages of Innovar Vet included occasional muscle twitching and incontinence. Lymphatic channels were visualized by injecting sub-dermally 0.5 ml of a mixture of equal parts of Patent Blue V[2] dye and 1% Lignocaine[3] into a toe web on the dorsum of each hind foot. After a few minutes the blue dye was transported in superficial lymph vessels which were clearly seen through the skin of the leg.

With local anaesthesia a 2 cm skin incision adjacent to a lymph trunk was made on the medial aspect of the distal hindleg. The blue dye was milked up the lymph trunks making them easily seen. The most suitable vessel for cannulation was distended by placing a firmly tied black linen suture around both the lymph channel and a small amount of surrounding soft tissue, near the upper end of the incision. Removal of this suture after lymphatic cannulation was facilitated by inserting it through the skin edge where it could be cut with minimal movement (Fig. 1A).

The animal was then kept immobile. A supine position with the legs extended was suitable to align the lymphography needle and lymph vessel. Alternatively the animal was placed in lateral recumbency, and lymphatic cannulation on one side was followed by turning the animal and repeating the procedure on the opposite leg. The fascial planes overlying the lymphatic were gently cut in the direction of the vessel using a sharp scalpel. Fragility of lymph vessels contraindicated attempting to isolate them completely by removing the surrounding fat. A suture was then placed around the vessel 1.5 cm distal to the occluding suture.

It was eventually used to tie the lymphography needle within the lumen,

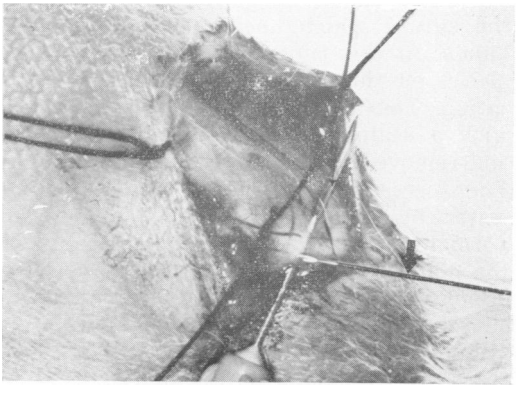

Fig. 1B. Rabbit Hindleg — Medial aspect — Dye in Lymphatics.

Note immobilizing needle behind lymphatic. ↓ Cannulating needle aligned with vessel. ↓

[1]Pitman-Moore Ltd., Don Mills, Ontario.

[2]Sterilab, Toronto, Ontario.

[3]Xylocaine, 1%, Astra Pharmaceutical Division, Mississauga, Ontario.

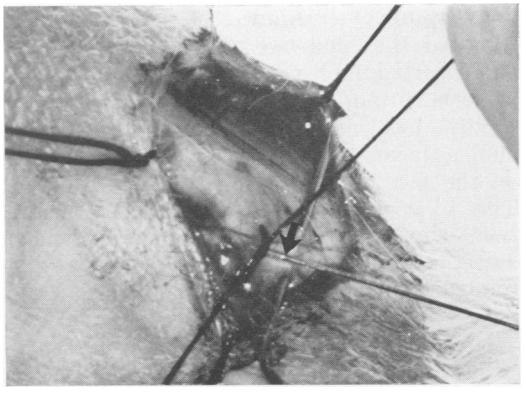

Fig. 1C. Rabbit Hindleg — medial aspect — Dye in Lymphatics.

The lymphography needle has been advanced into the lymph trunk. ⬇

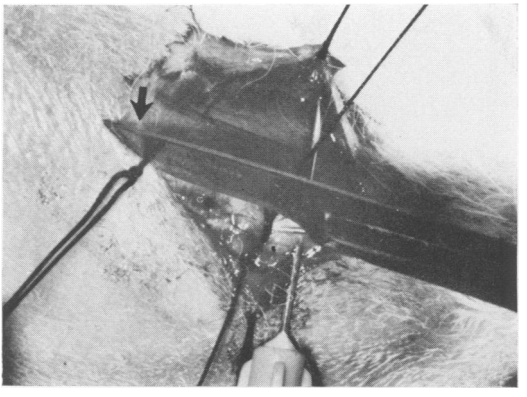

Fig. 1D. Rabbit Hindleg — Medial aspect — Dye in Lymphatics.

Removal of occluding suture. ⬇ Note tie suture has been tightened around the needle within the lymphatic.

and therefore a single loose knot was made with the ends left in an easily accessible position. Experience made it possible to often thread the lymphography needle up the lumen of the lymphatic for 0.5 to 1 cm, in which case no tie suture was required.

Prior to cannulation the lymph vessel was immobilized by a 25 gauge needle inserted from left to right behind its surrounding soft tissues (Fig. 1B). The lymphatic was kept perfectly still and slightly stretched, and by holding a 30 gauge needle with the left hand it was introduced into the lumen and advanced for approximately 0.5 cm with the right hand (Fig. 1C). The operator had to ensure that no tension on the connecting tube caused withdrawal or displacement of this needle when the hands were removed to tighten the suture which anchored it within the lumen. At this time small wet gauze swabs placed on the tubing facilitated immobilization. The occluding suture was then cut with a scalpel adjacent to the skin edge and removed without movement (Fig. 1D). The success of cannulation could then be tested by injection of a small amount of normal saline with minimal pressure on the plunger of the syringe. A few tiny air bubbles through the lymphatic or distension of the lymph trunk meant an intact system through which radiographic contrast material could be introduced.

Radiography — Ultra Fluid Lipiodol[4], a 37% iodine-containing mixture of the esters of natural poppy seed oil, is the most suitable contrast medium for lymphography. The total dose of 0.15 ml UFL per kg body weight was divided equally between the two limbs. Because a slow rate of injection, approximately 1 ml per ten min was most suitable, an inexpensive syringe holder was made to allow constant pressure by a lead weight on the plunger of a 10 or 20 cc syringe (Fig. 2). A 2 lb lead weight on the plunger of the syringe resulted in an injection pressure at the end of the needle of approximately 50 mm of mercury or less, levels often reached during rapid passive movement and exercise in the intact animal (12). Immediately after the contrast medium had been introduced, the needles were removed and the incisions closed. It was important to leave lymph vessels intact by careful removal

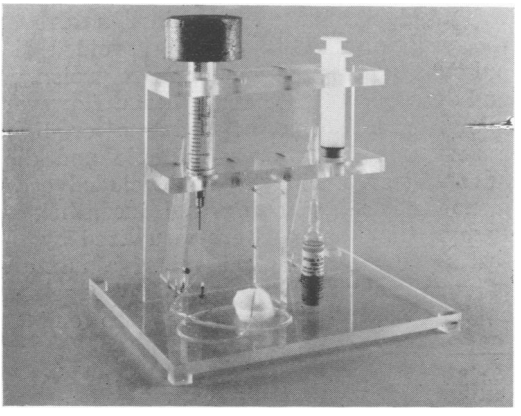

Fig. 2. Simple syringe holder with two pound lead weight on plunger.

[4]Denver Laboratories (Canada) Limited, 451 Alliance Avenue, Toronto 334, Ontario.

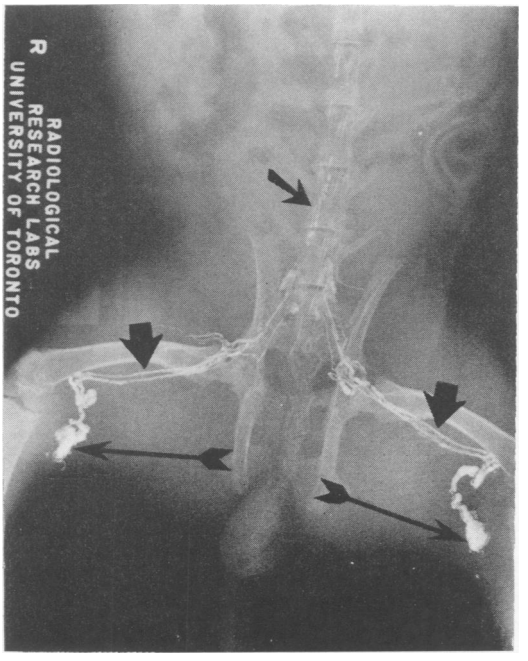

Fig. 3. Rabbit — Ventrodorsal Radiograph — Pelvis and Hindlimbs.

— Popliteal lymph nodes.

— Lymphatics ascending to inguinal region.

— Para-arotic lymph trunks.

of sutures to avoid subsequent lymphedema and slow wound healing.

A ventrodorsal radiograph of the abdomen, pelvis and limbs of the animal showed lymph trunks from both popliteal nodes to the para-aortic regions (Fig. 3). Suitable exposure factors were approximately 90kv and 200 Mas using a 0.3 mm focal spot and 36″ film focus distance for a 3 kg rabbit or 5 kg dog.

Non-screen RPM[5] fine grain film gave excellent radiographic definition in small laboratory animals, but a cassette and grid are recommended for larger subjects. Simple primary magnification techniques gave useful additional enhancement of radiographic detail (2).

After twenty-four hours UFL had left the lymph vessels and was trapped within the parenchyma of regional nodes which could be seen on ventrodorsal, oblique and lateral radiographs. Homogeneously opacified lymph nodes with a slightly granular texture and smooth contour were seen in the normal mammal (Fig. 4). These nodes remained radiopaque for several months and could be observed on subsequent radiographic examinations to detect changes in the contrast patterns indicating progressive disease. Slow loss of the opaque medium over the ensuing months was a normal finding, after which a second examination could be performed when further study was necessary.

Precautions — Aseptic surgical technique was essential to prevent wound infection and skin sutures were removed after approximately five days. The most important complications described shortly after the introduction of lymphography were due to deposition of excessive amounts of oily contrast material in the vascular bed of the lung (5, 13). This resulted in a chemical pneumonitis (10). Such sequelae in our studies were almost totally avoidable with good technique. ^{131}I labelling of UFL has shown that a minute amount of oily contrast medium is filtered in the vascular bed of the lung after all lymphograms (18) and causes slight alteration in respiratory function (9). However, using

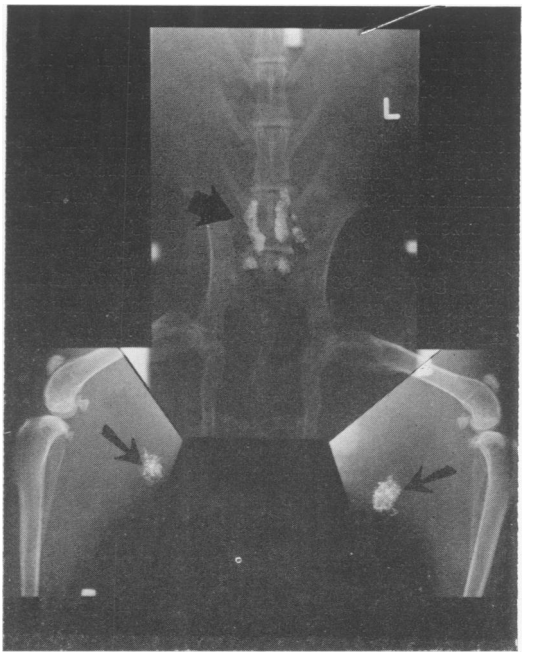

Fig. 4. Rabbit — Ventrodorsal Radiography — Pelvis and Hindlimbs.

— Popliteal lymph nodes.

— Ilio-lumbar lymph nodes.

[5]Rapid Processing Mammography (Kodak Ltd.).

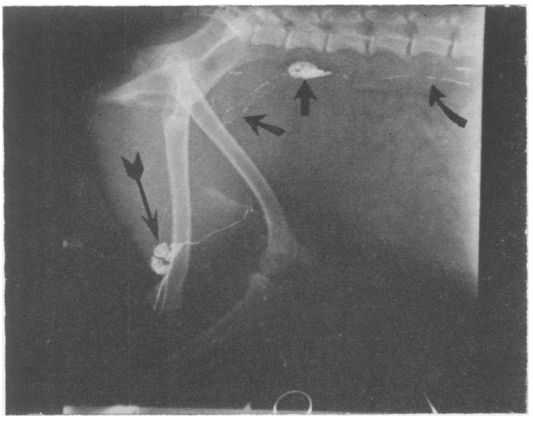

Fig. 5A. Dog — Lateral Radiography — Pelvis and Hindlegs.

— Lymph vessels from hind limb.

— Popliteal lymph nodes.

— Ascending vessels in lumbar region.

— Lumbar lymph nodes.

— Para-aortic lymph trunks.

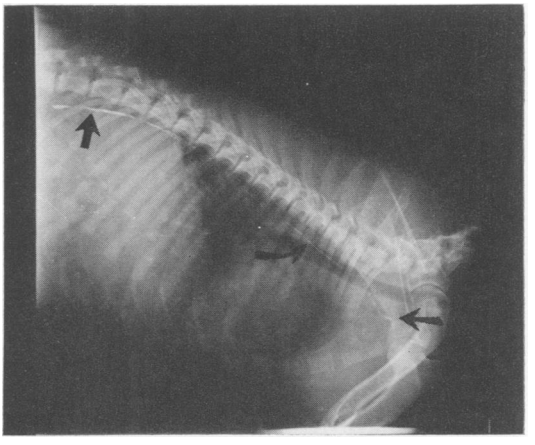

Fig. 5B. Dog — Lateral Radiograph — Thorax and Abdomen.

— Upper para-aortic trunk.

— Thoracic duct.

— Termination of thoracic duct.

the dosage recommended, this was of no clinical significance and pulmonary changes due to break-down of UFL to free fatty acids usually result from poor clinical judgment, such as failure to reduce the dose in subjects with a decrease in lymphoreticular tissue due to administration of immunosuppressive drugs (3). Allergic reactions to iodine-containing contrast media (5), Patent Blue V, or Lignocaine (10), giving localized or generalized manifestations are rare with normal dosage, and did not occur in our experience.

Silicone Rubber Casting of Lymph Microcirculation

Lymphatic cannulation was performed by a method similar to that already described for lymphography. Microfil[6], a brightly coloured radiopaque silicone rubber compound, was introduced using a 30 gauge needle with a 4 lb weight on the plunger of the syringe (Fig. 2). This allowed a steady flow of the casting medium. Vessels and nodes in the popliteal, femoral and ilio-lumbar groups were filled in approximately 15 min (Fig. 8). The gel time of

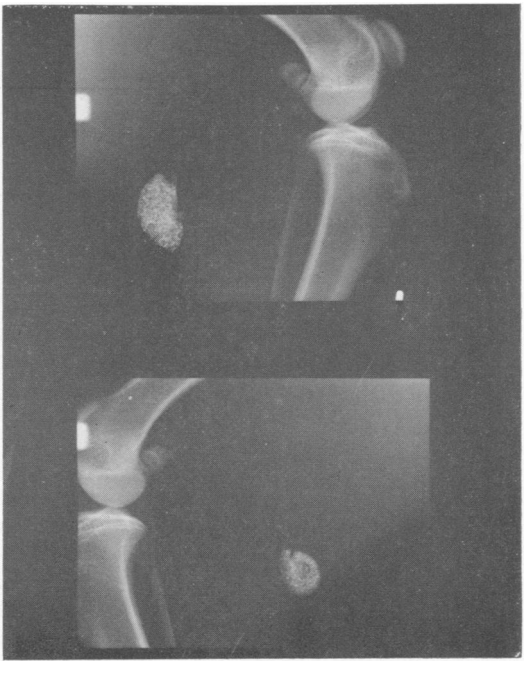

Fig. 5C. Dog — Lateral Radiograph — Hindlegs.

— Popliteal lymph node. Note homogeneously granular parenchyma. The right node (top) is somewhat larger than the left.

[6]Canton Biomedical Products Inc., P.O. Box 2017, Boulder, Colorado, U.S.A.

Fig. 5D. Dog — Ventrodorsal, Right Posterior-Oblique Radiograph — Pelvis.

— Pelvic and ilio-lumbar lymph nodes.

the radiopaque casting medium was approximately 100 min varying as the colour used. The nodes and vessels were removed by careful dissection once it was set. These were cleared in alcohol and methyl salicylate as follows: (this procedure was usually done two to three hours post-injection) 1. Tissue-containing node was immersed in saline for twenty-four hours. 2. On the following consecutive days specimens were dehydrated for twenty-four hours each in fresh ethyl alcohol solutions of 25, 50, 75 and 95% as well as absolute alcohol at room temperature. 3. The specimens were placed in synthetic methyl salicylate until the surrounding tissues became transparent. The casts were examined using a stereo-binocular microscope on the external or cut surfaces. A coloured cast of the lymphatic microcirculation within each individual lymph gland was revealed by sectioning. Minute detail of the sinusoidal system within the gland could then be examined with a stereo-binocular microscope or simple photographic enlargement.

RESULTS

LYMPHOGRAPHY

Radiographs taken during or immediately following injection of the contrast media, showed the normal trunks afferent and efferent to the popliteal and aorto-iliac glands in the rabbit (Fig. 3). A beaded appearance of vessels due to normal valves was seen and from one or two large calibre efferent popliteal trunks, several smaller lymphatics ran alongside the major vessels to the level of the pelvic brim. At a higher level two or more periaortic channels conduct the contrast material to the thoracic duct which was not normally visualized using the dosage described. After 24 hr the lymphadenogram (Fig. 4) displayed single unilateral superficial popliteal lymph nodes. Small groups of femoral glands were visualized. At the level of the

Fig. 6. Rhesus Monkey — Ventrodorsal Radiograph — Pelvis.

— 24 hour film showing femoral, iliac and aortic lymph glands with homogeneously opacified parenchyma.

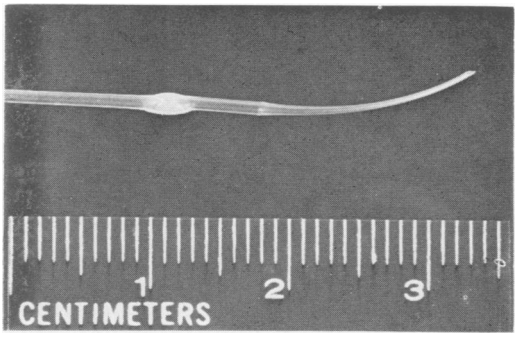

Fig. 7. Polyethylene catheter for lymphatic cannulation.

pelvic brim, two or more larger ilio-lumbar nodes were projected over the sacrum on each side.

In the dog (Figs. 5A, B, C, D) the appearances were similar to those of the rabbit except that small para-aortic nodes at the upper lumbar level were also opacified. In unusual circumstances, such as suspected injury to the thoracic duct, larger amounts of contrast material may be used. Normally this is to be avoided as UFL which enters the thoracic duct will eventually be deposited in the lung.

In the rhesus monkey, one or two long saphenous trunks were visualized following lymphatic cannulation in the hind leg (Fig. 6). These normally branched in the upper thigh into numerous afferent vessels to small groups of femoral and iliac lymph nodes. Efferent trunks led to peri-aortic channels and thence to the cisterna chyli at the level of L1 above which the thoracic duct conducts lymph to the junction of the jugular and subclavian veins. At 24 hr the vessels were empty and radiopaque lymph nodes (the lymphadenogram - Fig. 6) were seen in femoral, iliac and para-aortic groups. The radiographic appearance of these nodes was similar to that in man. Clearly demarcated margins with slightly mottled parenchyma and radiolucent defects due to hila were easily identified in normal glands.

Silicone Rubber Casting of Lymph Microcirculation

The normal cast of rabbit lymph nodes studied showed a marginal sinus of approximately 50 to 100 μ in diameter from which numerous intermediate sinusoids led around the follicles to a sponge-like network in the centre of the node (Fig. 9). Lymph canniculi efferent to the system at the hila of individual nodes led directly to one or two larger major trunks through which flow occurred to adjacent nodes or vessels. The minute detail of intranodal sinusoids of extremely small size (approximately 20μ) demonstrated the large surface area of the intranodal lymphatic microcirculation.

The use of such a cast to study changes due to an immune response in the popliteal lymph node regional to an injection of the antigen Keyhole Limpet Hemocyanin[7] is illustrated (Fig. 10). Microfil was injected to elicit possible changes in lymphatic structures during the time when antibody is known to be produced by the rabbit (22, 23). New lymph vessel formation within the germinal follicles (angiogenesis) was clearly demonstrated in a popliteal node regional to an injection of the antigen after a period of eight days. This interesting finding was not detectable by either *in vivo* or *in vitro* radiography of whole lymph nodes by current techniques (Fig. 11). However, the radiopacity of the silicone rubber allowed comparison *in vivo* of radiographs of the lymph nodes with the true microstructure *in vitro*.

[7]Biomarine Laboratories, Venice, California, U.S.A.

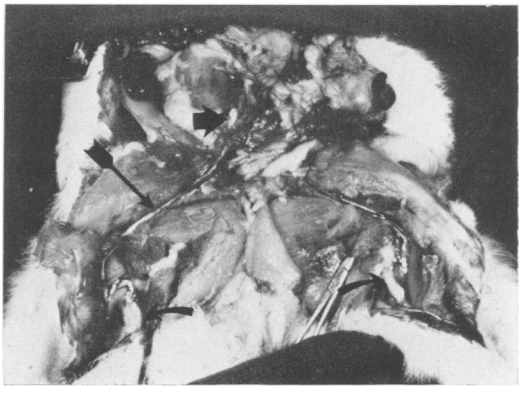

Fig. 8. Rabbit — Photograph During Necropsy of Dissected Hindlimbs and Abdomen.

— Radiopaque silicone rubber in lymphatic system.

— Popliteal lymph glands.

— Femoral trunks.

— Lumbar lymph nodes.

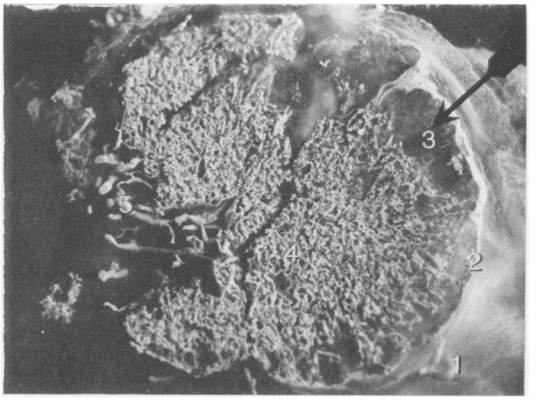

Fig. 9. Cast of the popliteal lymph node in the rabbit.

1 Afferent lymph trunk.

2 Marginal sinus.

3 Spaces for follicles.

4 Network of sinusoids in medullary parenchyma.

5 Efferent lymphatic canniculi and vessels.

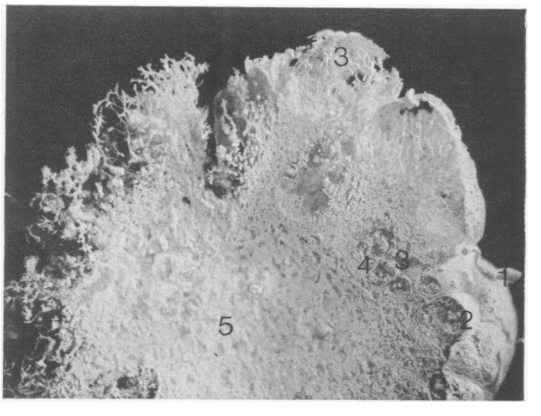

Fig. 10. Cast of rabbit popliteal lymph node regional to antigen.

1 Afferent lymphatic.

2 Marginal sinus.

3 Note new lymphatic sinusoids and follicles.

4 New follicle formation.

5 Increase in medullary sinusoidal system.

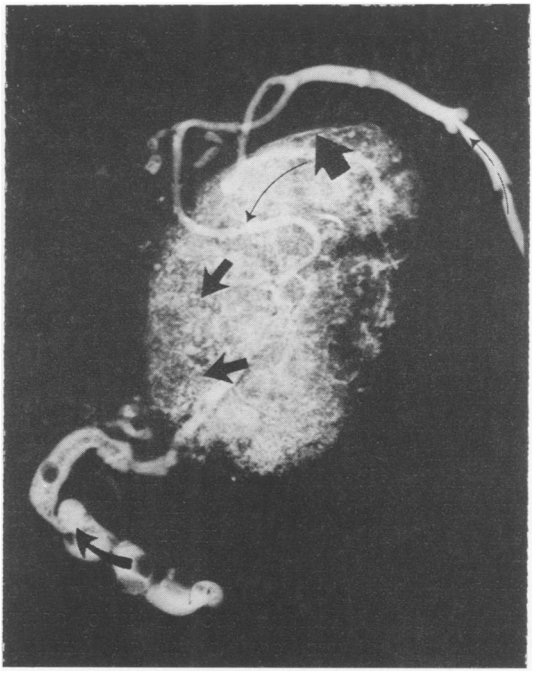

Fig. 11. Radiograph on high resolution plate of silicone rubber cast of rabbit lymph node.

— Afferent lymphatic with valves.

— Marginal sinus.

— Individual follicles.

— Large calibre efferent lymph trunk.

DISCUSSION

Minor variations in the instrumentation and technique of lymphography will prove equally successful according to the preference of individual operators (20). A magnifying binocular is often useful. Some may prefer polyethylene catheters of similar size to the 27 and 30 gauge needles used for lymphatic cannulation in man (Fig. 7). In our experience this decreased the success rate unless a more expensive operating binocular was available. After some experience, the technique described should result in successful examination in the vast majority of subjects. The simplicity and low morbidity of such radiographic studies of impalpable lymph nodes in comparison with extensive diagnostic laparatomies indicate possible applications to diagnostic difficulties in valuable animals or in detecting carriers of endemic

lymphoreticular disease in groups of animals.

Present knowledge of lymph node anatomy has been derived from serial histological sections of whole node slices or camera lucida pictures (6). Arterial casts and partial lymphatic casts by direct injection of nodes have also provided useful information (19). The method described differs from previous studies as it allows comparison of three dimensional detail within whole lymph nodes with radiography *in vivo* or *in vitro* following their removal. This technique seems particularly applicable for demonstrating the microcirculation of nodes in different regions which are already known by T3 thymidine uptake studies to have functional differences (1) of basic importance to the understanding of immune mechanisms. Our preliminary studies, which show that lymphatic sinusoidal growth into active germinal centres occurs in an immune response, delineate formation of an important new transport mechanism during this predominantly humoral reaction. This is not unlike angiogenesis in developing neoplastic disease (8).

Many features of microcirculation in disease, particularly in malignancy, are at present unknown. These may be demonstrated by sequential injection in the live animal of the afferent lymphatics and arterial supply to lymph nodes giving a simultaneous view of both lymphatic sinusoids and blood supply. The radiopacity of the silicone rubber compounds described makes them particularly suitable for experimental examination of *in vivo* radiographic appearances and the true microcirculation. This principle applies not only to lymph nodes but to all organs and avoids some of the pitfalls inherent in attempting comparison of radiographs with serial histological sections.

ACKNOWLEDGMENTS

The authors wish to express their appreciation for the technical assistance of Mrs. Christine Oliver for the preparation of the specimens and radiographs. The photographs were by the Department of Medical Illustration of the University of Toronto, and this manuscript was typed by Mrs. Glenys Hodgkinson and Miss Cathy Gammon.

Appreciation is also expressed to Dr. L. R. Christensen, Director, Division of Laboratory Animal Science, for his co-operation in providing equipment and facilities.

REFERENCES

1. **ANDREASEN, E. and J. OTTESEN.** Studies on the lymphocyte production: investigations on the nucleic acid turnover in the lymphoid organs. Acta physiol. scand. **10**: 29-32. 1945.
2. **BOOKSTEIN, J. J. and E. VOEGELI.** Critical analysis of magnification radiology. Radiology **93**: 23. 1971.
3. **DAVIDSON, J. W.** Pulmonary complications of lymphography. Letter to the Editor. New Engl. J. Med. **285**: 237-238. 1971.
4. **DAVIDSON, J. W. and E. A. CLARKE.** Radiographic Features of Hodgkin's Disease. Lymphology. In press. 1972.
5. **DOLAN, P. A.** Lymphography: Complications encountered in 522 examinations. Radiology **86**: 876. 1966.
6. **DRINKER, C. K. and M. E. FIELD.** Lymphatics, Lymph and Tissue Fluid. Baltimore: Williams and Wilkins. 1933.
7. **DRINKER. C. K. and J. M. YOFFEY.** Lymphatics. Lymph and Lymphoid Tissue. Their Physiological and Clinical Significance. Harvard University Press. 1941.
8. **FALKMAN, J., E. MERLER, C. ABERNATHY and G. WILLIAMS.** Isolation of a tumor factor responsible for angiogenesis. J. exp. Med. **133**: 275-288. 1971.
9. **FRAIMOW, W., S. WALLACE, P. LEWIS, R. R. GREENING and R. T. CATHCART.** Changes in pulmonary function due to lymphangiography. Radiology **85**: 231-241. 1965.
10. **FUCHS W. A., J. W. DAVIDSON and H. W. FISCHER.** Recent Results in Cancer Research: Lymphography in Cancer. New York: Springer-Verlag. 1969.
11. **FUNAOKA, S.** Mechanismus der Lymphbewegung. Arb. 3rd Abt. Anat. Int. Kaiserl. Univ. Kyoto. Series D. Untersuchungen uber die Physiologie der Lymphbewegung. Heft. 1. Die Rontgenographie des Lymphgefasses. Kyoto. p.l. 1930.
12. **HALL, J. G., B. MORRIS and G. WOOLLEY.** Intrinsic rhythmic propulsion of lymph in the unanaesthetized sheep. J. Physiol., Lond. **180**: 335-349. 1965.
13. **KOEHLER, P. R., W. A. MEYERS, J. R. SKELLY and B. SCHAFFER.** Body distribution of ethiodol following lymphangiography. Radiology **82**: 866-871. 1964.
14. **KINMOTH, J. B.** Lymphangiography in man. A method of outlining lymphatic trunks at operation. Clin. Sci. **II**: 13. 1952.
15. **KINMOTH, J. B. and G. E. TAYLOR.** The lymphatic circulation in lymphoedema. Ann. Surg. **139**: 129. 1954
16. **KUISK H.** Technique of Lymphography and Principles of Interpretation. St. Louis, Missouri: Warren H. Green Inc. 1971.
17. **PROKOPEC, J., V. SVAB and E. KOLIHOVA.** Lymphogrie and Lymphadenographie inder Klinischen Praxis. 9th Internat. Cong. Radiol. Munchen. Abhandlungen. B. Rajewsky, ed. Stuttgart, Germany: Georg Thieme Verlag. 1959.
18. **RICHARDSON, P., E. H. CROSBY, H. A. BEAN and D. DEXTER.** Pulmonary oil deposition in patients subjected to lymphography: Detection by thoracic photoscan and sputum examination. Can. med Ass. J. **94**: 1086-1091. 1966.
19. **SUSHKO, A. A.** Morphology of lymph flow in man. Eksperiment Meditsina Kiev. 1-9. 1937.
20. **SUTER, P. F. and R. W. GREENE.** Chylothorax in a dog with abnormal termination of the thoracic duct. J. Am. vet. med. Ass. **159**: 302. 1971.
21. **TJERNBERG, B.** Lymphoraphy. An animal study on the diagnosis of V2x carcinoma and inflammation. Acta radiol. (Stockh.) Suppl. 214. 1962.
22. **WALLACE, S., L. JACKSON and R. R. GREENING.** Cinical applications of lymphangiography. Am. J. Roentgenol. **88**: 97. 1962.
23. **WU CHIN-YU, B. CINADER.** Increase in Hapten-specific plaque-forming cells after pre-injection with structurally unaltered macromolecules. J. exp. Med. **134**: 693-712. 1971.