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M. Jack Keyl, M.D. Department of Physiology and Biophysics, University of Oklahoma Health Sciences Center, Box 26901, Oklahoma City, Oklahoma 73190 USA

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Direct Transtissue Intra-Organ Lymphography;Technique and Results

P. Gibert*, B.J. Lee**

* Attending Surgeon Hospital Maciel, Hospital Maciel, 25 de Mayo Montevideo, Uruguay
** Associate Attending Physician Department of Medicine, Memorial Hospital, New York,

Summary

Rodriquez Sica and *Sica's* technique of intra-organ lymphangiography is described and has been demonstrated to be effective in dogs. Ultrafluid liposoluble iodine dye is injected directly into the tissues at a slow rate, into areas where the capillary network is abundant, without any incision of the skin. The technique has been carried out in the head and neck, mammary glands, limbs, testis and rectum. Collateral channels, after obstruction of the thoracic duct, have been visualized. Lymphatics of intra-abdominal and retroperitoneal organs were seen. The possibility of using this technique in man is suggested, based upon the absence of lasting inflammatory reaction in the injected organ, and upon excellent tolerance of the procedure by experimental animals.

Lymphangiography has gained wide clinical use and in many instances can be considered a routine procedure. It is used as a diagnostic tool when the lymphatic system is primarily involved, and as a means of evaluating the extent of disease both in primary lymphatic conditions (1) and in those where the lymphatic system is secondarily involved by another pathologic process. The basic technique has been that developed by *Kinmonth* and described by him in 1952, 1954, and 1955 (2). In 1960 *Hreshchyshyn* and in 1961, *Wallace* made modifications in details. The modified *Kinmonth* technique is currently used in clinical practice. Its indications and the details of the procedure will not be discussed.

Kinmonth's method can be difficult to perform. A surgical procedure is necessary so as to insert a catheter into one of the minute lymphatic channels. This surgical procedure usually is not done without difficulty and even though there is little risk, it can cause anxiety in the patient. The dissection of the lymphatic vessels must be done with care. The catheterization of the lymph vessel can be difficult. The procedure has been largely limited to the lymphatic system in the extremities. There are many other areas, such as the mammary glands and the pelvis, where study of the lymphatic anatomy would be of fundamental importance. The Kinmonth technique is founded on the dissection and catheterization of the lymphatics

and most currently described modifications of the original Kinmonth technique have not eliminated these difficulties.

Studies have been done in an effort to obviate such disadvantages. Direct lymphangiography by percutaneous injection of contrasting dye has been investigated by several authors. In 1953, *Colette* (3) studied the fate of a hydrosoluble iodine dye (Umbradil) injected in the subcutaneous tissues in order to study the circulation of plasma and found that this procedure was innocuous in man. *Danese* in 1960 and *Arnulf* (4) in 1961, demonstrated the feasibility of lymphangiography by direct subcutaneous injection of the dye. Arnulf used colloidal thorium bioxide (Thorotrast) but radioactivity limited its employment to experimental animals. In 1962, *DiMatteo* (5) and co-workers used both hydro and liposoluble (Lipiodol) iodine dyes, injecting them under a blood pressure cuff at a pressure of 220 mm. of Hg. in order to avoid local diffusion of the dye. They obtained good radiographs of the lymphatic vessels and the lymph nodes. Their technique was obviously limited to the limbs. In 1962 and 1963, *Rodriguez Sica and Sica* (6,7,8) published in Montevideo their work on intra-organ lymphangiography by injection of an ultrafluid liposoluble iodine dye. They demonstrated that the dye was well tolerated by the tissues, so that it became possible to visualize almost all areas of the lymphatic system, and avoid the main limitations of *Kinmonth's* technique.

The work herein reported has been done following the technique of *Rodriguez Sica* and *Sica.* Modifications in some details were made because of differences in available material. Not only have two of the areas studied by *Rodriquez Sica and Sica* (testes and mammary glands) been reinvestigated, but possibilities of the technique have been assayed in other areas such as the tongue, lips and rectum.

Materials and Methods

Twelve dogs (ten male and two female), chosen at random, were used in this study. The weights varied from 12 to 20 pounds. One of the female dogs was lactating, 2 weeks postpartum. The animals were healthy, without any apparent distress, and had not been employed in previous experiments. They were used in 26 procedures, each dog being subjected to at least 2 procedures. Two dogs had undergone, 2 weeks previously, a surgical obstruction of the thoracic duct through a left thoracotomy.

The dog was placed on the x-ray table lying on its back, under general anesthesia. This was achieved by I.V. injection of pentobarbital sodium*, 1 cc./5 lbs. weight of dog, the routine general anesthesia used in experimental animals. It was necessary to anesthetize the dogs in order to keep them motionless for taking good x-ray films.

The dye used was Ethiodol**, 37% liposoluble iodine dye. This is equivalent to the ultrafluid Lipiodol used by *Rodriguez Sica* and *Sica.* Colloidal thorium bioxide (Thorotrast), considered to be the best contrasting dye, was not employed since its radioactivity precludes use in man.

Disposable 10 cc. syringes were used, connected to a 25 gauge needle through a plastic tubing. The most useful needle is a 25 scalp vein needle into which the plastic connection has been made. The same pressure injector used in Memorial Center for routine lymphangiography was employed in order to standardize the rate of injection.

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* Pentobarbital sodium, 65 mg./cc., for veterinary use. Philadelphia Laboratories, Inc.
** Ethiodol obtained from *Fougera & Company*, Inc. Hicksville, N.Y.

The skin of the area injected was thoroughly cleaned, the needle introduced deeply into the tissue, and the dye injected at a maximum rate of 0.5 cc./min. The volume injected depended upon the anatomic region studied, and upon the reason for each individual study.

Although *Rodriguez Sica* and *Sica* took the first x-ray film after injection of 1 cc. of dye, we arbitrarily decided to make this exposure 5 minutes after starting the injection. The second x-ray film was made 30 minutes after injection, the third 1 hour later, and the fourth 24 hours thereafter. The second and third films show the presence of dye both in the areas injected and in the lymphatic vessels connecting the area to the lymph nodes. The fourth shows more nodes and in better anatomic detail. The chest was included in the third and fourth films in order to provide a control of lung invasion by the dye immediately after the procedure and 24 hours later.

Areas Injected and Results

1) *Head* and *Neck:* Injection of the lip must be done at a low rate because the tissues are very soft and they become distended very easily. The dye filled lymphatics going directly to the superficial lymph nodes of the neck. The dogs were able to drink as soon as they awoke from the anesthesia.

The tongue offers considerable resistance to the injection. The dye remains for many days at the site of the injection and gradually infiltrates the muscle. The lymphatic channels pass close to the mandible and terminate deep in the neck. There was no inflammatory reaction 24 hours after the injection and the dogs showed no pain during drinking or eating.

Injection of the tonsil was attempted in order to study the possibility of direct injection into lymphatic tissues. There was always a considerable leak at the site of injection and the films showed dye in the larynx.The rate of injection must be very slow.

Figure 1 shows the deep and superficial lymph nodes of the neck. The tonsil is visualized and the dye infiltrating the tongue is seen. This film was taken 24 hours after injection of tongue and tonsil in the same dog.

2) Mammary Glands: Injection was done in the sub-areolar area, where the net of lymphatic capillaries is most abundant. When the fore gland is injected, the first film usually shows a tortuous lymphatic channel going to the lower axillary lymph node. The second film (Fig. 2) shows the upper and lower axillary lymph nodes and the dye diffusing from the mammary gland to the surrounding tissues. The third x-ray (Fig. 3) shows the lymphatic channel posterior to the upper axillary lymph node, and dye in the lung. When the injection is done in the hind mammary gland, the first and second films show lymphatic channels going toward the groin and the complex of the inguinal nodes. The control film, taken 24 hours after (Fig. 4) shows the low lumbar nodes and the remaining dye in the mammary gland. From either fore or hind glands, the dye diffuses profusely to the surrounding tissues, and is visible for more than one month afterwards.

3) Limbs: The fore limb was always sucessfully injected. For reasons unknown, 50% of the attempts to inject the hind paw failed. When the fore paw is injected, the first and second films show the lymphatic channels and the supraclavicular node. The channels are very tortuous; the node is large. The third and fourth films show the same node, which seems to be disconnected from the axillary nodes. When the hind limb is successfully injected (Fig. 5), the first three films show the channels in the periphery ending in the

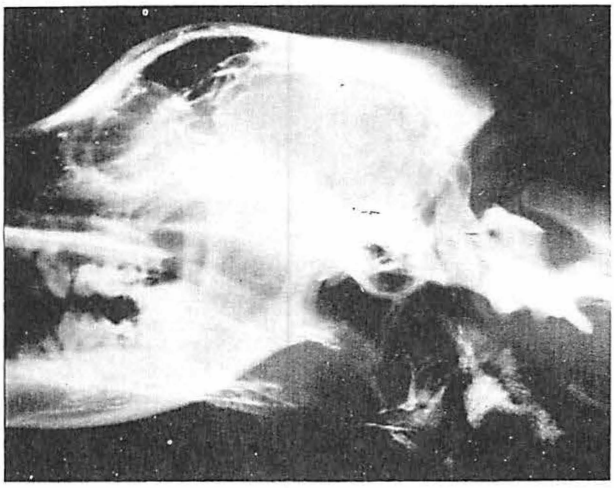

Fig. 1. Dog G86, male, 36.5 lbs., general anesthesia: Pentobarbital sodium. Injected in the left tonsil and left half of the tongue. This is a 24 hour control film showing the tonsil, remaining dye in the tongue and both superficial and deep upper cervical nodes.

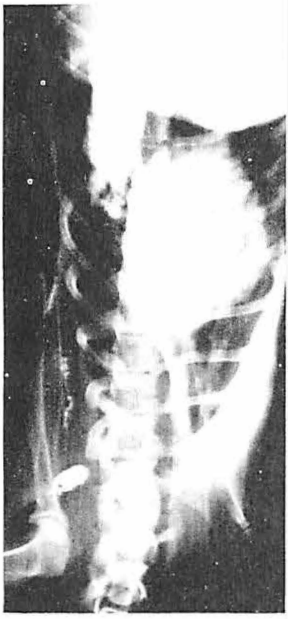

Fig. 2. Dog E712, female, 24 lbs., general anesthesia: Pentobarbital sodium. Injected in the left fore mammary gland. Film taken 30 minutes after starting the injection showing the dye in the mammary gland, the low axillary lymph node connected to the upper axillary lymph node by one lymphatic channel.

Fig. 3. Dog G153, female, 34 lbs., two weeks post-partum, general anesthesia: Pentobarbital sodium. Injected in the right fore mammary gland. Film taken one hour after starting the injection showing the dye in the mammary gland, the low and upper axillary lymph nodes connected by one tortuous lymphatic channel and the lymphatic channel starting in the upper axillary lymph node ending in what seems to be the great lymphatic vein. The right lung is completely filled with the dye.

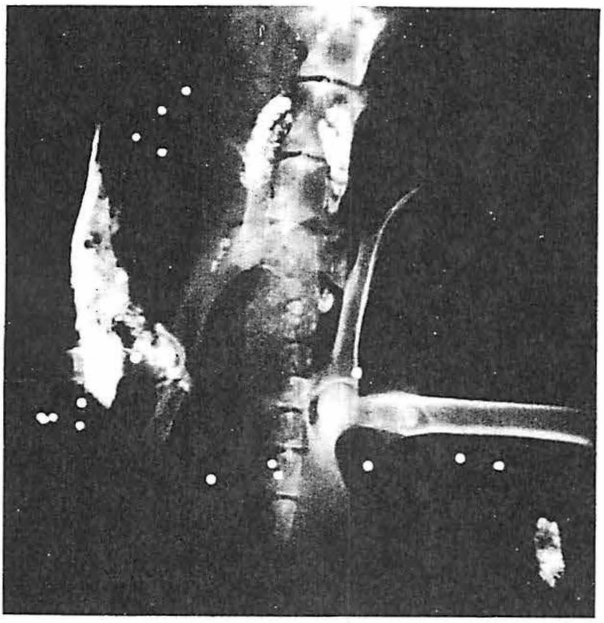

Fig. 4. Dog G99, female, 34 lbs., anesthesia: Pentobarbital sodium. Injected in the hind mammary gland. This is a 24 hour control film showing the hind mammary gland, external iliac lymph nodes, the low lumber nodes on the right side, and on the left the popliteal and low lumbar node from hind paw injection, done 24 hours previously.

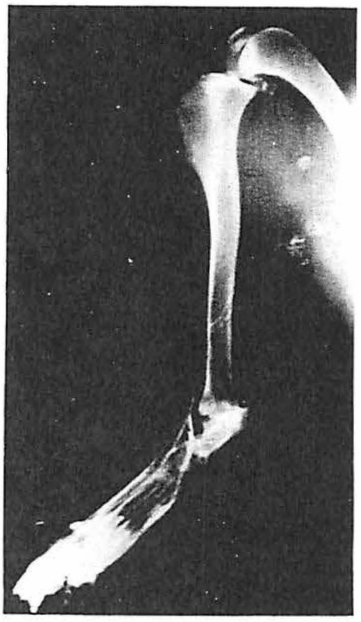

Fig. 5. Dog E712, female, 24 lbs., general anesthesia: Pentobarbital sodium.Injected in the right hind paw. This is a second film taken 30 minutes after starting the injection showing several channels ending in the popliteal node.

popliteal node. Sometimes channels starting in these nodes were seen in the third film The fourth film always shows the low lumbar nodes.

In both cases the dogs did not seem to have any impairment in walking; no sign of pain was noticeable 24 hours after the injection. The dye remains in the paws for more than 4 weeks.

4) Testes: These were injected with the needle deeply introduced toward the posterior fascia. This area was used both to study the testes and their specific lymphatic drainage, and also to investigate collateral channels formed after obstruction of the thoracic duct. For the latter studies, both testes were injected simulataneously. Intra-testicular injection is always successful, and either side may be injected without difference. The lymphatic channels are quickly seen, continuous if the injection is done at the rate of 0.5 cc./minute, and discontinuous if it is slower. Two to five channels are seen in the first film. In the second film the lymph nodes of the lumbar area are seen; in the second and third those at the level of the kidney are seen (Fig. 6). In the third film the nodes anterior to the cysterna chyli are filled with dye, as well as a recurrent node in the lumbar area. Generally, a unique channel leaves the main group at the level of the iliac crest and ends in the lowest lumbar lymph node at the edge of the pelvis. In the fourth film, the nodes of the precava or preaorta areas from the pelvis to the cysterna chyli are seen. In all the films the lymphatic capillary net of the testis is seen. The dye remains in the testes for more than two months.

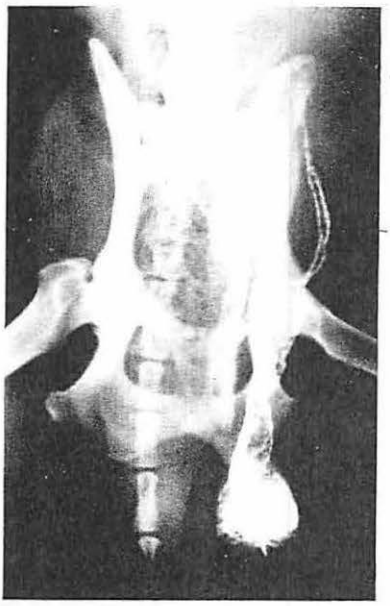

Fig. 6. Dog G86, male, 36 lbs., general anesthesia: Pentobarbital sodium. Injected in the left testis. The first film shows the testis and several lymphatic channels. The second film shows the first lymph nodes. The third film shows the second group of lymph nodes and a recurrent node in the lumbar area. The fourth film shows the lumbar nodes and the remaining dye in the testis and the lymphatic channels.

Histologic sections of testes were done one to two months after the injection, when the dogs were sacrificed for purposes other than the present investigation. The lack of local significant inflammatory reaction did not justify the sacrifice of an animal for the purpose of the histologic study of the tissues.

5) Rectum: The needle was introduced through an anoscope into the upper edge of the rectum, the only areas approachable without special needles. Three attemps at injection were made, the needle being introduced once in the anus and twice in the lower rectum. One of the latter was unsuccessful; the dye diffused into the tissues of the perirectum without filling any channel. In the other two, significant infiltration of the wall of the rectum was followed by visualization of one channel going to a lymph node in the presacral area. Films 24 hours later showed the low lumbar nodes. None of the three dogs showed any subsequent local discomfort and all were used in further investigations without difference from normal animals.

Regional nodes from different areas

A dog was injected in one side, and another area on the other side; the regional nodes corresponding to these areas are shown in the 24 hour control film. In Figure 4, the low lumbar lymph nodes are shown corresponding to the hind mammary gland and hind limb. With the same procedure it is also possible to visualize the regional lymph nodes of the neck.

Physiological modification of the lymphatic channels

Both hind and fore mammary glands of one female dog were injected while in lactation, three weeks post-partum. The lymphatic channels in the lactating mammary glands (Fig. 3) were twice the size of those in other areas of the same animal and twice the size of those in the mammary glands of a non-lactating female dog (Fig. 2). The injection was done easily, and the mammary gland showed no inflammatory reaction after 24 hours, having the same reaction as the nonlactating gland.

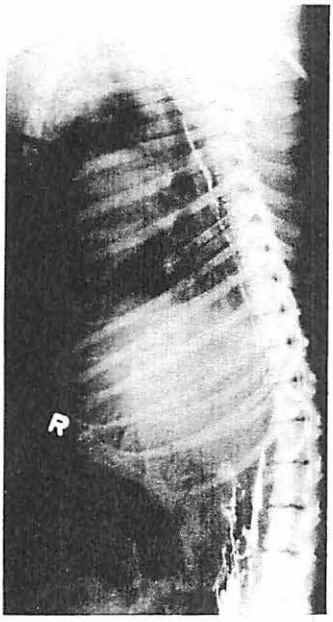

Fig. 7. Dog G336, male, 40 lbs., general anesthesia: Pentobarbital sodium. Injected in both testes. This is a second film taken 30 minutes after the injection showing a lateral view of the thoracic duct starting in the cysterna chyle. The lungs show more dye than in the usual procedure.

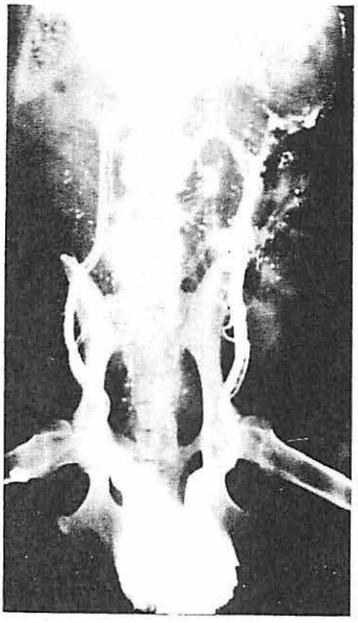

Fig. 8. Dog G268, male, 39 lbs., general anesthesia: Pentobarbital sodium. Injected in both testes. This is the fourth film taken 24 hours after starting injection showing the testes, the lymphatic channels, the lumbar nodes and the collateral circulation surrounding the kidney.

Study of the nodes and collateral channels after obstruction of the thoracic duct

Injection of a large amount of dye, 10 cc. in each testis, makes it possible to obtain a good view of the thoracic duct (Fig. 7). The dog tolerated 20 cc. of dye well despite significant opacification of the lungs. Two dogs were injected after obstruction of the thoracic duct. In theses it is possible to see collateral circulation (Fig. 8), surrounding the kidney and draining into the thoracic duct distal to the obstruction. With a film in the lateral position, the channels and nodes of intra-abdominal visceras are seen (Fig. 9) in the region of the celiac and superior mesentery nodes.

Anatomical and histological studies

Films taken two weeks to two months after injection always showed opacification due to residual dye. When surgically opened after more than two months, the testes contained dye collected in a cavity in the posterior half of the gland. No membrane was found. Histologic studies of these testes show normal spermatogenesis, with vacuolated interstitial tissues (Fig. 10) due to the remaining dye. The tubes of the epididymis are filled with a normal amount of normal spermatozoa.

Fig. 9. Dog G268, male, 39 lbs., general anesthesia: Pentobarbital sodium. Lateral view showing the kidney, the collateral circulation, the cysterna chyli and the obstructed thoracic duct, and nodes of intra-abdominal viscera.

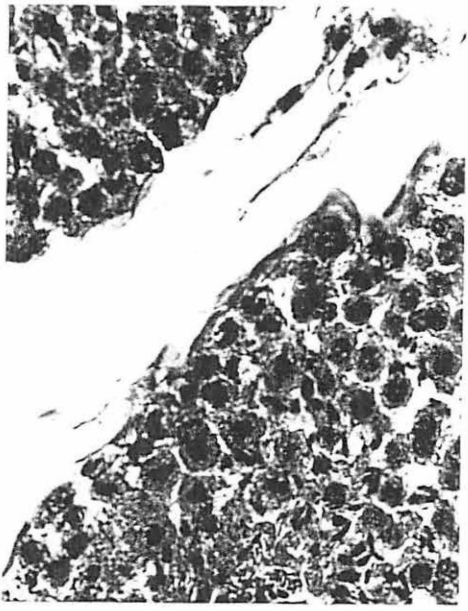

Fig. 10. Histological study of a testis of the dog G268, 2 months after the injection of the dye, showing normal spermatogenesis and vacuolated interstitial tissue.

Discussion

The visceral and tegumentary lymphatic divisions are formed by numerous "lymphatic units." Each of these has a similar structure and function. They are made of a capillary network, draining collecting vessels and regional node groups. The capillary network is formed by channels arising in the interstitial tissues. Their endothelium is in intimate relation with tissues and parenchyme from which they drain extracellular fluid. The collecting vessels drain the lymph and orient the lymphatic stream to the regional node center. This is the terminal pole of the lymphatic unit, represented by the lymph node, and can be considered to be the common pathway of units draining different territories. The lymphatic units are intimately connected to each other. The collectors have anastomoses which decrease in number as the flow goes to the regional center; these establish communication between the superficial and deep collectors and the viscera and tegumentary divisions. The connection between visceral and tegumentary lymphatic units which share a particular common pathway (*Rodriquez Sica* and *Sica*), is established in the regional center.

Collette, Arnulf, Danese and *DiMatteo* have already shown that when iodine dye is deposited in the tissues, it tends to concentrate in them and it is absorbed by the lymphatic capillaries. *Rodriguez Sica* believes that the richer the tissue is in lymphatic capillaries, the faster is the absorption of the dye. The trauma of the needle, seems to facilitate this

absorption. The progression of the dye is produced by two mechanisms. Initially, the interstitial hydrostatic pressure transmitted by the injection forces the dye into the collectors. When the hydrostatic pressure disappears, the normal mechanism of absorption by the lymphatic system continues to drain the radio-opaque dye from the tissues. It is important to inject at a rate not higher than 0.5 cc. per minute in order to avoid excessive interstitial pressure which tends to collapse the capillaries and collectors. The absorption of the remaining dye explains the visualization of the lymph nodes after injection, making possible the later demonstration of the regional center and centers connected to it.

The results obtained in this work indicate that this technique may be useful in studying the lymphatic system in experimental animals and in man. The anatomy of the normal lymphatic system has been studied but changes occurring in different or abnormal physiologic states have not been documented. It might be informative, for example, to inject a resting or lactating mammary gland during pregnancy or at different times in the oestrus cycle. One could also study the uterus at different times in the ovarian cycle, or in pregnancy. One could also study the possible collateral channels which open up with pathologic obstruction of the thoracic duct. Figures 8 and 9 show that it is possible to visualize some of the lymphatic channels and nodes draining intraabdominal and retroperitoneal viscera without performing a laparotomy. This would be useful in studying the lymphatics draining these organs, and also the fate of these lymphatics if the organ is grafted.

The next step is the utilization of this technique in man. The histologic studies of the testes done in this work, indicate that there is no lasting reaction in the tissue after injection of the dye. Tolerance for the injection and the absence of painful reaction in dogs infers that a similar absence of toxicity might be obtained in man.

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P. Gibert, M.D. Attending Surgeon Hospital Maciel 25 de Mayo Montevideo/Uruguay
J. Lee, M.D. Associate Attending Physician, Department of Medicine, Memorial Hospital New York, NY.