

Pedal Microlymphangiography in the Experimental Animal

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Summary

The technique of pedal microlymphangiography is described using a modified lymphangiogram needle, a dissecting microscope and special instruments. The method is applicable to small animal pedal lymphography and studies requiring endolymphatic injection.

Introduction

The technique of lymphography was introduced by Kinmonth et al. (1) for the clinical study of the lymphatic drainage of the lower limb; the method has since been used in many clinical settings.

Experimental lymphography (2) has allowed the study of lymphedema (3), solid tumor metastases and endolymphatic therapy (4). The technique described in this report is applicable to small mammals such as the rat.

Materials and Methods

Lymphangiogram Needles

Standard 30-SWG hollow steel needles (0.33 mm in diameter) in St. Thomas's Hospital Lymphography Sets (McCarthy Surgical, Ltd.) were modified using an electrolytic process (Fig. 1) (5). Needles, with attached tubing, were clamped in a jig (Fig. 1b) which was connected to the positive terminal of a 12 volt battery. The jig was raised and lowered into a bath containing a mixture of glycolic acid 28.2%, metaphosphoric acid 30.5% and sulphuric acid 41.3%. A copper cathode in the bath was also connected to the battery (Fig. 1a). The needles were immersed in the electrolytic acids for 4-5 second periods un-

til they were sharpened to 0.193 mm in diameter. The lumen remained the same size due to air trapping. Excessive exposure of the needle to the electrolytic process resulted in blunting. The needles were cleaned in an ultrasonic water bath, dried and gas sterilized.

Lymphangiogram Technique

Mature male Wistar (WAG) rats weighing at least 250 g were studied; normal animals and a group with lymph node metastases, induced by the injection of 5×10^6 viable Rd/3 tumor cells into the hind footpad seven days previously (6), were used.

The principles of the Kinmonth technique were applied by first obtaining a visual lymphogram following interstitial injection of 0.1 ml of 10% patent blue violet into the dorsum of the hind foot of animals anesthetized with diethyl ether vapour. The skin of the leg was incised over a blue stained lymphatic and exposed by sharp dissection using a Micro Snare Clutch Holder with surgical razor blade fragments and a Siso No. 3 Stainless Steel Watchmaker's Forceps under a Woolf dissecting microscope. After isolation of the lymphatic vessel from surrounding loose areolar tissue and fat it was dilated gently by using proximal occlusion (with an elastic band) and distal massage. A modified lymphangiogram needle was primed with Ultrafluid Lipiodol at 37 °C and connected to a Harvard electric infusion pump. Holding the needle with MATHALONE Forceps it was introduced into the lymphatic vessel. Care was taken to position the needle and tubing to allow it to lie without stress. Approximately 0.05 ml

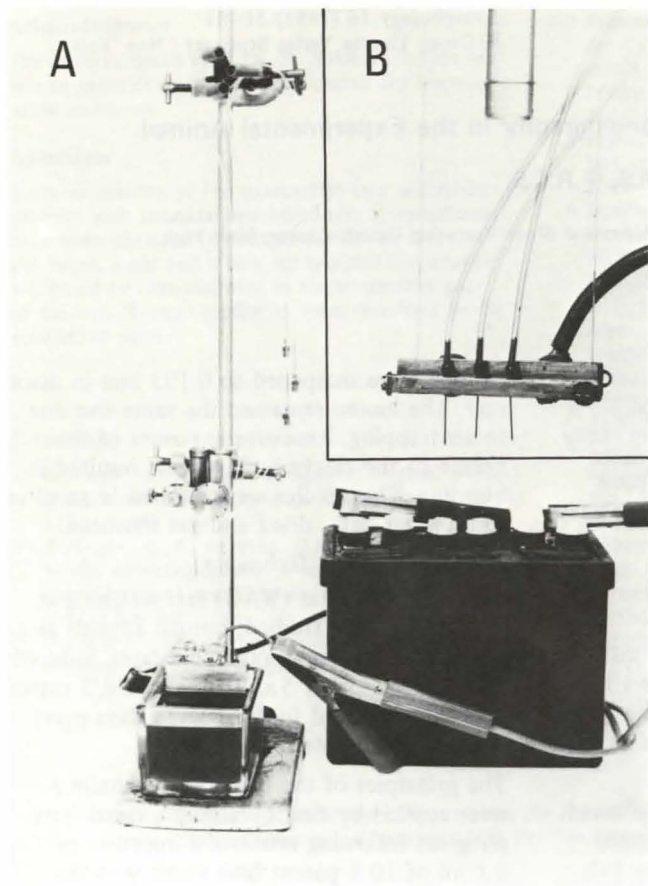


Fig. 1 Apparatus to make modified lymphangiogram needles. The whole apparatus, A, shows electrolytic acid bath with copper cathode connected to a 12 volt battery. Insert B shows close-up of needles in jig attached to positive terminal

of contrast material was injected at a rate of 0.01 ml/min. Radiographs of the rats were taken two hours after injecting using a Watson dental x-ray machine.

Results

Radiographs (Fig. 2a and b) demonstrate the lymphadenogram two hours after injection. The normal popliteal lymphadenogram (Fig. 2a) shows subcapsular sinus filling, retrograde lymphatic flow, a normal lymph node and an efferent lymphatic to the para-aortic chain. The presence of partial lymph node replacement by Rd/3 metastatic tumor is seen in

Fig. 2b where the lymph node is enlarged and there is a filling defect due to metastatic tumor in the upper pole.

Discussion

Microlymphangiography may be of value in experimental applications such as the study of solid tumor metastases in small animals or investigating endolymphatic therapy. The technique may also be used to collect lymph for transport studies. Alternative methods of microlymphangiography using the drawn-out glass tubing have not been successful (5).

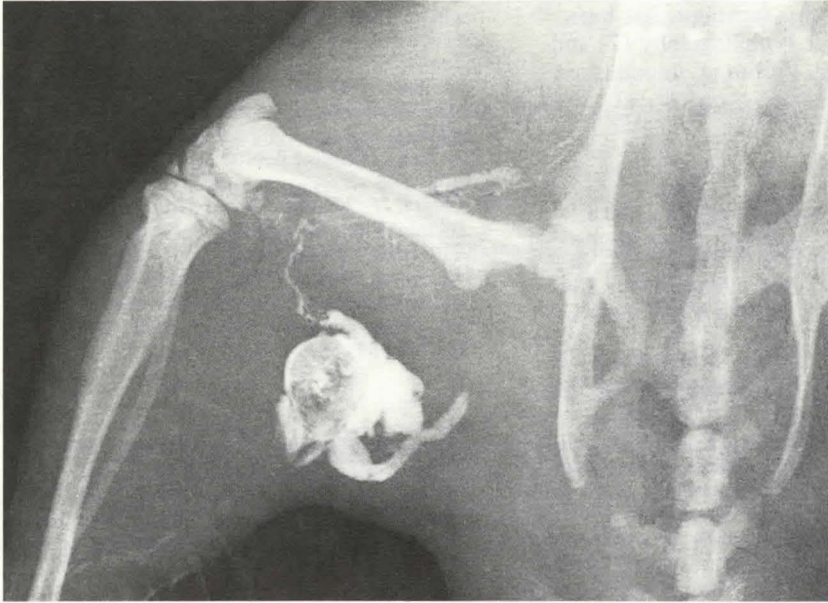


Fig. 2A



Fig. 2B

Fig. 2 Popliteal lymphadenogram in the rat showing
A: a normal lymph node with sub-capsular sinus filling, retrograde lymph flow with lymphatic distension and an efferent lymphatic to the para-aortic chain.
B: shows an enlarged lymph node partially replaced at its upper pole by Rd/3 metastatic tumor

In the clinical field this technique has been applied to mesenteric lymphography (7) and breast lymphography (Osborne, unpublished data).

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