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J. R. Casley-Smith, Electron Microscope Unit, Departments of Botany, Microbiology and Zoology, University of Adelaide

Studies of the Physiology of Lymphatic Vessel by Microcirculation Methods*

S. Godart

Chirurgie infantile. Hopital Universitaire Brugmann, Bruxelles.

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The study of the lymphatic system is difficult because of the tenuity of its vessels and of the transparency of lymph. MASCAGNI (6) and CRUIKSHANK (2) described the lymphatic collectors within the 18th century, although the knowledge of most peripheral lymphatics remained indefinite. Lymphography, developed by KINMONTH (4), initiated a renewed interest in the lymphatic system. Unfortunately, even though the lymph collectors and lymph nodes are thus shown, the peripheral channels are not demonstrated. In addition, the visceral lymphatics remain inaccessible with this technique.

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Hungarian (7) and Italian (1) authors used a method based on the ligature of efferent lymphatic vessels in order to dilate the lymphatics above the occlusion. These lymphatics then appear in microscopic sections. Unfortunately, this method is not physiologic. In order to study morphological and physiological aspects of the peripheral lymphatics we chose to observe them in the living animal using a transillumination method (2–4).

Methods

The living structures of small laboratory animals are transilluminated and studied under the microscope. The light from a Xenon source is introduced under the investigated organ of the animal, trough a fused quartz rod, being transmitted to the tip by internal reflection. The microscope is multidirectional and can be brought at right angles to the examined tissue.

The microscopic study is blurred by the respiratory movements bringing regularily the tissues out of focus. To avoid this difficulty, the animal is curarised and the rate of respiration reduced by a respirator. For observations at high magnification continuous oxygen flow replaces ventilation suppressing almost all movement. We prefer an avascular midline incision. If another incision is necessary, all vessels must be carefully ligated in order to avoid bleeding.

We performed 750 experiments on mice, rats and guinea pigs. In order to visualize the lymphatics dyes have been injected. *Patent blue violet,* the usual dye for lymphatic investigations, was used for most experiments. *Methylen blue, trypan blue and Chicago blue* were injected for hepatic experiments since patent blue is excreted with bile. *India ink* was prepared from Japanese carbon stone just prior to the experiment.

A typical experiment on the lymphatics of the skin:

The animal is anaesthetised with urethane. The abdominal skin is shaved, opened by a midline incision and separated from the muscle by gentle blunt dissection with the animal in lateral position. The skin is spread with two pins in order to examine its deep aspect. A very fine needle, mounted on a catheter is inserted into the hypodermic tissue and a very small amount of patent blue violet is injected (fig. 1).

A fast resorption into the blood occurs first; after 10 to 15 minutes filling of lymphatic vessels is observed cephalad to the site of injection. This process is slow and irregular, occuring by saccades. Once filled the lymphatic vessel can be studied easily. The following anatomic and physiologic conclusions are derived from our observations.

Results and discussion

Number and localisation of lymphatics

In the subcutaneous tissue of the abdominal wall, the lymphatic vessels are located along the main blood vessels. Only one or two of them are seen along the medial subcutaneous vessels and another two along the lateral vessels (fig. 2). The size of these lymphatics, in the mouse, is comparable to the size of the main artery (fig. 3). When the dye is injected in the *dermal tissue,* the observed lymphatics are different, probably arranged in a loose mesh. This mesh is still difficult to investigate for technical reasons.

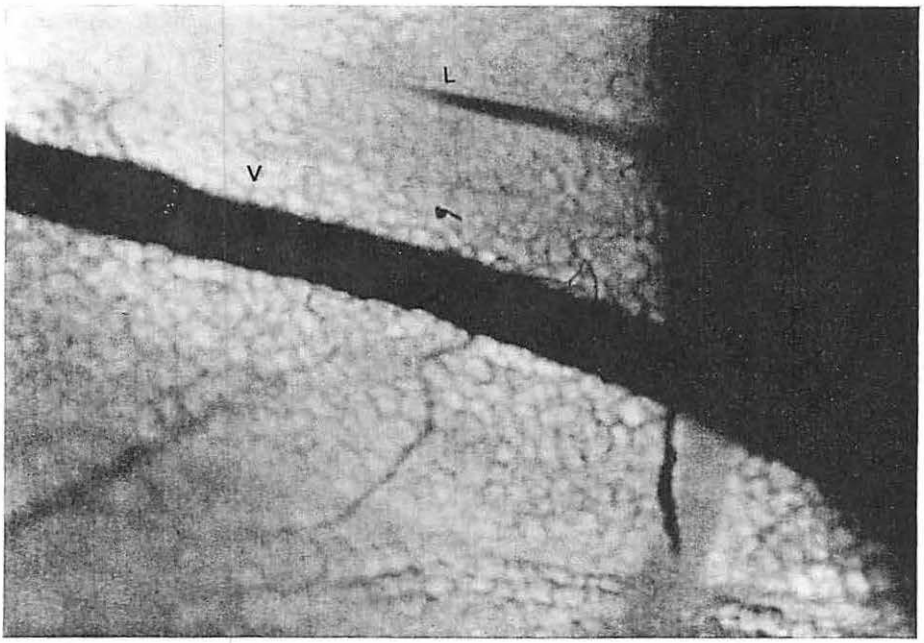

Fig. 1 Deep aspect of the skin (mouse). Patent blue dye has been injected into the subcutaneous tissue, visible at the right upper part of the figure. A lymphatic vessel (L), parallel to the vein (V) is slowly filled. The fine reticular structures seen are due to the subcutaneous adipose tissue.

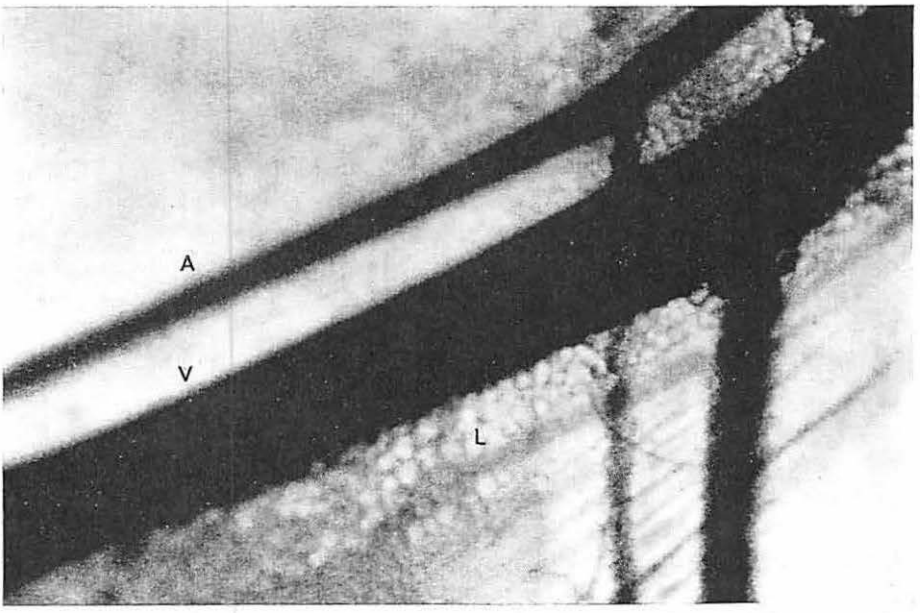

Fig. 2 Deep aspect of the abdominal skin (mouse). Medial artery (A), vein (V) and Lymphatic (L).

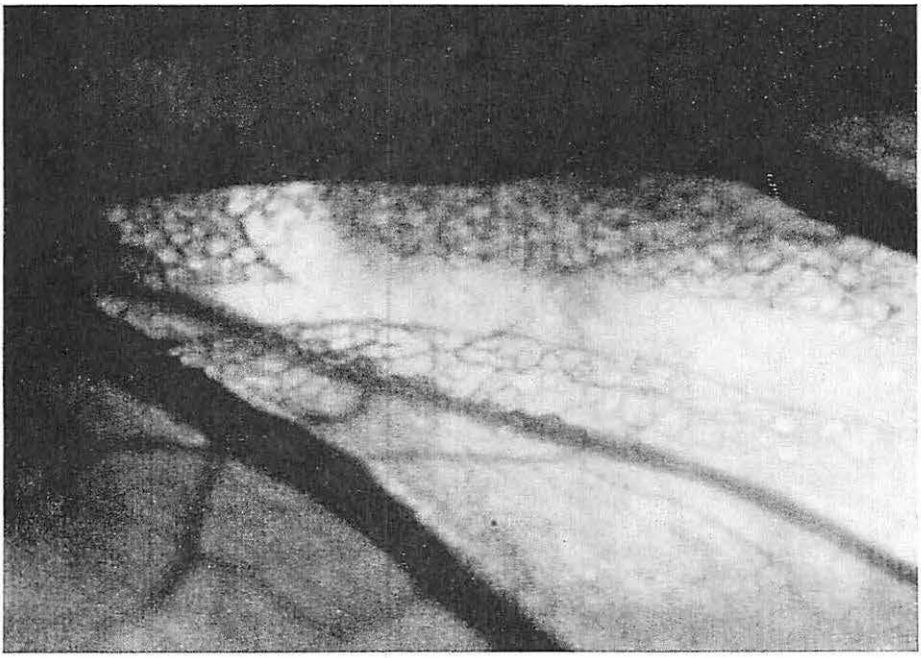

Fig. 3 The size of the lymphatic is comparable to the size of two arteries seen as black bands.

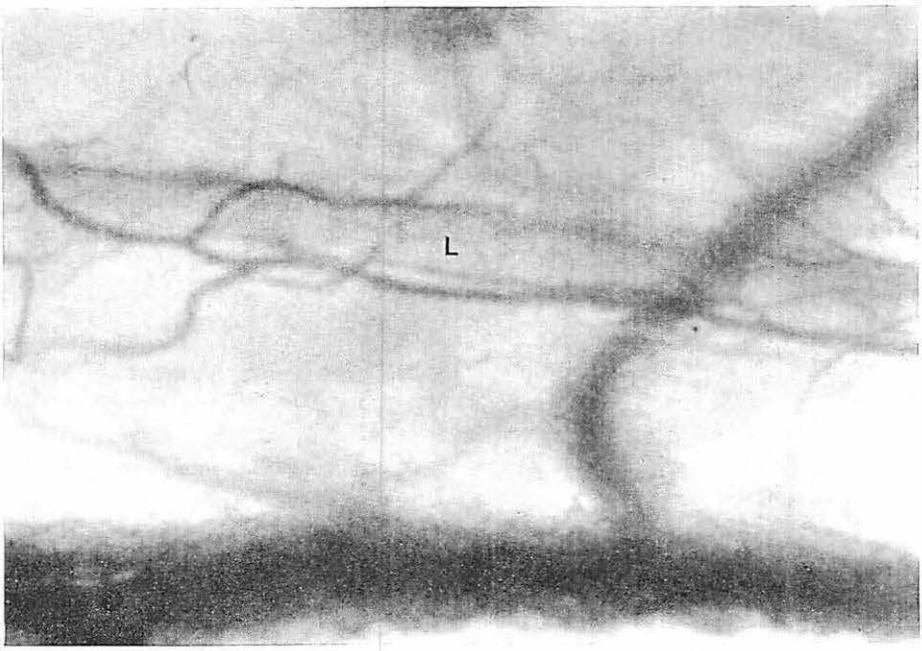

Fig. 4 Deep aspect of the abdominal skin (rat). The lymphatic (L) is surrounded by blood capillaries. It is much wider than the capillaries.

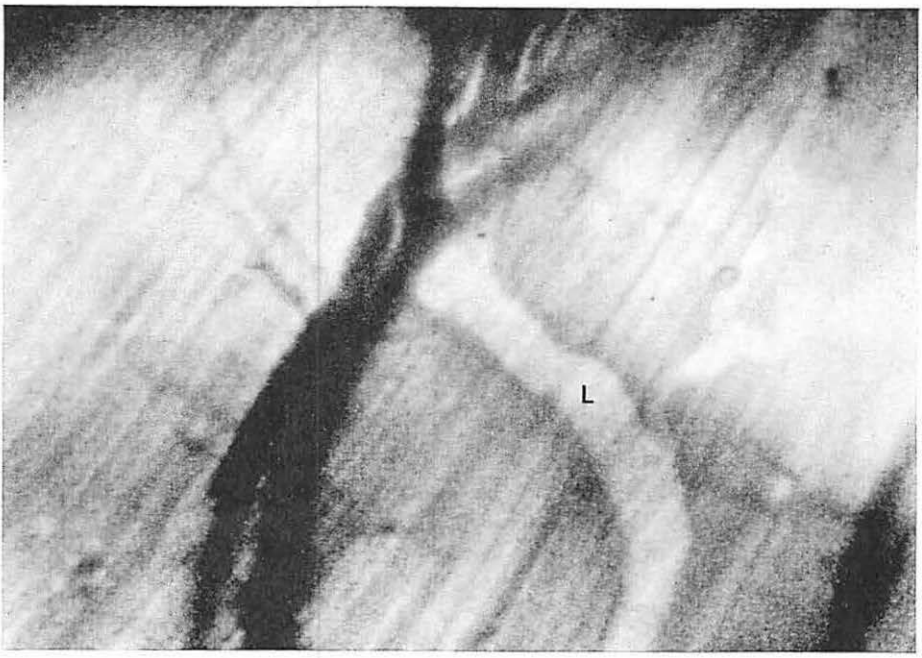

Fig. 5 Pleural aspect of the diaphragm (mouse). Patent blue, laid on the surface concentrates along the wall of the lymphatics (L). The parallel striation is due to muscle fibers. An artery and a vein are crossing the picture.

In the *muscle*, there are even less lymphatics than in the skin. No lymph vessel can be seen in the muscle tissue itself but a few trunks are observed in the connective tissue along the main blood vessels. It is interesting to note that if lymphatics are scarce at the periphery, they are numerous in the *hilum of the viscera*. The liver, spleen and pancreas have been investigated and in these *parenchymatous organs* the lymphatics remain in the connective tissue surrounding the blood vessels.

Calibre

All lymphatic vessels that we have observed in the living animal are of comparable calibre (around 50 μ). They do not taper off towards the periphery, as arteries do. The efferent vessels of a lymph node is slightly wider than the afferent collectors. Our most important observation is the fact that there are no lymphatic vessels of very small calibre (fig. 4); even the resorption plexus are made of vessels which are much wider than blood capillaries.

Resorption

The phenomenon of resorption was best observed on the pleural aspect of the diaphragm (fig. 5). After tracheostomy, the curarised animal is placed in a lateral position and the left hemithorax is opened. A minute drop of patent blue violet is placed on the pleura. Lymphatic vessels soon appear transparent on the dyed surface. Slowly, the dye

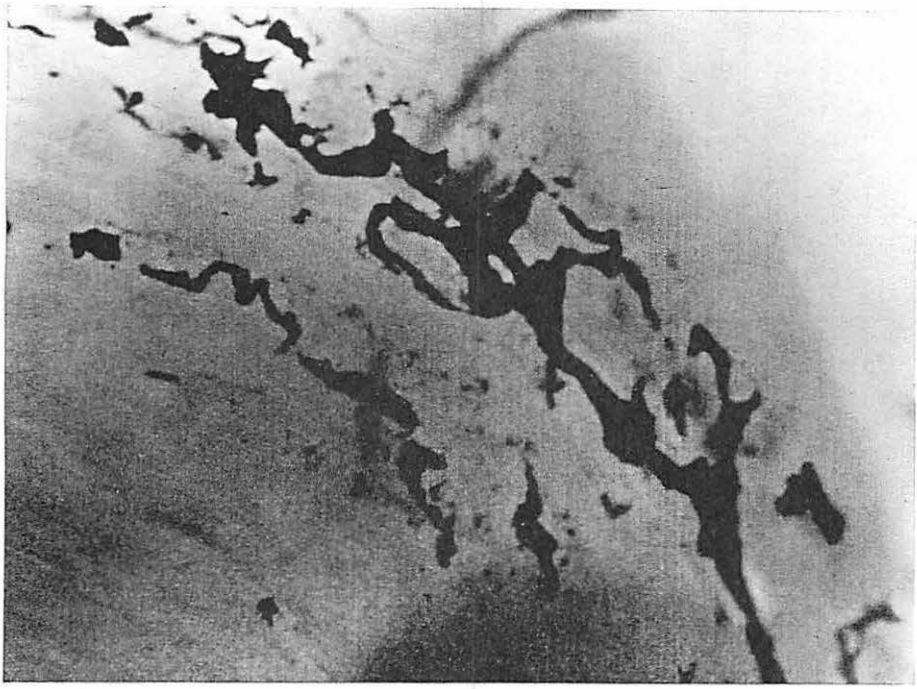

Fig. 6 Peritoneal aspect of the diaphragm (mouse). Lymphatic network impregnated with India ink injected into the peritoneal cavity.

concentrates along the lymphatic walls. Resorption takes place after approximately ten minutes. The dye progressively disappears from the surface, remaining only inside the lymphatic vessel. The concentration of the dye along the lymphatic vessels is probably due to the well known binding of this dye to proteins. The transport of proteins themselves could be studied in this manner.

Selective reabsorption

When India ink is injected into the peritoneal cavity, there is hardly any reabsorption by the parietal lymphatic vessels. Although bathed in the ink, the mesenteric lymphatic vessels do not reabsorb ink either. Meanwhile, a rich plexus is filled on the diaphragm (fig. 6). Communications exist between this plexus and the pleural lymphatic vessels.

Permeability of the lymphatic wall

Dyes injected in the interstital tissue are slowly removed by lymphatic vessels. Experiments on the bowel wall show that dye injected into the lymphatic reabsorption plexus (fig. 7), may diffuse out into the interstitial tissue. Thus the passage occurs in both directions.

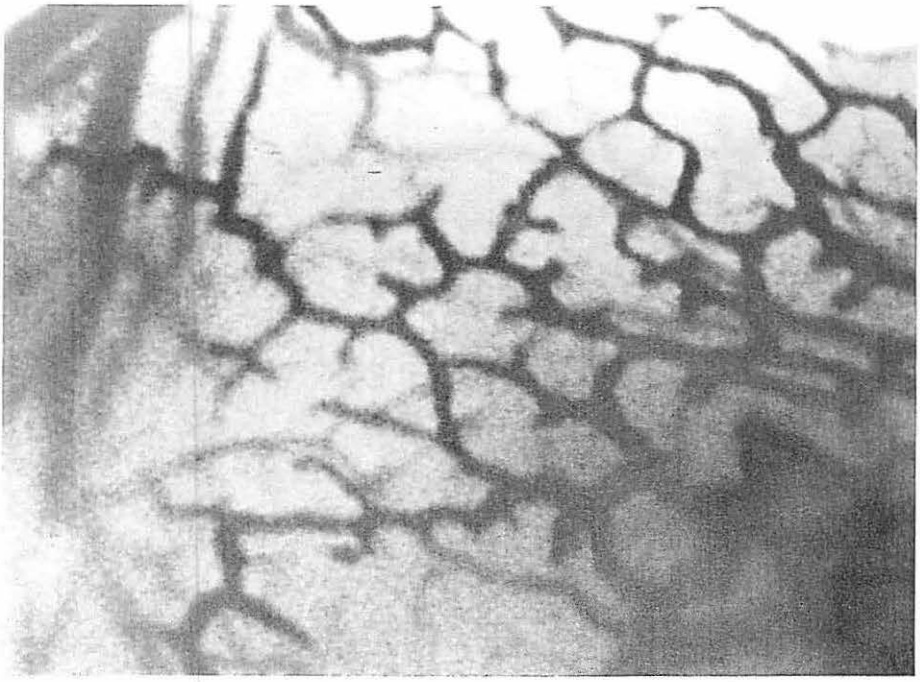

Fig. 7 Lymphatic network of the ileum (mouse). Patent blue was injected into the wall of the bowel at the right lower angle and is flowing to the left. Color filtering makes lymph vessels appear darker than blood vessels in this picture.

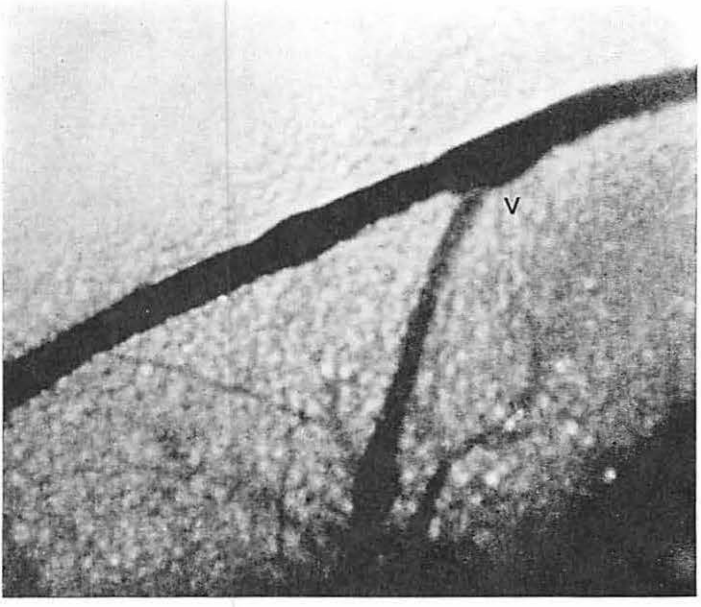

Fig. 8 Mesenteric lymphatic (mouse). The valve (V) is competent.

Lymph nodes

Contractions are observed along lymph collectors. They are forceful and take place simultaneously in a long segment which is emptied of its lymh in this way and may sometimes disappear from sight. After relaxation the vessel resumes its original size. The contractions are independant of heart beats and respiratory movements. The function of the valves can be clearly observed when the lymphatic vessels are filled with dye (fig. 8).

The contractions differ from one species to the other. In rats they are vigorous and numerous, in mice they are almost imperceptible.

Contractability of lymphatic vessels

The collectors filled from peripheral lymphatics lead the lymph towards the lymph nodes. Lymph follicles do not get stained. Dye starts to leave the node through the efferement vessel long before impregnation of the parenchyma is complete.

Summary

The lymphatic system was studied in mice and rats by injection of dyes into tissues and subsequent microscopic examination in vivo. Reabsorption of the dyes takes place through the walls of large calibre lymphatics arranged in networks of varying morphology according to the organ.

With this method contraction of lymphatic vessels, transport of lymph, valvular motion and filling of lymph nodes can be examined.

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S. Godart, M. D., Chirurgie infantile, Hôpital universitaire Brugmann, Bruxelles, Belgium