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THE METHOD OF D. D. ZERBINO FOR STAINING LYMPHATIC VESSELS

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A method for staining capillaries, blood vessels and lymphatic sinuses, according to the anatomopathologist *D. D. Zerbino* of Lvov, and personal results with this method are described.

The purpose of this paper is to report experience gained at the Department of Experimental Surgery and Department of Normal Anatomy in the routine use in research of a method for staining lymphatic vessels described by *D. D. Zerbino* (1, 2). The method is a modification of a previous method by *Gerota* and *Akilova* (3), consisting in selective staining of capillaries, blood vessels and lymphatic sinuses with Paris blue or Berlin blue. Very clear results can be obtained. In this study, human and animal material was stained.

DESCRIPTION OF THE METHOD

1. Filling of the lymphatic vessels. As soon as obtained, the specimen should be placed in 0.9% saline solution at temp. 37°. After 15 min it can be injected with the dye solution (v.i.) by means of syringe with a thin needle, No. 25 or No. 27. The injection should be made at an average rate of 1 ml per 5—10 min. The site of injection depends on the organ under study (Table I).

Three different solutions are used for staining lymphatic vessels: I — 1.0 g of Paris blue or Berlin blue and 20 ml chloroform, II — 1.0 g of Paris or Berlin blue in 100 ml of chloroform, III — 1.0 g of Paris or Berlin blue in 150 ml chloroform.

Paris blue and Berlin blue are oily suspensions of dyes used in artistic painting.

Table I. Sites of injection of dye in different organs for filling of the lymphatic vessels

Organ	Site of injection of the dye
Lymphatic node	afferent vessel or marginal sinus
Skin	between the epidermis and cutis
Lung	visceral pleura
Esophagus	mucosa or muscularis
Stomach	serosa, muscularis, mucosa
Small intestine	serosa, subserosa
Colon	serosa in the region of the tenia libera, submucosa
Liver	capsule, hilar vessels in retrograde direction
Pancreas	deeply into the tissue
Heart	epicardium, myocardium, endocardium
Trachea	submucosa
Diaphragm	tendinous portion

Solution I is used for staining lymphatic vessels with diameters over 1 mm, solution II for vessels with diameters 0.5—1 mm, and solution III for staining capillaries and lymphatic sinuses.

Exactly weighed amounts of the dye should be ground in a mortar with a small volume of chloroform for 20—30 min and then diluted as desired. The solutions should be filtered two or three times through paper.

2. Fixation of specimens. Specimens injected with the dye are fixed for 24 hr in 15% formalin solution. When staining large specimens, the arteries and veins should be filled with formalin separately. The specimen is then cut into smaller portion 2—5 mm thick, which are dehydrated in alcohol solutions of rising concentrations: 60% — 24 hr; 70% — 24 hr; 80% — 24 hr; 96% — 48 hr; 100% — 24 hr. The dehydrated specimen is placed in methyl salicylate or glycerin for 24 hr. The specimen is then transparent and can be inspected with a stereomicroscope. Methyl salicylate as well as glycerin are fixatives, in which the specimens can be stored for many months.

3. Preparation of histologic specimens. Histologic blocks can be made from preparations fixed in methyl salicylate. For this purpose, the specimens should be washed 4 hr in running water at temp. 40°, placed for 4 hr in 0.2% alcoholic solution of NaOH, and again washed in water for 2 hr. The specimen is then ready for typical histologic elaboration. Blue staining of the walls of lymphatic vessels is permanent and does not fade during staining of histologic sections.

DISCUSSION

In the method of staining lymphatic vessels described above, the blue dye forms a permanent thin layer on the internal surface of the vessels,

of which the lumen remains unstained. Observation with a magnifying glass against light clearly shows the shape of the vessel, thickness of its walls, and valves. Good translucence of the specimen allows studies of vessels in various layers. Histologic sections can be made after inspection with the magnifying glass.

Results obtained with the method are illustrated in Figs. 1—6.

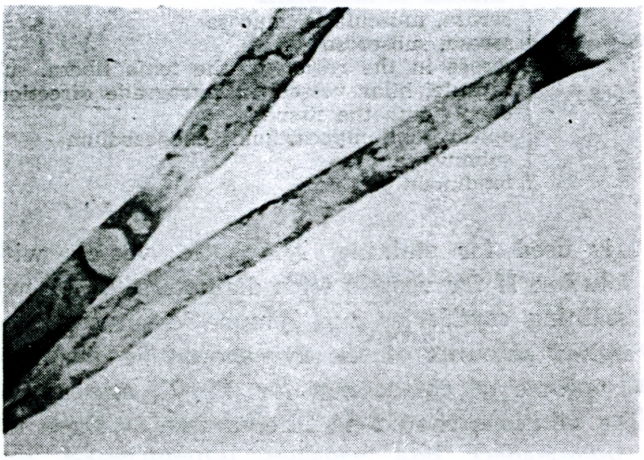

Fig. 1. Lymphatic vessels in the mesentery of the small intestine of a dog. Magn. approx. × 70.

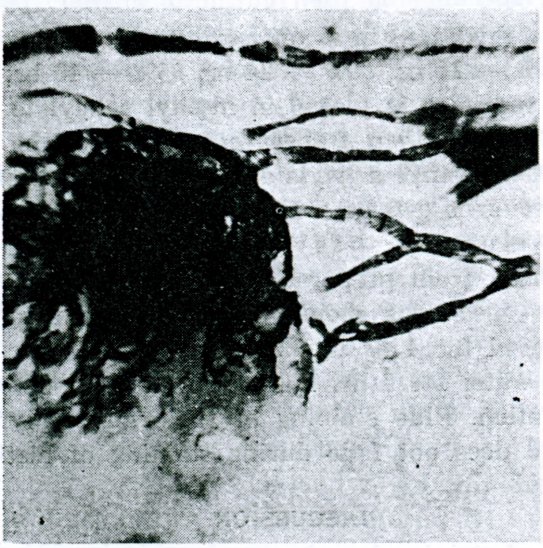

Fig. 2. Lymphatic vessels and sinuses of a lymph node in the mesentery of the small intestine of a dog. Magn. approx. × 70.

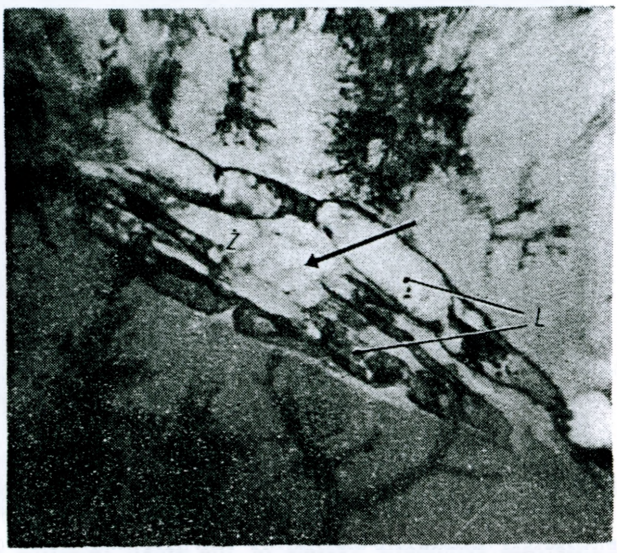

Fig. 3. Intrahepatic lymphatics accompanying branches of the portal vein in a dog. L — lymphatic vessels, Ż — vein. A network of small lymphatic vessels surrounds the vein (arrow). Magn. approx. × 70.

Fig. 4. Lymhatic vessels in the hepatic hilum in man. Numerous valves can be seen. Magn. approx. × 70.

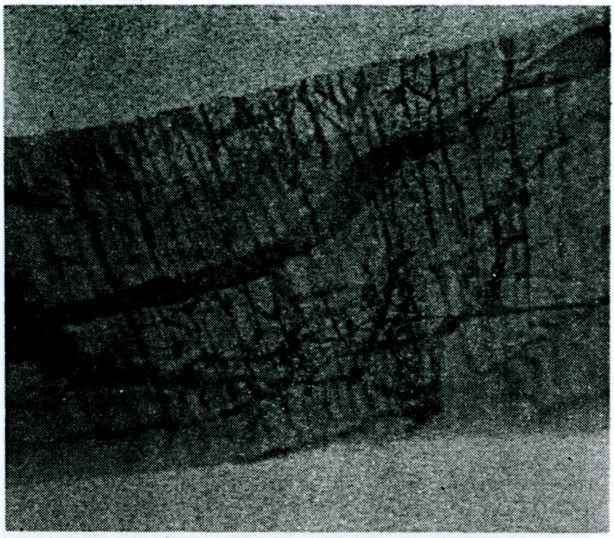

Fig. 5. Subserosal lymphatic vessels of the small intestine of a dog. Magn. approx. × 70.

Fig. 6. Subserosal lymphatic vessels of the large intestine of a dog. Magn. approx. × 70.

REFERENCES

1. *Zerbino, D. D.:* Nauchnye zapiski Cherniovitskogo Meditsinskogo Instituta. Vypusk 13. Cherniovtse 1960.
2. *Zerbino, D. D.:* Personal information, 1966.
3. *Zhdanov, D. A.:* Anatomiya limfaticheskoi sistemy. Medgiz, 1962.