

# Evaluation of several methods used in anatomical investigations of the blood and lymphatic vessels

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[Received 15 March 2002; Revised 17 April 2002; Accepted 17 April 2002]

*The aim of this review is to describe the advantages and limitations of several methods used in anatomical investigations of intravisceral blood and lymphatic networks. The microangiographic methods as well as corrosion methods are described. In conclusion the authors confirmed that the most useful way for exploration of the blood and lymphatic vessels is to prepare corrosion casts. This paper focuses on the scanning electron microscopic examination of vascular corrosion casts. This method allows the examination of the three-dimensional organisation of vessels, including the blood and lymphatic capillaries. Imprints of endothelial cell nuclei can be observed on the surface of the blood and lymphatic vessels.*

**key words:** anatomy, methodology, blood and lymphatic vessels

## INTRODUCTION

Since ancient times the vascular system has been the topic of much research. The last three decades have brought significant progress in both the preparation of highly infiltrative masses used for filling the vessel and the development of the already existing equipment used to expose the filled vessels. We would like to give a short presentation of a few methods used by us in anatomical investigations of the blood and lymphatic vessels, which enabled us to recreate the three-dimensional structure of the vascular network as well as distinguish the types of vessels forming those networks. This last statement is due to the very often occurring uncertainty over whether we are dealing in the investigated corrosion specimen with the casts of capillary, venous or arterial vessels or lymphatic vessels.

## Remarks about a few methods used in anatomical investigations

Because the preparation of specimens is easy and their cost is low, we have used most frequently the angiographic and microangiographic methods as well as several other methods, generally called corrosive methods. The additional advantage of those methods is the fact that the specimens can be examined a couple of days after their preparation. In our paper, we are also going to describe the method used to generate the so-called “transparent specimens”.

The blood vessels were rinsed with a physiological solution, NaCl, containing a small addition of heparin to remove the clots and blood remaining in the vessel. In some cases, in order to strengthen the vessel endothelium, we introduced a 3% glutaraldehyde solution. The angio- and microangiographic

specimens were prepared by filling the blood or lymphatic vessels with contrast agents used in x-ray research. These are available on the market as ready-made preparations containing barium compounds (such as Micorpaque, Unibaryt, Polibar ACB). In our research [2] we used the ready-made preparations as well as their 30 and 50 per cent dilutions. Contrast agents were introduced into the blood or lymphatic vessels; then we prepared sections, between 300 and 1000  $\mu\text{m}$  thick, and exposed them to the x-rays.

Although angiographic specimens give a good insight into the vessels' organisation they also have quite important disadvantages, which are:

- the impossibility of giving a stereoscopic view of the investigated vessel network structure;
- difficulties in distinguishing the type of vessels forming those networks;
- problems with ascertaining the diameter of each individual vessel.

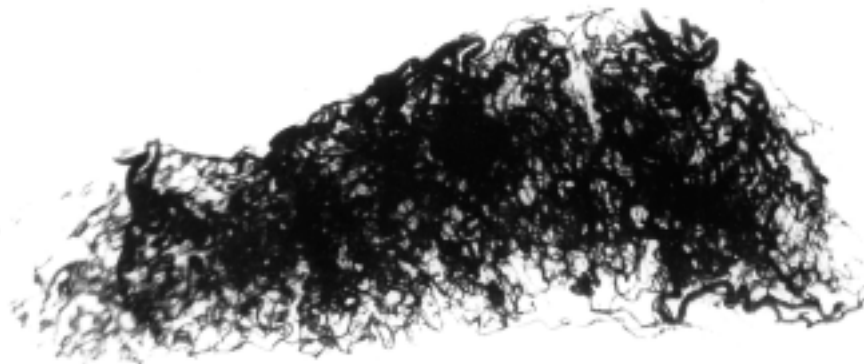
Regardless of the disadvantages listed above, the angiographic samples are extremely useful for the initial evaluation of the vessel networks' appearance and density, as well as ascertaining the passageway of the blood or lymphatic vessels in the investigated organ (Fig. 1). It is worth stressing that the contrast agents have a large penetrability ratio, particularly in the 30 and 50% dilutions. For the most part, this depends on the correct preparation of the suspension of barium sulphate particles, the diameters of which should not exceed 5–8  $\mu\text{m}$ .

Preparing vascular casts is the method we have used most frequently. We would like to stress once again that in each case the vessels were rinsed with a physiological NaCl solution and the endothelium was strengthened with a 3% solution of glutaraldehyde. We make this reservation as some of the researchers consider this type of conduct an unneces-

sary procedure. From our tests it ensues that the removal of clots and blood remains greatly improves the quality of the specimen cast. However, in our opinion the use of equipment allowing constant measurement of the pressure applied when filling the vessels is not necessary.

Similar to other researchers [1, 3, 5, 7, 11], we reckon that the sufficient criteria for a correct filling of vessels in any given organ are:

- When introducing a filling mass into an arterial vessel, the mass exists through the venous vessel (or vessels). This means that the specimen's capillary vessels have been filled correctly; it can also provide evidence of the presence of arterio-venous anastomoses.
- Whilst filling up the venous vessels (i.e. introducing the mass in reverse to the physiological blood flow direction) one must remember that the capillary vessels and arteries will be filled only in the areas where the injected veins do not contain valves. Therefore, not all types of vessels can be filled in every kind of organ.
- A good indicator of whether the vessel has been filled correctly is the enlargement of the capacity of the investigated organ and clear hardening. We should also observe the appearance of small surface vessels, often running just underneath the fibrous capsule, encompassing the organ; infrequently the mass outflows through damaged small superficial vessels.
- If the blood or lymphatic vessels have been injected with a coloured-filling agent, then the colour of the injected organ should have changed visibly. In the case of using a filling mass containing contrast agents, following the injection we can take an x-ray photo and evaluate the degree to which the vessel has been filled.

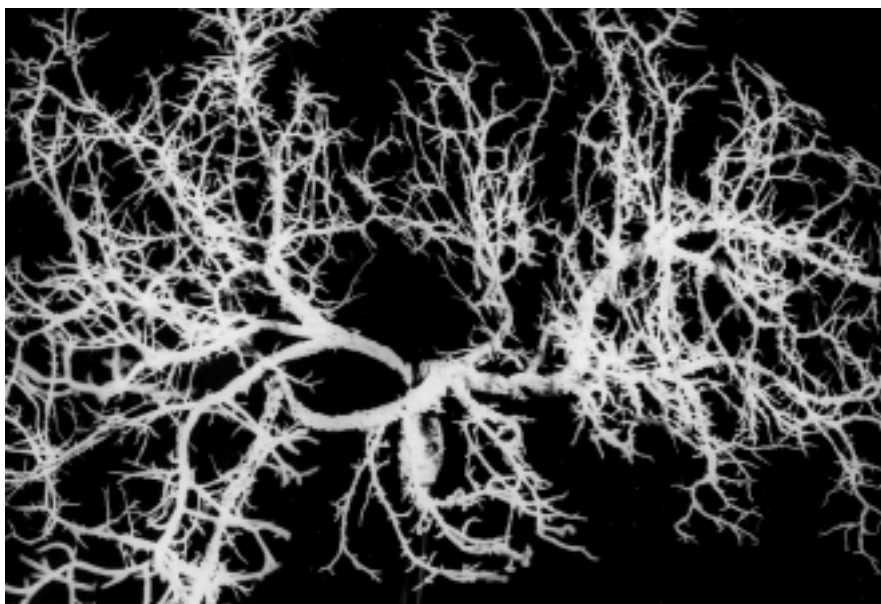


**Figure 1.** Microangiogram. Vascular network within the human epididymis. Blood vessels filled with Micorpaque®. Magnification — 5 ×.

The quality of the generated corrosion specimens depends not only on the degree of filling the vessel but also, or perhaps even mainly, on the type of mass applied. In our research we have used a few types of casting media, namely: the acetone solution of chlorinated polyvinyl chloride, latex, some kind of manufactured polyester resins, such as Polimal, Kallocryl M, Plastogen G and Mercor. Most of them have some deficiencies, which considerably affect an objective evaluation of the produced casts. For example, the casts made with the help of the acetone solutions of chlorinated polyvinyl chloride have uneven surfaces and the measurements of the vessels' length and diameter contain many mistakes due to this medium's high shrinkage ratio during polymerisation (Fig. 2). The latex materials are characterised by very good penetrability [4] and have a very small shrinkage ratio during polymerisation; however, most of the latex casts do not preserve their dimensional structure after completing the treatment of the investigated organ tissues. The length measurements in latex vascular casts can be very misleading due to the elasticity and expandability of the material used. For instance, the bibliography shows the results of conducting measurements of the testicular artery's length in farm cattle, quoted by some of the authors [7] in centimetres, whereas some authors using the latex in research evaluated the length of the same artery in metres [6]. Synthetic resins of the Polimal or Kallocryl type (to a lesser degree the

Plastogen G) lack sufficient penetrability due to the high viscosity ratio. When using those materials, we have encountered problems with filling the capillary vessels. The listed materials have an advantage of preserving the dimensional proportions of internal vascular networks up to the level of the capillary vessels (Figs. 3–4).

Mercor has proved to be the best available material used for producing vascular casts. In our view, this material meets most of the expectations which a morphologist would have with regards to a mass designated for filling up structures containing light. One of the few negative features of Mercor is the fact that it does not contain contrast agents and thus cannot be used simultaneously for the production of corrosive and angiographic specimens. The blood and lymphatic specimens casts made with Mercor very faithfully recreate the three-dimensional structure of a vessel network inside the organ (including capillary vessels) and enable the correct measurement of the internal vessels' length and diameter, as well as permitting the distinguishing of what type of vessels we are dealing with. This last-mentioned very important feature of the vascular casts depends on the ability to investigate their surface, where the replica of the vessels endothelium appears. Thanks to that, excellent effects are achieved when researching the micro-corrosive specimens using a scanning electron microscope. It is well known that there are significant differences in the



**Figure 2.** Corrosion cast exhibiting bile ductules in human liver filled with 20% acetone solution of chlorinated polyvinyl chloride.



**Figure 3.** Corrosion cast of testicular artery and pampiniform plexus within the bovine spermatic cord. Blood vessels filled with Plastogen G<sup>®</sup>. Magnification — 2 ×.



**Figure 4.** Corrosion cast of lymphatic vessels within the human spermatic cord. The lymphatic and blood vessels filled with Mercocox<sup>®</sup>. Magnification — 4 ×.

appearance of the endothelial lining of arterial, venous and capillary vessels [10, 11, 14, 15].

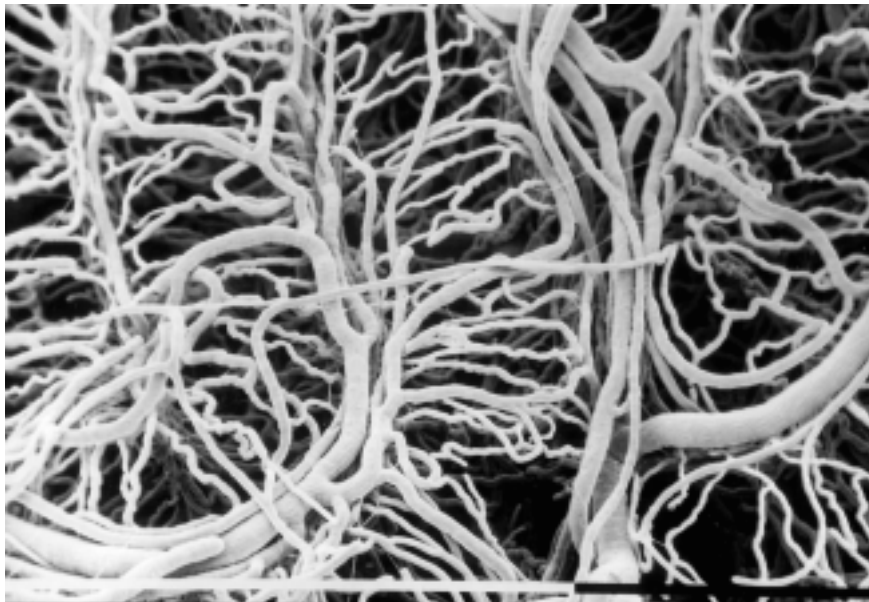
The scanning electron microscope has been successfully used in the research of corrosive specimens [1, 9–12, 14, 15]. Thanks to this method it is possible to give a nearly stereoscopic view of the vascular network structure in any freely chosen organ (Figs. 5–6). The way of preparing the corrosive specimens

and their further “treatment” up to the stage of installing the scanning microscope in the column and taking photographs is described in many text-books and essays [1, 5, 7–12], therefore we will not discuss it in this paper.

On the basis of images obtained by the scanning microscope we can establish what types of vessels the investigated vascular networks create. This is



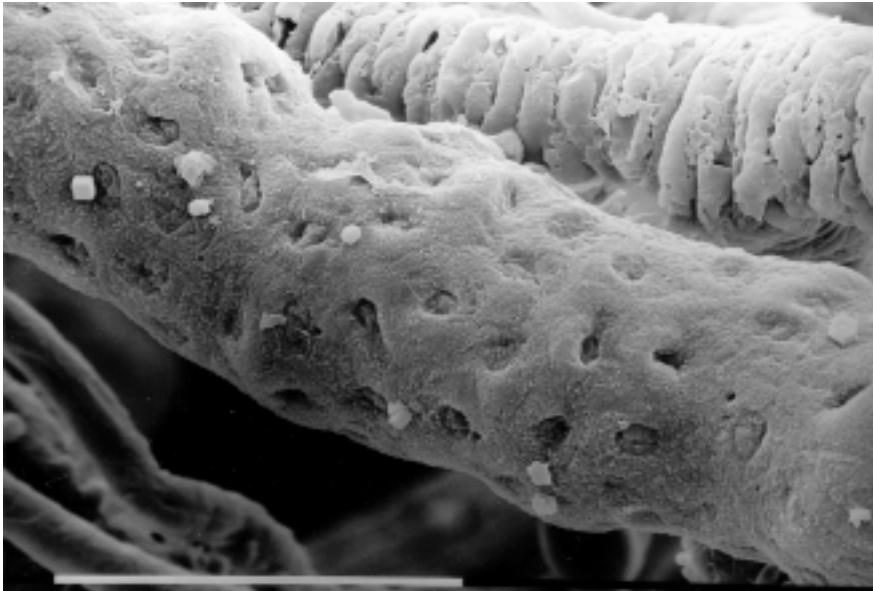
**Figure 5.** SEM-micrograph. Corrosion cast exhibiting capillary network within the human testis. The blood vessels filled with Mercox®. Scale bar — 0.1 mm.



**Figure 6.** SEM-micrograph. Corrosion cast exhibiting the vascular network in human epididymis. The blood vessels filled with Mercox®. Scale bar — 1 mm.

possible due to a different, specific layout of endothelial nuclei in the arterial, venous, capillary and lymphatic vessels [14]. Endothelial nuclei in the tissues of an arterial vessel are oval, often reminiscent of the shape of a plum stone and their long axis lies parallel to the long axis of the arterial vessel. We have also established that in the spiral vessels (such as a testicular artery in its end part) the endothelial

cells as well as their nuclei come close to round shapes, and even if they are slightly oval they never contain sharp edges; besides their layout is less regular than in arteries (Fig. 7). Images of the endothelial nuclei in the capillary vessels are very difficult to obtain. The lymphatic vessels have features differentiating them from the blood vessels. On the surface of a cast we can see large, round and oval



**Figure 7.** SEM-micrograph. Corrosion cast of a venule (note the oval or roundish-shaped replicas of the endothelial nuclei) and small arteriole (note the circular bands corresponding to the myocytes). The blood vessels filled with Mercox®. Scale bar — 0.1 mm.

shaped imprints of the endothelial cell nuclei as well as the blind ended branching, characteristic for small lymphatic vessels (as yet not containing any valves). We can also observe the extravasations casts of the mass filling up intercellular spaces. In the corrosive specimens of the collecting lymphatic vessels with a diameter significantly bigger than described previously, the main striking feature is the casts of the two, three and sometimes more valves.

It must be stressed that in order to obtain proper corrosive specimens, suitable for scanning microscope examinations, they must be filled with masses exactly recreating the drawing of the vessels' wall, directed towards its light and the media used must guarantee the preservation of dimensional conditions. For morphological evaluation of the media used in our research we have taken advantage of computer technology potentials.

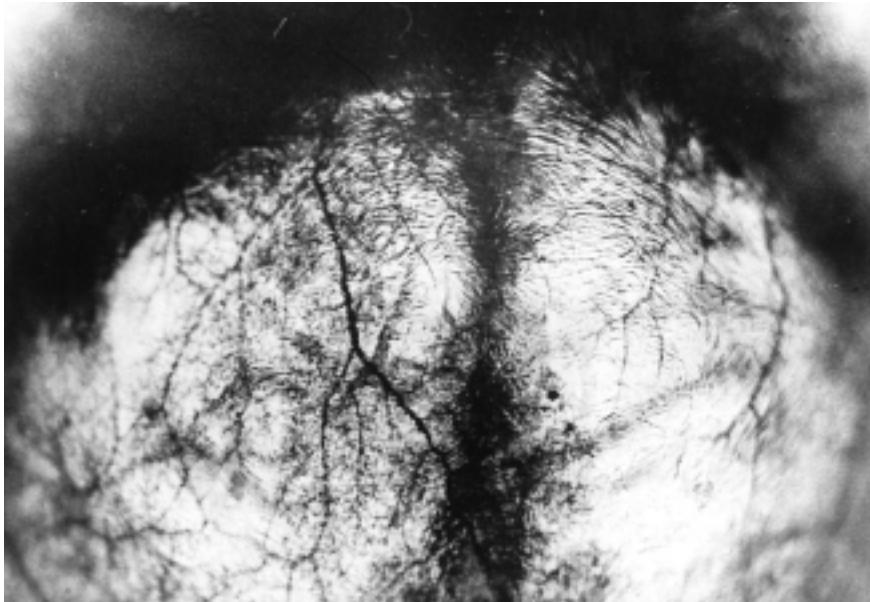
Finally, we would like briefly to touch upon the use of the so-called "transparent specimens". In other words, those are specimens where after filling the blood or lymphatic vessels with suitable contrast agents, the tissues of the investigated organ are treated with chemical substances, turning the tissues transparent (Fig. 8). In short, the process begins with dehydrating the investigated organ and then placing it in methyl salicylate. The "transparent specimens" permit the investigation of vascular networks inside the organ without removing the tissues, thus permitting the establishment of the to-

pographic relations of individual structures inside the investigated organ. This is however conditioned by the size of the investigated organ (or its parts), which cannot be too big since the chemical agents' infiltration inside the "transparent" tissues is limited and varies depending on the type of investigated organs (i.e. bones or solid or tubular organs). This technique has been known for a long time [13], however the introduction of new filling masses has brought considerable advantages here as well.

Amongst such masses are various kinds of Microfil. These masses have exceedingly good penetrability and are easy to use. Some of them contain small additions of contrast agents, which permit the conduct of comparative angiographic investigations, using the same material. Their disadvantage is, as in the case of Mercox, their high price as well as the fact that they are not suitable for corrosion.

## CONCLUSION

In conclusion, we would like to state that always more than one method should be used in anatomical investigations of the vascular system. In our view, the best solution is to use the angiographic and corrosive methods together, whereas observations made using a scanning electron microscope are extremely valuable for exposing the blood network of an organ. Of course, this does not mean that we reject or do not appreciate other investigation methods, such as the classical methods of preparation or histologi-



**Figure 8.** "Transparent specimen". Skull of newborn; branches of supratrochlear artery.

cal or histochemical methods, however the aim of this paper is only a brief presentation of simple methods, allowing the evaluation of angioarchitecture in individual organs.

### ACKNOWLEDGEMENT

Scientific Grant NR 502-11-616 (from the Medical University of Łódź) supported this paper.

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