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Issue 98-7, Sept. 1998**

# **Vascular Corrosion Casting Can Provide Quantitative as Well as Morphological Information on the Microvasculature of Organs and Tissues**

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## **Introduction:**

Complete casts of the vasculature of organs and tissues are obtained by infusing low viscosity resins into the vasculature and allowing the resin to polymerize. Dissolving away the surrounding tissue with alkali leaves a model of the intricate, three-dimensional distribution of vessels in that tissue, which is not easily obtainable by any other means, and which can then be studied with scanning electron microscopy (SEM). Because well prepared casts appear to faithfully replicate the true vascular anatomy of organs including the dimensions of vessels and details of imprints of the endothelial cells lining their lumens, they must also contain quantitative information about that vasculature<sup>1</sup>.

## **Methods**

The species of interest is anesthetized (60 mg pentobarbital/kg body weight; *i. p.*) and anticoagulated (700 U heparin/kg; *i. p.*) 30 minutes before use. The abdominal cavity is opened and the aorta (or an artery supplying the organ of interest) is cannulated and the

blood is flushed from the organ with saline or Ringer solution (37° C, at 80-100 mm Hg). Resin (Mercox/catalyst, 10/0.3; or Mercox/methyl-methacrylate/catalyst , 8/2/0.4; Ladd Research Industries, Burlington, VT ) is infused through the same cannula until the onset of polymerization (usually 10 minutes). The resin-filled tissue is immersed in hot water (50° C) for one hour to complete resin curing. Tissue is removed by maceration in alternating rinses of 5% KOH and hot water, and the resulting casts are cleaned in formic acid (15 minutes) and distilled water, dried by lyophilization, and mounted on stubs for routine SEM. Casts are sputter coated with gold and viewed at 10 kV in the SEM. For vascular volume determination, resin filled tissues are weighed before and after maceration, and vascular volumes are calculated from tissue and resin densities. Vessel measurements and counts are made from micrographs. Fixation of the tissue with dilute aldehyde fixatives prior to casting enhances preservation of details of the endothelial cells as imprints on the casts, but may prolong the maceration process.

## **Results and Discussion:**

High quality casts display the 3-dimensional arrangement of the vasculature of organs (Figs. 1, 2, 3) including arteries, capillaries, and veins and their valves, and may exhibit details of the endothelium on the cast surfaces including imprints of endothelial nuclei (Figs. 3, 4, 5) and endothelial cell borders. From these imprints one can observe the orientation of the endothelial cells with regard to the vessel axis, determine the number of endothelial cells per unit area, and from this calculate an average luminal surface area for the endothelial cells. In arteries, endothelial cells are usually very much elongated, lying parallel with the long axis of the vessel (Figs. 3,4), in contrast to those in veins and capillaries which are more rounded and less highly oriented (Fig. 5). For example, in the cast of an arterial surface from a duckling eye shown in Fig. 4, there are on the average 3.03 endothelial cells per

1000  $\mu\text{m}^2$  and, therefore, each cell covers an area of about 328  $\mu\text{m}^2$  of the vessel wall. In this case nuclear imprints average 16  $\mu\text{m}$  in length, whereas in the cast of the choriocapillaris of the duckling eye shown in Fig. 5, they are more rounded and average 13  $\mu\text{m}$  in length.

If one is reasonably certain that all vessels in a tissue have been filled with resin (and it is often difficult to make this assumption with real assurance), vascular volume estimates can be made from tissue and cast weights and densities<sup>2,3</sup>. Resin density was determined to be 1.28 g/ml from polymerized resin blocks and a reported value of 1.05 g/ml was used for tissue density. Table I shows some example measurement made from corrosion casts from rat heart and hamster lung under several different conditions. It is worth noting that the values for capillary diameter and intercapillary distance measured from casts of rat heart very closely match those determined *in vivo*.

## **Summary and Potential Other Applications of Corrosion Casting.**

Many other examples of the use of vascular corrosion casting for obtaining quantitative information are found in the literature, but for simplicity this report is limited to our own work. While the primary application of vascular corrosion casting has been and likely will remain in the elucidation of the 3-dimensional distribution of vessels in tissue and organs, high quality casts also yield valuable quantitative information about that vasculature. In addition, I see no obvious reason why the corrosion casting method could not be used to describe or measure the internal volume of any hollow or porous structure or material as long as methods are available to remove the surrounding material and leave the cast intact. Applications might include determining the core of a mold, or the shape or volume of the lumen of a tube, or demonstrating the distribution of interconnected spaces within a penetrable substance

in materials science. Similarly, the volumes of hollow organs such as the urinary bladder or ureter could also be measured. Incorporation of marker substances in the casting resin (*e.g.* isotopes, heavy metals, electron dense materials, photosensitive materials, or fluorescent substances) to enhance quantitative measurements are also being considered in some laboratories.

**Table I: Example Measurements from Corrosion Casts**

<b>Parameter</b>	<b>Hamster Lung*</b> (inflated)	<b>Adult Rat Heart*</b> (3-5 mo.old, right ventricular free wall)	<b>Geriatric Rat Heart*</b> (19-20 mo. old, rightventricular free wall)
<b>Capillary diameter</b>	---	$5.6 \pm 1.9 \mu\text{m}$	$5.7 \pm 2.3 \mu\text{m}$
<b>Intercapillary distance</b>	---	$14.9 \pm 4.9 \mu\text{m}$	$12.7 \pm 5.6 \mu\text{m}$
<b>Capillary density</b>	---	---	$22.2/10^4 \mu\text{m}^{2**}$
<b>Vascular volume</b>	$11.33 \pm 1.17\%$	$10.01 \pm 1.06\%$	$9.10 \pm 1.67\%$

\* values are means  $\pm$  sd<sup>2,3\*\*</sup>mean from 17 samples

## Literature Cited

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2. Hossler, F.E., Douglas, J.E., and Douglas, L.E., 1986 Anatomy and morphometry of myocardial capillaries studied with vascular corrosion casting and scanning

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3. Hossler, F.E., Douglas, J.E., Verghese, A., and Neal, L. 1991 Microvascular architecture of the elastase emphysemic hamster lung. *J. Electron Microscopy Tech.* **19**:406-418.

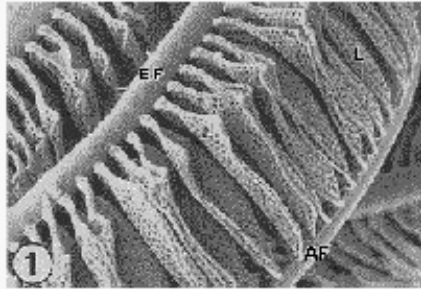


Figure 1. Vascular corrosion cast of a portion of a hybrid striped bass/white bass gill. AF, afferent filamental artery; EF, efferent filamental artery; L, capillary beds of secondary lamellae. x154

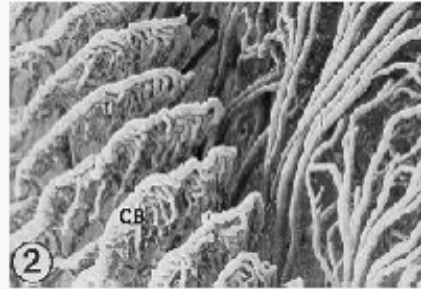


Figure 2. Vascular corrosion cast of a portion of a duckling eye showing capillary beds of ciliary bodies (CB) and the posterior iris (I). x98

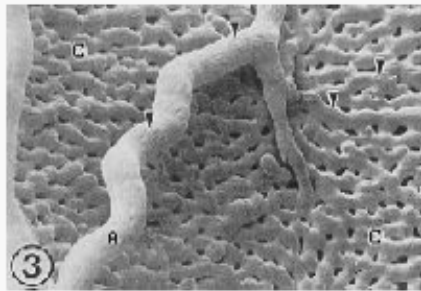


Figure 3. Vascular corrosion cast of a portion of a bull frog eye showing an artery (A) supplying the choriocapillaris (C). Arrowheads, endothelial imprints. x113

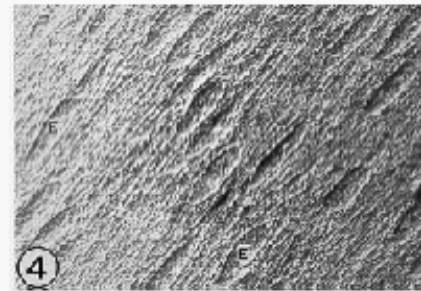


Figure 4. Surface of a corrosion cast of an artery in the duckling eye showing endothelial imprints (E). x1145

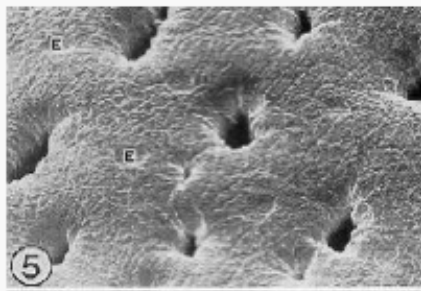


Figure 5. Vascular corrosion cast of a portion of the choriocapillaris of the duckling eye. E, endothelial imprints. x559

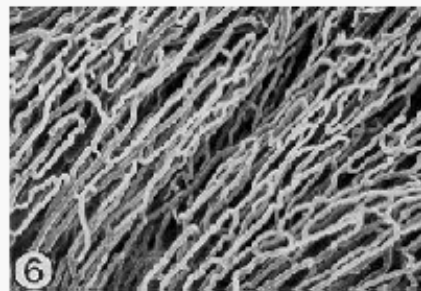


Figure 6. Vascular corrosion cast of a portion of the endocardial surface of a ventricle from an adult rat heart showing capillary beds of the trabecular muscles. x247