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DIFFERENT FORMS OF CORROSION CASTS

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Abstract

We have previously described artifacts in corrosion casts prepared with Mercox®, diluted with methylmethacrylic acid (MMA). Using scanning electron microscopy (SEM) of casts and light microscopy (LM) of tissue sections, we found new forms of artifacts in casts prepared with both undiluted and MMA-diluted Mercox®. When undiluted Mercox® was used, most of the casts did not show artifacts. Artifacts were rarely seen and comprised empty casts consisting of a rim, while endothelial cell nuclear imprints and other vascular wall structures were replicated. Other casts sometimes showed vessels with a fine granular surface or some with conglomerates in their internal structure, however, surrounding vessels were well cast. When using MMA-diluted Mercox®, beside casts consisting only of a rim of resin, casts with granular surface, or casts composed of conglomerates, we found casts which were partially collapsed, casts with artifacts on many vessels, casts which only rudimentarily mimicked vascular structures, and finally casts which did not reveal any vascular structures. Light microscopy confirmed these findings and showed that the number of artifacts increases toward the periphery of an individual microvascular bed, demonstrated in intestinal villi as a model. The number of artifacts was higher when MMA-diluted Mercox® was used, regardless of the tissue or species used. Casts which did not replicate vascular structures were never found when undiluted Mercox® was used. Therefore, we recommend the use of undiluted Mercox® for the preparation of vascular corrosion casts.

Key words: Corrosion casting, artifacts, Mercox®, methylmethacrylic acid, microscopy, scanning electron.

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Introduction

Different resins have served as casting media to prepare vascular casts in the past. Methylmethacrylate was first used by Murakami (1971) to prepare microvascular casts suitable for scanning electron microscopic (SEM) studies. By this method, replicas of vascular beds can be viewed three-dimensionally. Ever since, the method has been applied to many basic or clinical problems (Nowell and Lohse, 1974; Miodonski *et al.*, 1976, 1981; Hodde *et al.*, 1977; Hodde, 1981; Christofferson and Nilsson, 1988; Lametschwandtner *et al.*, 1990; Aharinejad and Lametschwandtner, 1992). Methylmethacrylate compounds can be prepared and used according to Murakami's (1971) protocol. A commercially available, ready to go resin, which contains a methacrylate compound is Mercox®. This low viscosity resin passes through the capillaries and, depending on the amount of the catalyst added, polymerizes within a short time (Lametschwandtner *et al.*, 1990; Aharinejad and Lametschwandtner, 1992). It can be either used undiluted or diluted with monomeric methylmethacrylic acid (MMA; Lametschwandtner *et al.*, 1990; Aharinejad and Lametschwandtner, 1992; Aharinejad *et al.*, 1993; Aharinejad and Böck, 1993a,b). The casts obtained are mostly of high quality, and the details of the vascular luminal wall are replicated. However, we have previously reported that dilution of Mercox® with MMA may lead to phase separation (Aharinejad and Böck, 1993a, b). An unidentified component of the mixed casting medium penetrates into endothelial and blood cells, mummifies them, and makes these cells resistant to maceration (Aharinejad and Böck, 1993a). Moreover, this mechanism may be responsible for occurrence of plastic sheets observed on the surface of elastic arteries (Aharinejad and Böck, 1993b). Therefore, we recommended to rather use undiluted Mercox® because casts prepared with undiluted Mercox® have less artifacts.

The present study seemed to be urgent because researchers applying the corrosion casting method to microvascular research are interested in casts of high quality. Such casts are those with smooth surface and

replicated reliefs of vascular wall components, e.g., endothelial cells, smooth muscle cells, or pericytes (Miodonski *et al.*, 1976, 1981; Christofferson and Nilsson, 1988; Lametschwandtner *et al.*, 1990). Other structures adhering to the cast's surface have been considered as artifacts (Aharinejad and Böck, 1993a,b). Meanwhile, we sometimes observed artifacts in our casts prepared with MMA-diluted Mercox®, which differed in shape from those we previously described. We analyzed casts prepared with undiluted Mercox® to see whether the same artifacts could be found in them. This search was only positive for certain artifacts. Hence, we analyzed the morphology of these artifacts more thoroughly, combining SEM of vascular corrosion casts with light microscopy (LM) of tissue sections. It should be emphasized that only the forms of casts were of primary interest for the present approach.

Material and Methods

Scanning electron microscopy of vascular casts

For analysis of casts prepared with undiluted Mercox®, we used the following organs: the lung, intestine, and pancreas. Casts prepared with MMA-diluted Mercox® were obtained from the same organs, scapular flaps, and heart. The organs were obtained from humans, rats, and cattle.

Rats were anesthetized with sodium pentobarbital (intraperitoneally, 40 mg/kg body weight), the abdomen or thorax was opened by a median cut, and the thoracic or the abdominal aorta, as well as the caudal or inferior vena cava were cannulated with a plastic catheter (Argyle 0.8 x 19 mm, Sherwood Medical, St. Louis, MO, USA). The cannulas were connected to a two-way connector (LS2, B. Braun-Melsungen, Germany), and the systemic (via the aortic arch) and pulmonary circulatory system (via the caudal vena cava) were rinsed with 42°C heparinized Tyrode solution (5,000 IU/l) until the efflux of the inferior vena cava, incised just beneath the right atrium (as the systemic circulatory system was perfused); or the efflux of the abdominal aorta incised at the level of renal veins (as the lungs were perfused), was clear. In cattle lungs, we injected the lavage medium and the resin through the pulmonary artery and drained the efflux by the pulmonary vein. For human pulmonary flaps, we cannulated the scapular circumflex artery and drained the efflux by a vein at the pedicle of the flap.

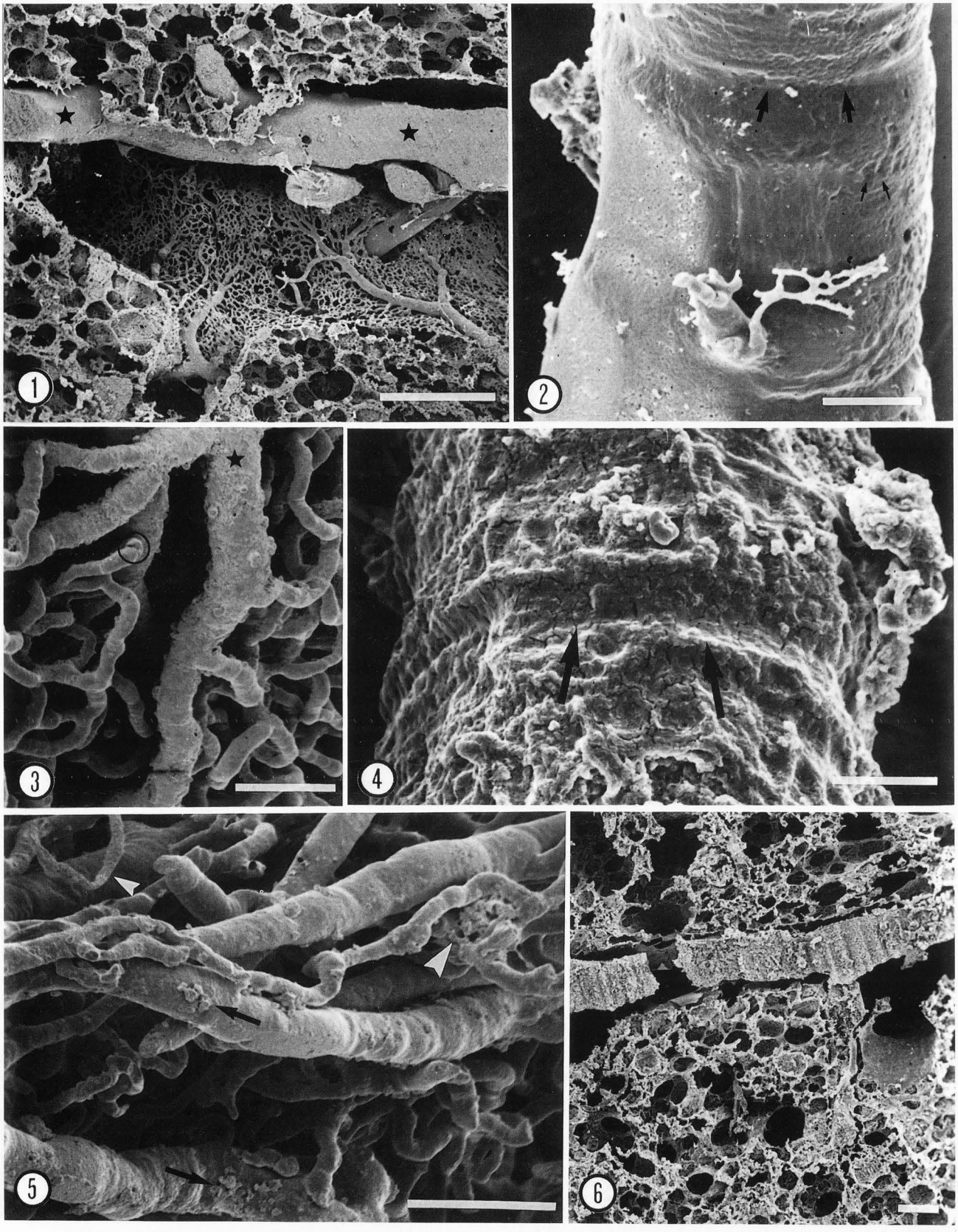
Protocol 1. We injected Mercox® (Dainippon Ink & Chemicals, Tokyo, Japan) diluted with a mixture of monomeric MMA and catalyst (v/v:4/1). Twenty milliliters MMA was mixed with 0.75 g catalyst (55% benzoyl peroxide).

Protocol 2. Ten milliliters Mercox® was mixed with 1 g catalyst (Aharinejad *et al.*, 1993).

For casting cattle lungs, a segment of the lung was obtained immediately after the animal was killed in the Department of Anatomy, Veterinary University of Vienna. Human hearts were obtained from fresh cadavers within 6-24 hours after death. Same lavage and casting procedures were applied as described above. We used 120 ml lavage medium and 40 ml Mercox® for cattle lungs and human hearts.

Animal bodies, cattle lungs, and human hearts were left at room temperature for 2 hours, then put in a 60°C water bath overnight. The organs were isolated and then macerated in a 5% KOH solution at 40°C for 8 hours, before the maceration solution was renewed and maceration was allowed to continue for another 2 days. Then, the specimens were rinsed with tap water for 30 minutes, followed by cleaning in 5% formic acid solution at room temperature for 30 minutes, and several passages of distilled water, 2 minutes each. Casts were frozen in distilled water and then freeze-dried. The specimens

Figures 1-6. Casts prepared with MMA-diluted Mercox®. **Figure 1.** Sectioned microvascular cast of the rat lung. A large artery is cut longitudinally in the center of the micrograph (asterisks). Note that the artery is homogeneously filled with resin and that its surface is smoothly outlined. The surrounding capillary networks (bronchial beneath the artery, and alveolar atop it) have a regular form. Bar = 500 μm. **Figure 2.** Isolated cast vein from the rat lung. At the right side of the micrograph, the relief of sphincters (large arrows) and endothelial cell nuclear cells (small arrows) can be seen. However, at the left side, the cast surface is flattened and no vascular wall reliefs can be identified. Bar = 50 μm. **Figure 3.** Cast microvascular bed of the rat exocrine pancreas. Note the venule which exhibits a fine nodular surface (asterisk), while the surrounding capillaries are smoothly outlined. Note that at the merging site of a capillary with a venule, the cast is partially broken and seems to be empty (circled). Bar = 50 μm. **Figure 4.** Isolated cast vein from the rat lung. The surface of the cast is rough, but the relief of a sphincter (arrows) can still be identified. Bar = 25 μm. **Figure 5.** Cast microvascular bed of the rat exocrine pancreas. Note the artifacts on the surface of two vessels (arrows). A capillary is flattened (small arrowhead), on the surface of another one, leakage of resin is seen, where the resin is inhomogeneously polymerized (large arrowhead). Bar = 50 μm. **Figure 6.** Cast rat lung. A large vein is cut in the center of the micrograph, exposing the inhomogeneous internal structure of the vessel. The surface of surrounding capillaries are rough. Note that the structural details of the organ can still be identified. Bar = 125 μm.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were coated using evaporated carbon for 3 seconds and then sputter-coated with gold for 600 seconds (Aharinejad *et al.*, 1989, 1990). The cast preparations were mounted onto specimen stubs with silver paste using the conductive bridge method of Lametschwandtner *et al.* (1980). Some of the cast specimens were frozen in distilled water and cut at -20°C with a specially adapted circular saw into 1-2 mm thick slices (Aharinejad *et al.*, 1991; Aharinejad and Lametschwandtner, 1992). Observation was performed with a Cambridge 90 M SEM operated at an accelerating voltage of 10 kV and working distance of 60 mm.

Light Microscopy

After injection of Mercox® and polymerization of the resin, small blocks were obtained from the lungs and intestines. The specimens were immersion fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.4, for 2-4 hours, washed in the same buffer and postfixed in 1% osmium tetroxide for 4 hours. Cast tissue blocks were dehydrated in a series of ethanol solutions with graded concentrations of ethanol, and then embedded in Spurr's medium. In addition, small pieces of cast preparations were immersed after maceration, in 1% osmium tetroxide for 4 hours and embedded in Spurr's low viscosity medium. Sections were cut on a Reichert OmU2 ultramicrotome at 1 μm thickness. The sections were stained with alkaline Toluidine blue O. Specimens were observed with a Leitz Aristoplan microscope.

Results

We considered following structures or blood vessels as artifacts. **1.** Surface morphology: not smooth, porous or nodular, absence of endothelial or muscular cell imprints. **2.** Cast form: not cylindrical or oval, flattened or partially collapsed, partially empty casts (incomplete filling with resin). **3.** Leakage of resin. **4.** Internal structure of cast: not smooth, porous. **5.** Combination of these criteria. These criteria were applied to casts prepared both with MMA-diluted and undiluted Mercox®.

SEM of casts perfused with MMA-diluted Mercox®

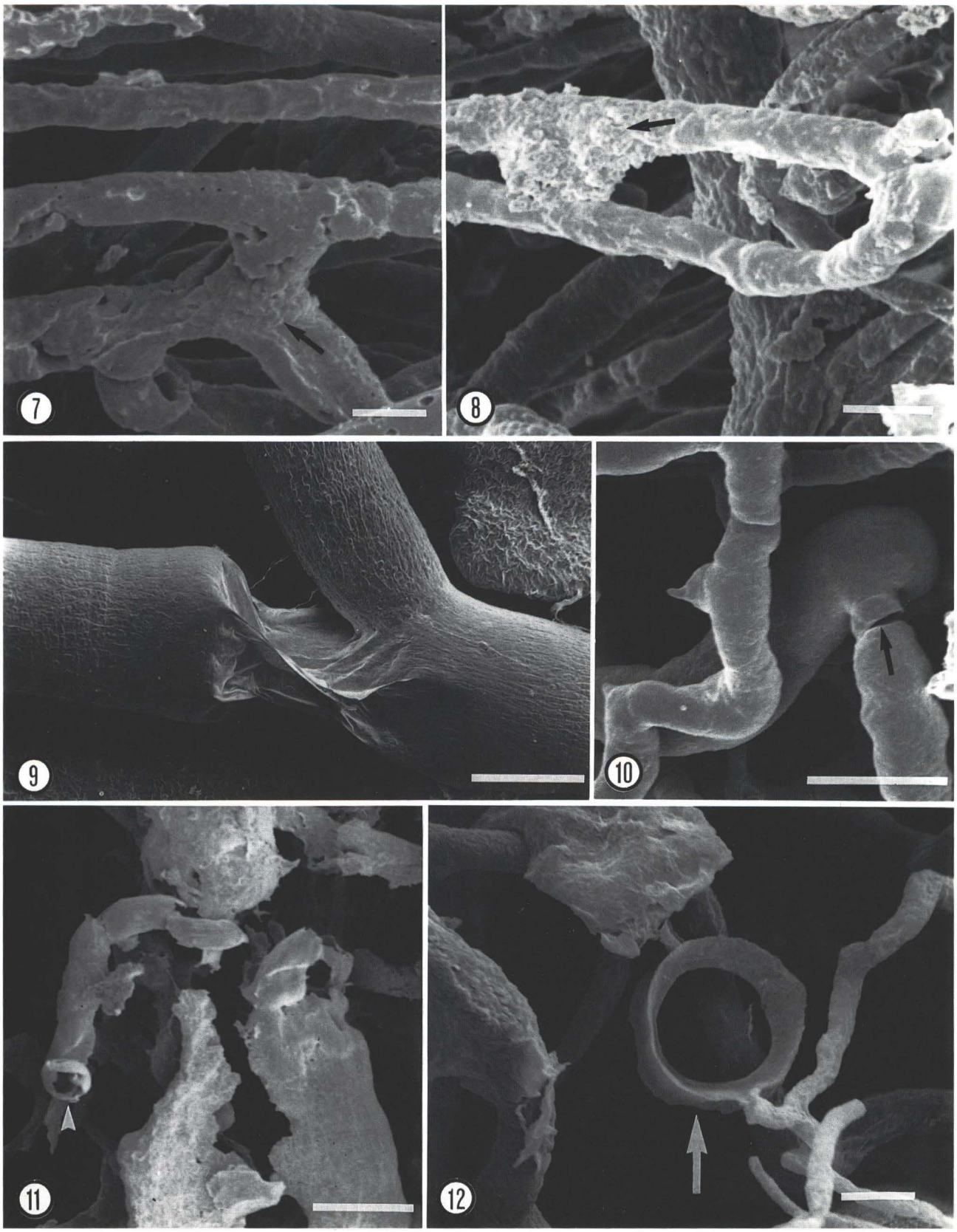
Casts with no artifacts in or on the surface of a large vessel, and with no or few artifacts in the surrounding microvasculature. The surface of the majority of casts prepared with MMA-diluted Mercox® had no artifacts. The internal structure of individual cast vessels, as viewed in sectioned casts, was also in most of cases homogeneous (Fig. 1). Normally, if a large vessel was found which revealed a smooth surface and a homogeneous internal structure, then the surrounding microvascular cast was also of a good quality and artifacts were rare in these areas. The area surrounding a large cast vessel without or with few artifacts was approximately 1,500 X 1,500 μm (Fig. 1).

In casts with otherwise fully regular vessels, only a segment of a large vessel showed partially a porous surface. Such casts were rarely seen. The circumference of the cast could be divided in two segments. On half the circumference, the surface was more or less well cast and showed the endothelial cell nuclear imprints. Even the relief of the smooth muscle cell sphincters were identified. The other half of the vessel circumference was, however, flattened and revealed neither the endothelial cell nuclear imprints nor the indentations of smooth muscle cell sphincters (Fig. 2).

Casts with artifacts on parts of a large vessel but with no artifacts in the surrounding microvasculature. Sometimes, a large vessel, preferentially veins, was found which showed a fine nodular surface; however, the surrounding capillaries were devoid of artifacts. In such cases, the cast surface became gradually smooth as the diameter of the vessel decreased (Fig. 3). One got the impression that the large vessel could be distinguished from the surrounding capillaries by its nodular surface.

Casts with artifacts on the whole surface of a large vessel but with no artifacts in the surrounding microvasculature. These vessels revealed a totally inhomogeneous internal structure and their surface was rough and nodulated. The vascular wall components were partly replicated, i.e., smooth muscle cell sphincter reliefs, but imprints of endothelial cell nuclei were not replicated (Fig. 4).

Figures 7-12. Casts prepared with MMA-diluted Mercox®. **Figures 7 and 8.** Human cardiac microvascular bed. The cast's surface is totally rough. The internal structure of the casts comprises large or fine conglomerates of resin (arrows). Bar = 10 μm. **Figure 9.** Cast artery from the rat small intestine. The surface of the cast is smoothly outlined and reveals the endothelial cell nuclear imprints. Note the collapsed segment of the branching site of the vessel. Bar = 100 μm. **Figure 10.** Microvascular bed of the rat exocrine pancreas. The vessels are well perfused, but at the merging site of a capillary into a venule (arrow), the cast capillary seems to be empty. Bar = 25 μm. **Figures 11 and 12.** Cast human cardiac microvasculature. **Figure 11.** Vessels can only rudimentarily be identified and are often empty (arrowhead). Bar = 50 μm. **Figure 12.** In other parts, cylindrical vessels are missing and, most probably, merely a part of the cast vessels is present as a ring (arrow). Bar = 10 μm.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Casts with artifacts on the surface of one or more large vessels with few capillaries surrounding them also showing artifacts. The lumen of large vessels was homogeneously filled with resin, however, their surface was sometimes porous, and in circumscribed areas, leakage of the casting material was seen. Capillaries were most smoothly outlined but some of them were flattened; or an extravasation was seen at their branching or joining sites (Fig. 5).

Casts with artifacts on the surface of a single large vessel and many artifacts in the surrounding microvasculature with still some smoothly outlined capillaries. The internal structure of the cast large vessel was porous. Capillaries had a wrinkled surface and their interior was often porous (Fig. 6).

Casts with artifacts on both the surface of all large vessels as well as on the surface of the majority of capillaries, but with still some regular capillaries. In these cases, the resin revealed an inhomogeneous internal organization, composed of conglomerates. As the lumen of vessels were only partially filled with these conglomerates, it was readily possible to get insight into the cast interior structure. The surface of the vessels was correspondingly rough. Small holes, 1-2 μm in diameter, were regularly found in such casts (Fig. 7). In circumscribed areas, the casting material extravasated and formed fine conglomerates which changed into rough ones, following the course of the same vessel (Fig. 8).

Casts with otherwise fully well replicated details but with a circumscribed, collapsed segment of the vascular cast. In these vessels, the endothelial cell nuclei were well replicated, and, beside the collapsed segment, they were well perfused with the casting medium (Fig. 9). In some casts, vessels were broken at circumscribed areas. In these regions, casts impressed as empty cylinders (Fig. 10, also see Fig. 3).

Casts with many artifacts; capillaries partially cast, and large vessels only partially perfused. Such specimens showed numerous artifacts, the microvascular organization of the organ under consideration could hardly be identified. Vessels only partially occurred as cylindrical structures, were empty and the relief of endothelial cell nuclei or other vascular wall structures could not be identified on their surface (Fig. 11). In other regions, large vessels were rudimentarily cast. These regions often showed only a part of the larger vessels, present as solid rings (Fig. 12).

SEM of undiluted Mercox®-perfused casts

Casts of a high quality with no artifacts. Most of casts prepared with undiluted Mercox® had no artifacts. (Fig. 13).

Figures 13-18. Casts prepared with undiluted Mercox®. **Figure 13.** Rat lung microvasculature. No artifacts are present. Bar = 100 μm. **Figure 14.** Rat lung microvasculature. Note that a large artery with a smoothly outlined surface, shows many sites which seem to be empty (arrows). Bar = 1 mm. **Figure 15.** An artery from the rat lung which merely consists of an envelope. Endothelial cell nuclear imprints are replicated (arrows). Bar = 125 μm. **Figure 16.** A vein from the rat lung. Also this vein only consists of an envelope and the relief of endothelial cell nuclei are replicated (arrows). Bar = 25 μm. **Figure 17.** A vein from the cattle lung with a fine porous surface. Multiple sphincter indentations are present. The cast internal structure is granular (arrow). Bar = 50 μm. **Figure 18.** Cast human cutaneous tissue. A valve is replicated on the surface of a vein (arrow), but the neighboring vein shows no structural details of the vascular wall. The latter vein is composed of fine resin conglomerates. Bar = 25 μm.

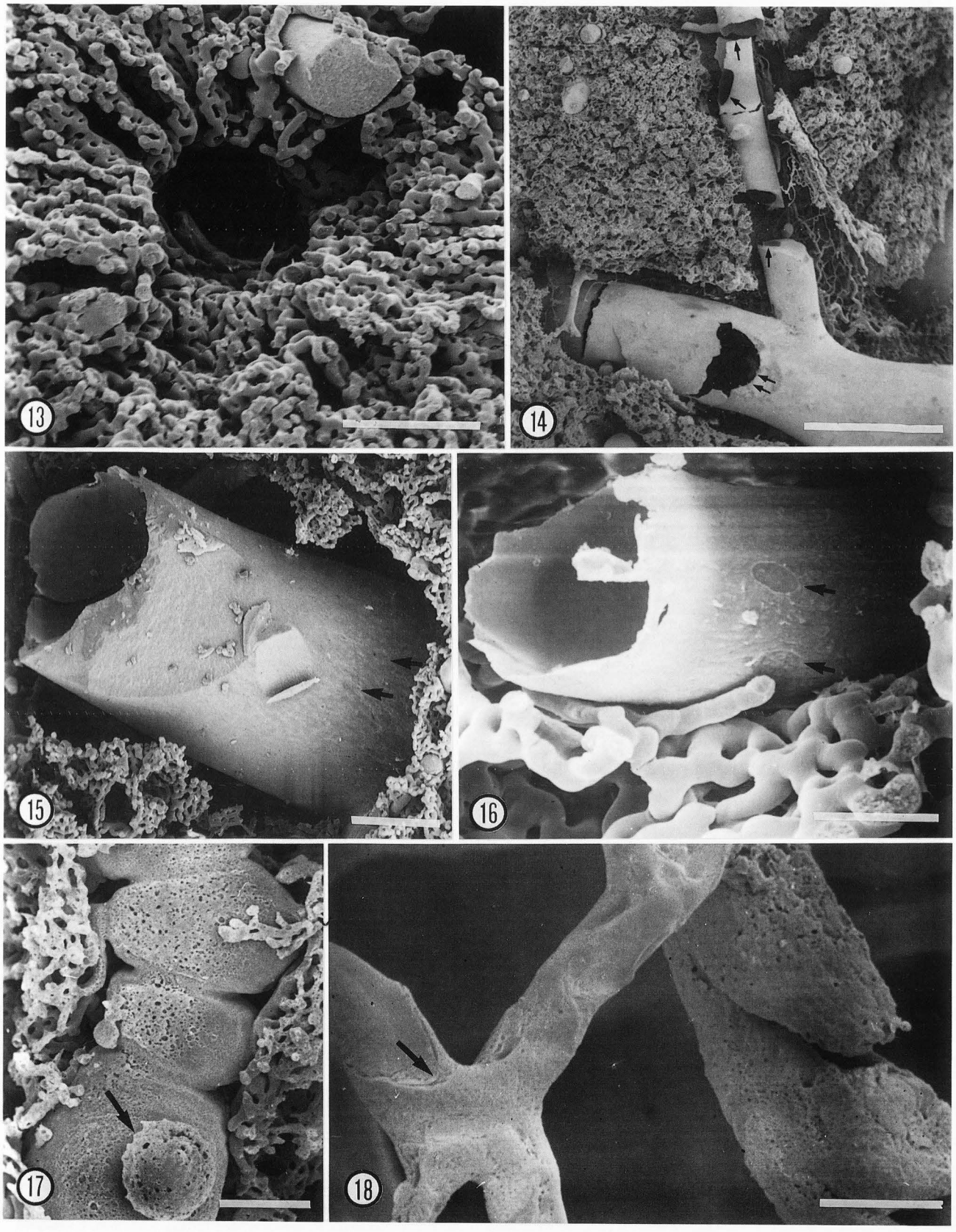
Specimens with otherwise well cast vessels but showing large empty vessels. These vessels were over longer distances devoid of other forms of artifacts described above and showed only in circumscribed areas, sometimes in regular intervals, fractured sites due to the partially incomplete filling of the vascular lumen (Fig. 14). Both arteries and veins showed this phenomenon. The surface of such vessels showed the elongated endothelial cell nuclear imprints arranged along the long axis of the vessel, characteristic for arteries (Fig. 15), or the roundish endothelial cell nuclear reliefs without any recognizable arrangement, characteristic for veins (Fig. 16).

Casts with a fine porous surface both on large vessels and capillaries. In these casts, the endothelial cell nuclear imprints and the relief of the vascular wall smooth muscle cells could be clearly identified (Fig. 17).

Casts with vessels which were partially provided with a rough surface and an inhomogeneous internal structure. Such vessels were only seen in material obtained from the human flaps. They did not show the endothelial cell nuclear imprints or reliefs from other vascular wall elements. Nevertheless, they were accompanied by relatively smoothly outlined surface, which showed the imprints of vascular wall structures, e.g., valves (Fig. 18).

Light microscopy

In unmacerated intestinal casts prepared with diluted Mercox®, it was readily possible to follow an individual villus, and judge on the filling of vessels from the bottom of a villus toward its tip. Sectioned specimens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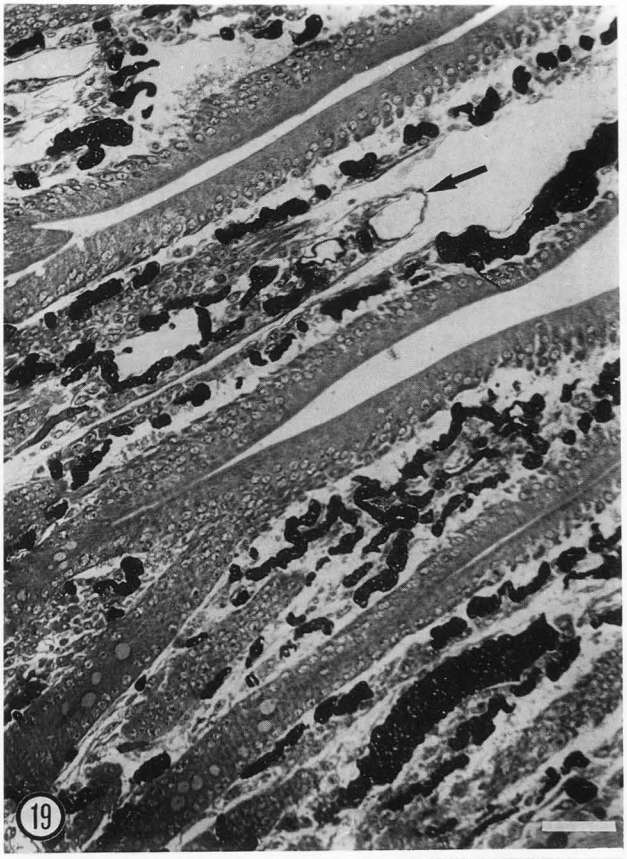
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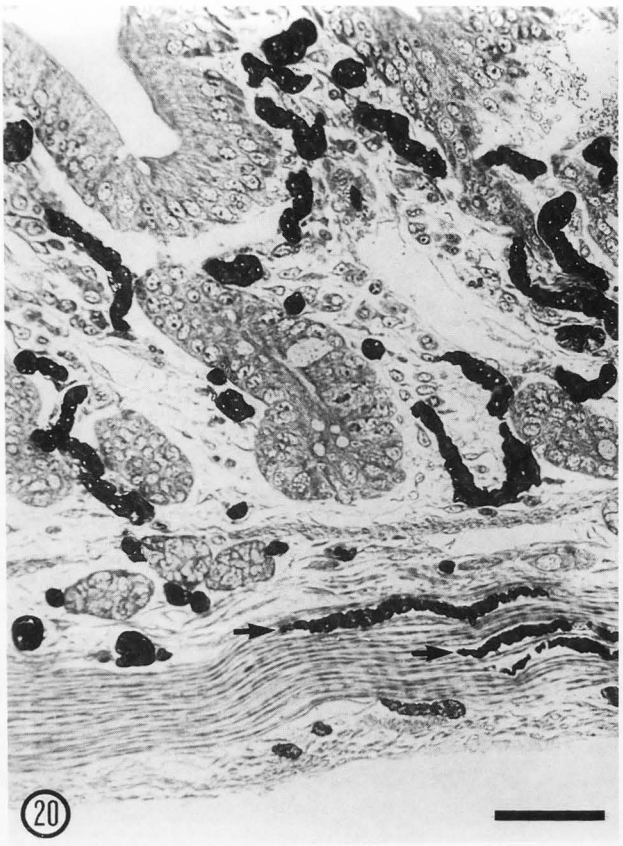
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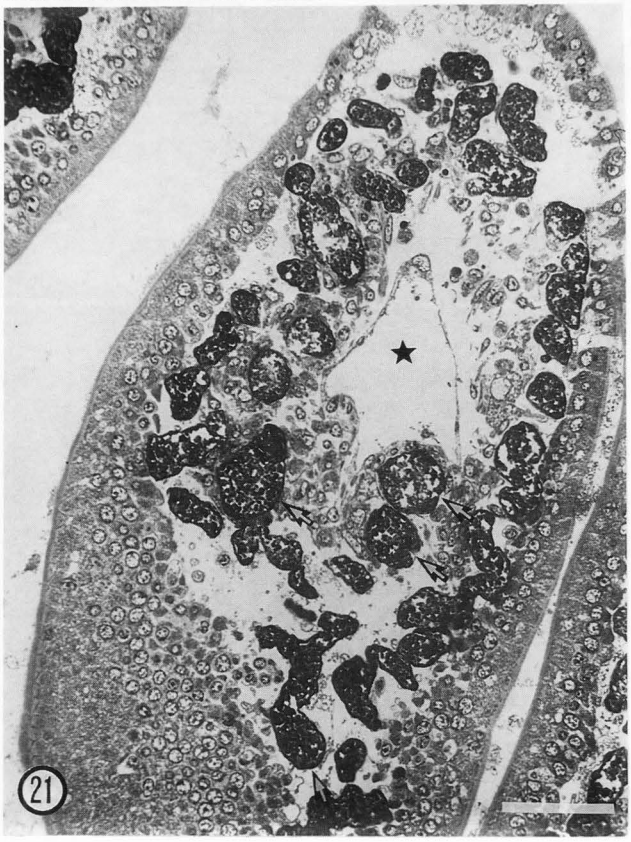
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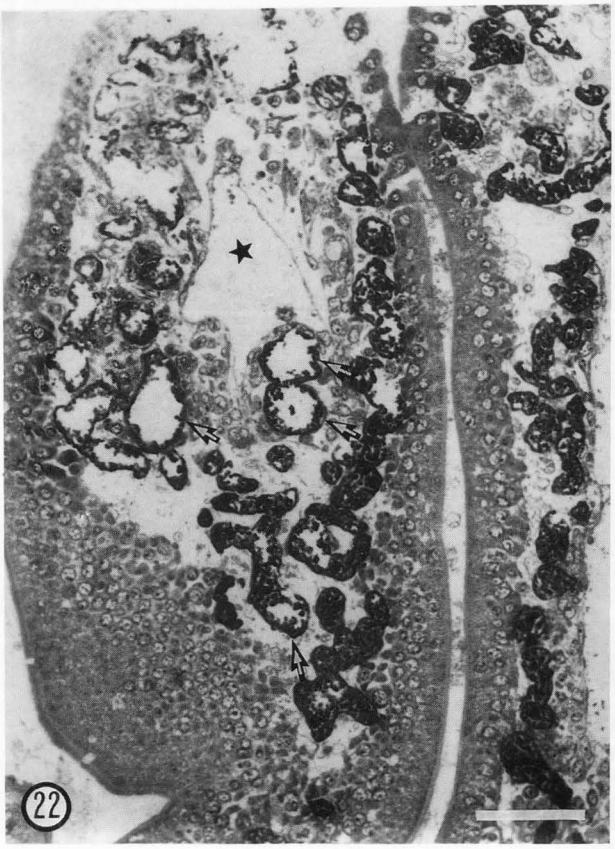
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Figures 19-22. Figure 19. Light microscopic images of sectioned cast rat intestines, MMA-diluted Mercox®, unmacerated. Vessels are homogeneously filled with resin. Sometimes, at the tip of a villus, vessels are partly cast and comprise a thin envelope (arrow). Bar = 50 μm. **Figure 20.** Also this image shows completely filled vessels. Note that the vessels in the muscular layer have a cork-screw shape, indicating the contracted condition of the muscle (arrows). Bar = 50 μm. **Figure 21.** In this micrograph, vessels are completely cast (arrows) at the bottom of the villus but incompletely filled toward the tip. A central, unperfused lacteal is marked with asterisk. Bar = 50 μm. **Figure 22.** This image shows the consecutive section of the same area shown in Figure 21. Those vessels which are completely cast in Figure 21 but incompletely filled in this image are shown by arrows. Some incompletely cast vessels are surrounded by an envelope which is, however, thicker than that shown in Figure 19. Moreover, the inner surface of such envelopes is not smoothly outlined. Bar = 50 μm.

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showed that the majority of casts were homogeneously filled with the resin (Fig. 19). The surrounding tissue appeared normal. Sometimes, however, some vessels were only partially cast in that they revealed a thin rim, obviously formed by the resin. These vessels were preferentially located at the end of an individual villous microvascular bed (Fig. 19). At higher magnification, muscular vessels were found which showed a wrinkled surface characteristic for cork-screw-shaped muscular capillaries (Fig. 20). Beside these forms of cast vessels, some specimens revealed casts the lumen of which was partially filled with resin conglomerates. The number of these vessels increased from the bottom toward the tip of a villus (Fig. 21). Another type of cast vessels had a rim of resin thicker than that shown in Figure 19. Moreover, the rim's surface was not smoothly outlined but was covered with fine resin granules (Fig. 22). The lumen of these vessels was better perfused than those shown in Figure 19 but less than those shown in Figure 20. Also, the number of such vessels increased toward the tip of the villus. In other specimens, we found vessels with a thick, smoothly outlined surface closely related to otherwise well perfused vessels (Fig. 23). Along the course of a villus, rarely some vessels occurred in which partly and fully perfused segments changed into the other (Fig. 24).

Macerated lung specimens showed differently perfused casts. Some vessels were almost homogeneously filled with the casting medium, whereas others, seen in the same section, were not homogeneously filled (Fig. 25). Both types of vessels revealed a delicate rim. At higher magnification, it became clear that the thickness of the rim varied along the vascular circumference (Fig. 26). Moreover, the rim was occasionally discontinuous, sometimes with a larger (Fig. 27) and sometimes with a smaller gap (Fig. 28). Erythrocytes were occasionally interspersed in the casting medium (Figs. 27, 28).

Discussion

Our observations on sectioned polymerized casting material, both prepared with undiluted and MMA-diluted Mercox®, reveal sometimes an internal granular structure of the casts. In SEM of corrosion casts, where the casts are sometimes fractured, a correlating internal granular composition can be seen (Aharinejad and Böck, 1993a). When comparing fractured surface of casts prepared with diluted or undiluted Mercox®, such internal granular structure is more pronounced when diluted Mercox® is used. However, in both cases, the external surface of the casts appears smooth in SEM. This surface is produced by a rim of more compact polymerized resin as shown in tissue sections. This observation shows the possibility that the internal structure of well prepared casts may be inhomogeneous (Aharinejad and Böck, 1993a,b). In other words, although a smooth surface of cast can be seen in SEM, the organization of the polymerized resin inside the vessel might not necessarily be homogeneous.

The mentioned inhomogeneity reaches different stages. It may result in casts which are empty and merely consist of a rim described above. Investigation with SEM may show a normal appearance of such segments, while sometimes, "collapsed" segments of vascular casts are observed. This phenomenon can be understood when considering the possibility of incompletely perfused tubular casts. As shown in LM of sections, in some vessels, perfused and unperfused segments changed into the other. In this manner, where the vascular lumen is partially perfused, the cast can appear collapsed. Such forms of casts were more frequently seen in casts prepared with MMA-diluted Mercox®. Collapsed casts may distinctly occur. As a consequence, in SEM, normal and shrank appearance of casts may be observed along the vascular course. The occurrence of the rim described above indicates that it is made up of a hydrophilic compound of the casting material. When casts show a rough surface in SEM, the external rim is evidently missing and the granular internal organization is consequently exposed.

All described phenomena of varying internal composition of polymerized casting material may be caused by phase separation of the casting resin during perfusion. It is assumed that phase separation takes place as resin flows through the vessels. It is most prominent in most peripheral segments. Centrally located arteries are more

closely positioned to the perfusion site and therefore, constantly receive new injection material. In this manner, casting material is constantly renewed and material which shows phase separation is shoved toward the periphery. Peripheral capillary segments receive therefore, less and less new resin bolus because of decreasing perfusion volume due to the larger luminal total capacity of capillaries compared to that of their supplying arteries. Moreover, the capillary bed receives that part of perfusion resin which had the longest time to allow phase separation. This mechanism can be convincingly demonstrated in intestinal villi. The tips of the villi constitute the terminal vascular bed, and accordingly show more frequently artifacts, while at the base of the villi the vessels were mostly filled with resin. All these complications may have two reasons: (1) The desired short polymerization time; and (2) the small amount of casting resin which can be perfused within short time. Both factors are closely interrelated. Within a given short period of time only a small amount of casting resin can be perfused by a given perfusion pressure. Therefore, the terminal vascular bed is not perfused with repeatedly renewed resin but only filled with a single bolus or not cast at all. Phase separation primarily takes place in these peripheral vascular segments and the artifacts described will be found more frequently there.

Another interesting aspect of artifacts is that they also more frequently occur in human material when MMA-diluted Mercox® is used. We perfused the human scapular flaps with undiluted Mercox®, whereas the human hearts were perfused with MMA-diluted Mercox®. This phenomenon shows that the postmortem thrombi, which mechanically occlude the vascular lumen, cannot explain this difference. We may conclude that phase separation of MMA-diluted Mercox® is potentiated in post mortem tissues. But this point needs further analyses.

The key question which arises is how to avoid these artifacts. One way would be to repeatedly perfuse a definite vascular bed until a complete perfusion and filling of all segments is reached (Ohta *et al.*, 1990, Okada *et al.*, 1993). This procedure needs prolongation of polymerization time and increased amounts of casting resin. Another possibility would be to perform prefixation of the microvascular bed, which decreases the permeability of the vascular wall. But we should not overlook the fact that prefixation, at least partially, only preserves endothelial cells from being penetrated with an unidentified component of MMA-diluted Mercox® (Aharinejad and Böck, 1993a,b). Therefore, the cause of phase separation should be avoided first. In other words, we may not prefix vessels because we know that the subsequent procedure, i.e., casting with MMA-diluted Mercox®, will cause artifacts. We may rather use undiluted Mercox® from the beginning because phase separation does not occur, and therefore artifacts are less frequently seen. In addition, this procedure minimizes expense.

Figures 23 and 24. Light microscopic sections of cast rat intestine, perfused with MMA-diluted Mercox®, unmacerated. **Figure 23.** Note that two large vessels (asterisks) are surrounded only by a smooth-surfaced envelope; other vessels do not. Bar = 50 μm. **Figure 24.** A longitudinally sectioned vessel is partly completely and partly incompletely filled with Mercox® (white arrows). Black arrows mark lymphatics which contain delicate resin granules. Bar = 50 μm.

Figures 25-28. Sections of cast rat lung, perfused with MMA-diluted Mercox®. **Figure 25.** Overview shows that the vessels are either homogeneously (long arrows) or inhomogeneously (short arrows) filled with resin. Bar = 50 μm. **Figures 26-28.** At higher magnification, erythrocytes (arrows) become visible which are interspersed inside the lumen. **Figure 26.** A delicate rim of resin surrounds almost the whole circumference of the vessel. The lumen is, though inhomogeneously, completely filled with resin. Bar = 20 μm. **Figure 27.** This vessel shows a large gap within the rim which surrounds it. Bar = 20 μm. **Figure 28.** The rim of resin encircling this vessel has a small gap. Interestingly, the resin inside the lumen is firmly attached to the rim and did not leak out of the vascular lumen. Arrows mark erythrocytes in Figures 27 and 28. Bar = 20 μm.

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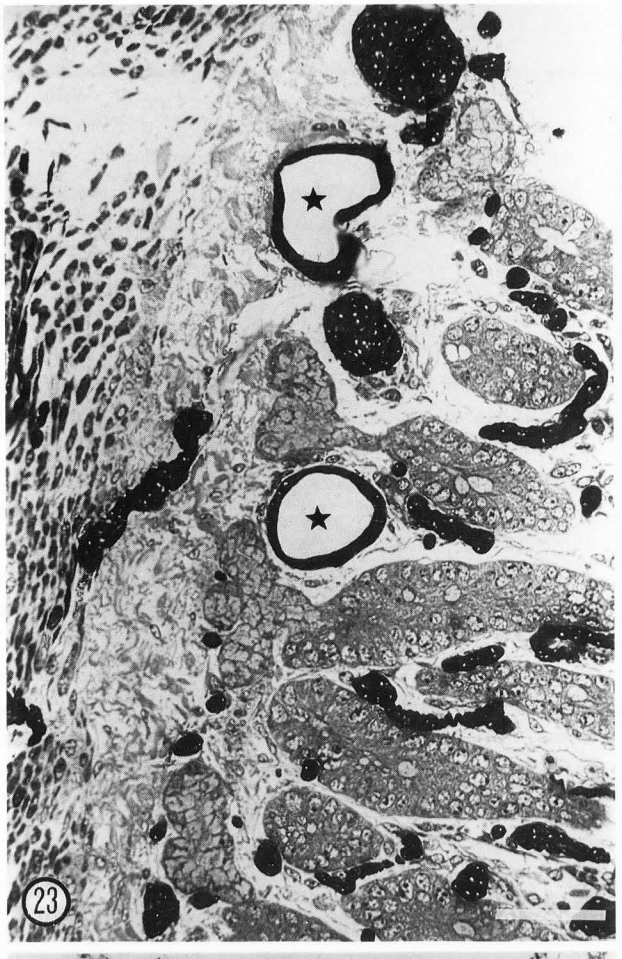
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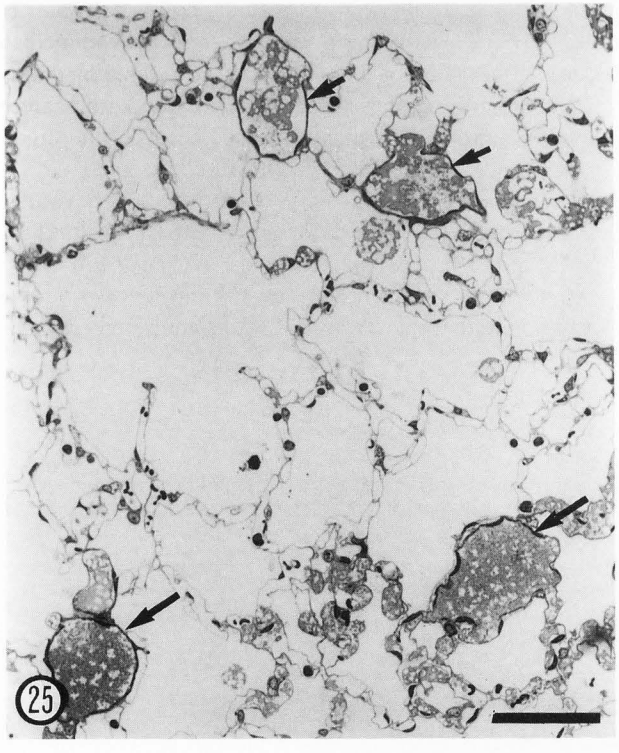
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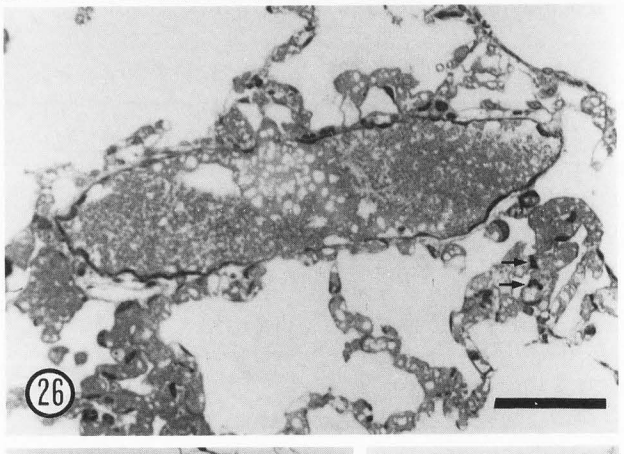
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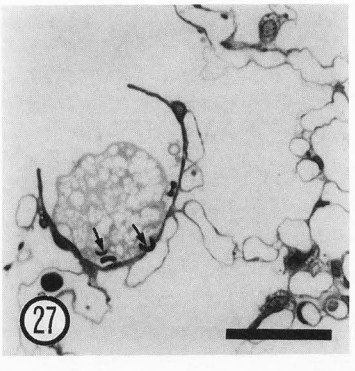
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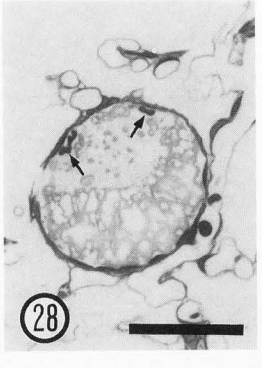
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Discussion with Reviewers

A. Castenholz: Did you apply a resin with other physical and chemical properties than Mercox® and compare the results concerning the artifacts?
Authors: We are comparing three other resins at the moment. These studies are not finished yet.

A. Miodonski: Do you think the described gaps within the superficial rim of resin (Figs. 27 and 28) are true features (if so, we probably could expect leakage of resin into the tissue), or they could be produced during sectioning with a microtome (I think that such a material is rather hart and therefore brittle).
Authors: The casting material could have leaked out of the vascular wall, and this material could be lost during maceration. Therefore, we can not exclude the leakage possibility. It appears rather unlikely that sectioning might have caused these gaps, because the sections cut are very thin (1 μm), and second because the cast is compactly surrounded by the Spurr medium. But we cannot conclusively exclude this possibility too. Finally, it might be possible that the vascular lumen is partially filled (partially filled lumen and partially formed rim) and the resin inside the lumen is firmly attached to the rim.

Y. Ohta: I would like to propose that the following medium to be mixed with Mercox®: methylmethacrylic acid + 10% benzoyl peroxide heated slowly in a water bath up to 80°C, kept for 5 minutes, and then immediately cooled in ice bath (Ohta *et al.*, 1990). This medium results in an oligomer, which, once mixed with Mercox®, will not produce artifacts. This is because the oligomer can hardly be separated from Mercox®.
Authors: We do not have experience with your proposed mixture. This is because heating the mixture of MMA and benzoyl peroxide to 80°C may cause explosion. At the moment, we are examining casts provided by you and prepared according to your suggestions.