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QUANTITATIVE MEASUREMENT FROM VASCULAR CASTS

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Abstract

A review of quantitative measurements show casting materials shrink from 0.2 - 20% and have viscosities ranging from 1.4 - 100,000 centipoise. Blood vessels have highly variable mechanical properties. Some microvessels are very stiff having little change in dimensions with pressure. Larger vessels generally change diameter significantly but show highly variable changes in length with pressure. Perfusion fixation does not fix the dimensions of blood vessels. Dog carotid arteries well fixed with glutaraldehyde at physiologic dimensions retain ≈20% of their elastic recoil circumferentially and ≈ 30% longitudinally. We recommend vascular casting as a method of accurately measuring the vasculature if care is taken to use low shrinkage casting resins and maintain physiologic transmural pressures for the duration of any casting procedure, even if prefixation is used. We measured a ≈10% error in our method of measuring both the size and location of periorificial atherosclerotic lesions from aortic casts. Little is known about how vascular smooth muscle tone changes during casting.

Key Words: Scanning Electron Microscopy, Vascular Casts, Shrinkage, Vascular Distensibility, Glutaraldehyde Fixation.

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Introduction

Vascular corrosion casting has been used extensively to determine vascular anatomy and morphology. The use of vascular corrosion casting have been reviewed by Hodde and Nowell (1980), Lametschwandtner (1984),Schraufnagel (1987). By comparison, quantitative studies of vascular casts are few and recent. They include measurements for the purpose of modeling the vasculature (Zamir et al, 1983; Zamir and Chee, 1987; Potter and Groom, 1983; Canham et al, 1984), measurement of pathologic change (Gatton and Sale, 1986; Zeindler et al, 1989) and measurement of endothelial cell impressions related to atherosclerosis (Kratky and Roach, 1983; Cornhill et al, 1980) and blood flow (Legg and Gow, 1982).

In this review we discuss some of the factors affecting quantitation. In the process we present some previously unpublished data on shrinkage of casting materials and dimensional changes of arteries as well as error measurement in our method of measuring atherosclerotic lesion location on the surface of casts.

The first step is making an accurate replica of vascular lumina. Factors affecting the dimensions of cast vessels include; shrinkage or distortion of the casting material, changes in the dimensions of vessels due to inappropriate perfusion/transmural pressures, and active changes in vessel dimension due to cellular elements (endothelium, smooth muscle). Measurements of vascular variables such as lengths, diameters, branching geometry, and surface features such as cell dimensions have been made from these casts.

In all cases measurements are made from a two dimensional image which is projected from the 3D object by means of light optical methods or scanning electron microscopy. No analysis of errors for any of these methods has been presented in the literature. We present an analysis of error for our own method and discuss sources of error.

General Properties of Materials

Methacrylate based plastics (acrylics) are the most common polymers used for vascular corrosion casting today. The liquid resins come in a wide range of viscosities depending on the degree of pre-polymerization (Table 1). The solid resin resists the basic or acid macerating solutions, though the surface of the cast can be degraded after extended times in the more concentrated solutions (Weiger et al., 1986). The acrylic casts do not appear to deform though brittleness can be a problem.

Silicone rubbers have been used to cast large arteries, specifically because of their low shrinkage (Legg and Gow, 1981; Rayman et al,1985). They are highly viscous but can be thinned sufficiently to fill the microvasculature (Nowell and

Table 1. Shrinkage of Casting Materials on Setting and Initial Viscosities

Material	% Volume Shrinkage on Setting	Method	Source	Viscosity centipoise (cp)	Method	Source
Methacrylates						
Mercox Cl-2B	8.0±0.8	Density (Pres.)	Weiger et al 1986	35 cp	Oswald	Weiger et al 1986
Mercox Cl-2B	9.0±0.5	Volume in Solid Container (Pres.)	*			
Mercox Cl-2R				53 cp	Oswald	*
Mercox Cl-2B +MMA (4:1 Vol)	12 ±0.8	Density (Pres.)	Weiger et al 1986	12 cp	Oswald	Weiger et al 1986
Batson's	6-10% linear	In Glass Tube (Atm.)	Zamir and Chee 1987	100 cp		Kardon and Kessel 1980
Batson's	13.4±0.4	Volume in Solid Container (Pres.)	Kratky and Roach 1984	520 cp	Oswald	*
Batson's				261 cp	TFR Rheometer	Nopanitaya et al 1979
Batson's +MMA (2.4:1 Vol)	20 ±0.7	Volume in Elastic Artery (Pres.)	Kratky and Roach 1984	71 cp	Oswald	*
Batson's +MMA (2.4:1 Vol)	15.8±0.4	Volume in Solid Container (Pres.)	Kratky and Roach 1984	71 cp	Oswald	*
Batson's +MMA (1.1:1 Vol)	16.0	Volume in Solid Container (Pres.)	Legg and Gow 1981			
Batson's + Sevriton (2.8:1 Vol)	17 ±0.7	Volume in Solid Container (Atm.)	*	69 cp 21 cp	Oswald TFR Rheometer	* Nopanitaya et al 1979
Poly MMA	17.8±0.8	Density (Pres.)	Weiger et al 1986	14 cp	Oswald	Weiger et al 1986
Poly MMA				4 cp	Oswald	Gannon 1981
MMA+HMA	20.4±0.8	Density (Pres.)	Weiger et al 1986	1.4 cp	Oswald	Weiger et al 1986
Tensol Cement #7	12		Bugge 1963	950 cp		Bugge 1963
Silicone Rubbers						
Reprosil	0.15		Legg and Gow 1981	10^5 cp		Legg and Gow 1981
JRTV (Dow Corning)	>0.5	Volume in Solid Container (Atm.)	*	80,000 cp		Dow Corning Sheet # 61-080A-76
JRTV + 200 fluid 20cs (1:0.16)	1.5	Volume in Solid Container (Atm.)	*			
Epoxies						
Epoxy QCl257 (Chemque)	5.0±0.4	Volume in Solid Container (Atm.)	*	790 cp	Oswald	*
Araldite	3.2 3.5% Linear	Density In Polyethylene Tube	Hanstede and Gerritis 1982a			
Metal						
Gallium	-3.2		Meiselman and Cokelet 1975	2.2 cp		Meiselman and Cokelet 1975

Abreviations: MMA- Methylmethacrylate, HMA- Hydroxypropylmethacrylate, Atm.- At atmospheric pressure, Pres.- At physiologic pressure.

* Previously unpublished data measured by the authors. Shrinkages were measured in 1 ml syringes using the scale on the syringe for lengthwise change and a series of calibrated wires were used to measure the gap between the solidified plastic and the syringe wall. Volume Shrinkage =(Initial Volume - Final volume)/Initial Volume. Viscosities of Batson's mixtures were measured without promoter added and Mercox without catalyst otherwise mixtures were complete. Viscosity was measured at 23° C with an Oswald viscometer calibrated with glycerine. Resins were mixed according to manufacturer's instructions or the literature reference.

Lohse, 1974; Sobin et al, 1962; Demis and Brim, 1965). On maceration in acid or base small vessels degrade, breaking up or deforming (Nowell and Lohse, 1974; authors' unpublished observation). Large vessels, macerated for a minimum time, are grossly unaffected but do not show endothelial cell impressions by SEM. Legg and Gow (1982) have shown arterial endothelial impressions on undigested silicon rubber casts.

Epoxies such as araldite, which has sufficiently low viscosity to fill the microvasculature, is corrosion resistant, and produces rigid casts (Hanstede and Gerritis, 1982a), are known for their relatively low shrinkage on setting. Rayman et al (1985) used an epoxy to produce a hollow cast for flow studies. Meiselman and Cokelet (1975) used Gallium for vascular casting, a metal which melts at 30°C and expands slightly on solidification. It has a high surface tension making filling of capillaries difficult and requires enzymatic digestion (noncaustic).

We have not included other materials, such as latex, for which we have no quantitative data on shrinkage.

Shrinkage of Casting Materials

The shrinkage of a casting material can vary depending on the physical environment. Kratky and Roach (1984) showed that a Batson's plus methylmethacrylate (MMA) mixture had 20 ± 0.7% volume shrinkage in an elastic container (artery) and 15.8 ± 0.4% in a solid container (syringe)(see Table 1). It is unknown if the area to volume ratio which is high for microvessels and low for large vessels affects shrinkage. Weiger et al (1986) measured the combined shrinkage of all the vessels of the rat, excluding the largest, by comparing the density of the liquid resin and the hardened cast. Their value of shrinkage, 8.0 ± 0.8%, is not apparently different from the measurement of 9.0 ± 0.5% which we made in a 1 ml syringe (see Table 1). This suggests that shrinkage is not greatly affected by surface area to volume ratio. We have observed that Batson's mixtures shrink during the entire setting time; when liquid, semi-solid, and during hardening, with the bulk, 82 ± 8%(SD), (from 4 measurements of Batson's plus MMA 2.4:1) occurring after the semi-solid point (unpublished data). Depending on whether shrinkage is measured in an open system, where casting material can flow in to replace volume lost during the early polymerization, or in a closed system, where shrinkage is measured on a fixed volume, one would expect different results.

The effect of shrinkage on dimensional measurement will depend on how the material shrinks. Kratky and Roach (1984) have shown that casts of well tethered segments of excised sheep femoral artery had 6 times as much shrinkage in diameter as in length while an untethered artery cast had similar shrinkage in length and diameter (volume shrinkage was the same for both cases). If a cast shrank isotropically then one could easily correct all dimensions by multiplying by a constant. For Mercox which shrinks 8-9% in volume one would increase all measurements by 3% linearly and for Batson's mixtures which shrink 15-20% in volume increase by 6-7% linearly.

No measurement of shrinkage anisotropy in vivo has yet been made. Gattone and Sale (1986) made measurements of kidney arteriole and glomerulus diameters in vivo compared to Batson's casts and reported no significant differences. However the measurement errors were greater than the 6-7% reduction expected due to shrinkage.

Viscosity of Casting Materials

The viscosity measurements in Table 1 are only a guide to the initial property of the casting materials. Viscosity can vary greatly with temperature (e.g., the viscosity of glycerine decreases by 56% from 20° to 25°C). These polymers likely display different viscosity with different shear rates (Non-Newtonian behaviour). Thus one instrument will measure a different viscosity from another. The properties of these materials may vary from batch to batch depending on the degree of polymerization or age. During casting the viscosity will be increasing so that no casting material will mimic the fluid mechanics of blood.

Table 2. Recoils* of Fresh and Glutaraldehyde Fixed Dog Carotid arteries when released from In Vivo Length and Transmural Pressure.

	Fresh	Fixed
Length	35 ± 9 % n = 11	10 ± 3 % n = 7
Diameter	52 ± 3 % n = 17	8 ± 3 % n = 8

*Means ± Standard Deviations calculated as (initial length - final length)/initial length

Vessel Dimensions and Transmural Pressure

The distending pressure at which a resin is injected is potentially the greatest source of variation in vessel dimensions. Lengths and diameters can more than double when pressures are raised from zero to physiologic values. Olson (1983) reported large qualitative changes in the dimensions of trout gill vessels cast at different pressures. We have measured the length of dog common carotid arteries in situ and after excision and calculated recoil (see table 2, unpublished data). These arteries were cleaned of nerve and vein, and cut into two lengths equivalent to 5-6 cm length in situ, cannulated, and placed in a saline bath with one fixed end and one end which could be adjusted to change the artery length. The arteries returned to their in situ length at between 100 and 200 mmHg transmural pressure. Output from a constant infusion pump and a pressure transducer were used to create pressure volume curves on an X-Y recorder while the carotids were cycled from -100 to +200 mmHg. The volumes were measured at 120 mmHg (systolic) and where the curve first reached 0 mmHg. Diameter and diameter recoil on reducing pressure from 120 to 0 mmHg were calculated (see table 2). Vessels such as the carotid which are well tethered do not change significantly in length with pressure (Patel et al; 1964), though length will change with posture. The canine aortic arch and pulmonary artery, which are poorly tethered, change length 5.3% and 11.6%, respectively, during the cardiac cycle (Patel et al, 1961; 1964). Some gut vessels are free to change length also. The dog aorta and pulmonary artery change their diameters 8.5% and 20%, respectively, during the cardiac cycle (Olson and Shelton, 1972; Shelton and Olson, 1972). The large central elastic arteries are known to be more distensible in diameter than large muscular arteries but both have similar Young's modulus in length (Petersen et al, 1960, Tanaka and Fung, 1974). Wiederhielm et al (1964) measured significant pulse pressures in the frog mesenteric microcirculation but observed no diameter changes. Lamport and Baez (1961) found no diameter change with pressure in rat precapillary vessels and little distensibility of arterioles in the physiologic range but vessels recoiled to about 50% of their diameter or collapsed at low pressures. Skalak and Schmid-Schonbein (1986) found the fully relaxed microvessels of the rat skeletal muscle were quite

stiff, with venules stiffest, capillaries intermediate and arterioles least stiff. Their data indicate that these vessels would experience 1 - 10% diameter reduction when pressures are dropped from physiologic to atmospheric values. They also report that the rat skeletal muscle microvessels are stiffer than the mesenteric microvessels measured by other authors in rat, cat, and frog. Veins are very distensible in the physiologic range, more so in length than in diameter, but are stiff at higher pressures. (Bocking and Roach, 1974; authors' unpublished data).

To produce accurate casts it is important to monitor the pressure in at least two locations in the system being cast. The pressures between any two locations can be reasonably extrapolated as if a single uniform fluid fills the entire bed given that one knows the physiologic pressure distribution in the vasculature.

Dimensional Stability of Perfusion Fixed Vessels

Fixation by aldehyde perfusion is used as an adjunct to casting or in alternate methods of preparing vessels. Before casting, perfusion fixation hardens delicate vessels (Nelson, 1987). Perfusion fixation is generally the first step in preparing uncast vessels for scanning electron microscopy (SEM), light microscopy (LM) or transmission electron microscopy (TEM). These can then be dehydrated and coated for SEM or dehydrated, then embedded in wax, epoxy, or methacrylate resins for sectioning, and TEM or LM.

Tindall and Svendsen (1984) reported that the in situ rabbit aorta expanded significantly (about 20% by our calculation) during two hours of pressure fixation with 2% glutaraldehyde solution. By contrast, Hirsch et al. (1981) reported (by different methods) that the in situ rabbit aorta contracted in diameter up to 20% during one to twelve hours pressure fixation with 2.5% glutaraldehyde. Most likely these diameter changes were caused by variation in the transmural pressure during fixation. Neither intra-aortic pressure or extra-vascular tissue pressure were measured in these two studies. Pressure fixation is known to cause edema (Lee, 1987, Hirsch et al., 1981, authors' unpublished observation) resulting in increased tissue pressures and reduced transmural pressure. When infusing rabbit aorta through the femoral artery Zarins et al (1980) found the infusion (reservoir) pressure was two to three times higher than the aortic pressure. We (Kratky and Roach, 1988) observed infusion pressures up to 80% higher than aortic during infusion of the rabbit aorta via the carotid even after blocking much of the microvasculature with microspheres. This pressure drop decreased during the two hour fixation due to increased peripheral resistance, likely due to edema.

After pressure volume (P-V) data were taken from the fresh excised dog carotid arteries (described earlier), the arteries (still held at in situ length) were fixed with 2% glutaraldehyde or 4% formaldehyde in isotonic phosphate buffer, ph 7.2, at 120 mmHg transmural pressure for 7 1\2 min., 15 min., 45 min., 3 hrs., 10 hrs., 1 day, or 3 days. The outside diameter of 6 arteries fixed in 4% formaldehyde for 3 hours or more was measured from photographs at the beginning and end of each experiment. No significant change in diameter was measured. Diameter increased by 0.01 ± 0.06 mm (standard error of the mean). We feel diameter changes during perfusion fixation will be negligible if transmural pressures can be held constant. There was no apparent change in the P-V curves from 3 hrs. on, thus the average recoil was calculated for fixation of 3 hrs. or longer (see Table 2). Diameter recoil was calculated as before and recoiled length with no transmural pressure and no lengthwise tension. These data show the dog carotid artery still has significant elasticity after glutaraldehyde fixation and would likely have a luminal volume reduction of approximately 24% on excision after 3 hrs. or more of careful perfusion fixation at physiologic transmural pressures.

Further reductions in tissue dimensions occur during preparation of tissue for microscopy. During freeze drying, Campbell and Roach (1983) showed purified elastin shrank 6.9% linearly, and Boyde and Franc (1980) showed fixed mouse liver shrank 7.3% linearly. These shrinkages were isotropic and associated with 20% volume reduction. Boyde et al (1977) found embryonic mouse and pig brain tissues shrank 30-50% by volume during critical point drying. Iwadare et al. (1984) reported 13% linear shrinkage (equivalent to 34% by volume) of bovine kidney and liver tissue during dehydration and paraffin infiltration. Some of this shrinkage would be reversed during the expansion that occurs when paraffin sections are floated onto slides. Hanstede and Gerritis (1982b) reported ≈ 8% linear shrinkage of fixed liver tissue during dehydration, infiltration, and polymerization of water-soluble resins. However the net change after processing sections onto slides was -1% to +4% change in linear dimensions, with slight anisotropy in the cutting direction vs. normal to the cutting direction. (Equivalent to 3% reduction to 12.5% increase in volume). The reduction in luminal dimensions of blood vessels on excision would be compounded with further changes during processing for microscopy.

Vascular Smooth Muscle Activity During Casting

There have been no direct measurements of the effect of casting materials on the vasoactive state of blood vessels that we are aware of. Motti et al (1987) reported that 3 out of 80 rat brain vascular casts had localized arterial constrictions which they postulated were caused by vasospasm in response to over-distension of these vessels during casting, though pressures were not measured. Olson (1980) cast the trout gill vasculature after preperfusion with one of three vasoactive agents: epinephrine, acetylcholine or nitroprusside. Qualitatively the distension of the central shunt vessels varied greatly as did the constriction of vascular sphincters, apparently showing three different vasoactive states. Schmidt et al (1983) found accordion pleated arterial vessels in norepinephrine contracted spleens, where none were found in distended spleens. These types of experiments suggest some portion of the preexisting vascular state is preserved during casting. Experimentally determining to what degree the vasomotor state is preserved would be difficult, especially since pressure effects would have to be accounted for.

Measurement from Casts

Measurement of surface contours of casts from stereo pairs of micrographs (stereophotogrammetry or stereometry) was applied by Cavanagh et al (1977) to measure diameters and volumes of gill vessels. Most authors have opted not to use stereometry presumably because specialized equipment is required (Boyde 1981; 1986) and the measurements are time consuming. Instead geometrical simplifications have been used, e.g., if segments of vessel are assumed cylindrical then the diameter can be measured from a micrograph taken at any orientation. If more information is required then mounting the specimen on a stage which has calibrated translation and rotation in all three dimensions, as Zamir et al (1983) has done, allows one to determine the axis of any vessel segment and view the vessel at right angles to be certain of the diameter even if it is elliptical. As well, angles of branches can be measured in three dimensions. Even stages with limited rotational and translational freedom can be used if the specimen is appropriately mounted (Kratky and Roach, 1983). Similar simplification is used to measure the size and morphology of endothelial cells from the surface of casts. If

Table 3. Measurement Error for measurement of Atherosclerotic Lesion Location (Polar Coordinates r,θ) and Size (Area) from rabbit aortic casts.

	Mean Radius (mm)	Error* r (mm)	θ (mm)	Mean Area (mm^2)	Error+ (mm^2)
Right Renal Map	3.13	0.32	0.31	13.7	0.83
Intercostal Map	0.81	0.070	0.071	1.0	0.14

*Errors are average standard deviations of the 10 positions coordinates (r,θ) measured 4 times.
+Errors are the standard deviations of the area measured 4 times.

the region micrographed is small compared to the vessel diameter then the region can be treated as a flat surface. (Kratky and Roach, 1983; 1987) Micrographs made normal to the surface can be measured directly, those at a measured tilt angle can be corrected by expanding the dimension in the direction of tilt by l/cos(Tilt Angle) (Kratky and Roach, 1987). When mapping the location of atherosclerotic lesion on the rabbit aorta around aortic branch junctions we (Zeindler et al, 1989) treated the aorta as a cylinder with circular branch orifices. A row of micrographs was taken of the central portion of aorta parallel to the aortic axis for each 30° rotation of the aorta around its axis. These micrographs were joined to form a map of the surface, with the orifice at the center and lesion visible around the orifice. Polar maps were made of the lesions with the center of the orifice as the center of the map. Lesion length versus polar coordinate location was measured and plotted. Lesion area and orifice area were also measured from these maps.

Error of Measurement for the Method of Mapping Lesions on Aortic Casts by SEM

We (Zeindler et al, 1989) made two assumptions in producing maps of the aortic surface: 1) the surface was locally flat, however some curvature was present at the edges of the micrograph, and 2) the aorta is cylindrical. This is not true near branch junctions. In addition errors can be introduced by the SEM. We have measured errors of up to 5% in the magnification, as determined by Philips SEM 501 micron markers, when compared to a calibration grid. At low magnification (<1000x) the edge of the photomicrograph will have lower magnification than the center. The CRT may introduce a distortion. The large focal depth of the SEM can also introduce error. In practice we focused at a high magnification to reduce the focal depth. The Philips SEM 501, which we used, calculates a magnification correction factor based on the focusing coil voltage. If an automatic magnification correction is not available a calibration curve can be made by measuring focusing coil voltage as a calibration grid is measured at different depths of focus. We usually move our specimen until the correction factor is 1.00.

When we made the composite scanning electron micrographs of the aortic surface, some mismatch occurred at the edges of the micrographs for the reasons outlined above. To quantify the error of measurement we produced the map of the 3rd right intercostal junction 4 times and the right renal junction 4 times, repeating all steps. The area of the lesion was measured on each map and the location of 10 points in the lesion at each of the two branch sites was measured in polar coordinates with the center of the orifice as r = 0, θ = 0 . Results of the error measurements are in table 3.

Conclusion

Our quantitative analysis of dimensional changes during preparation of blood vessels for measurements by other than in vivo methods shows vascular casting to be potentially as good or better than other methods. In vivo methods will have measurement error as well, but must be used as a standard against which other methods are checked. The evidence suggests vascular casts can produce very accurate reproduction of in vivo vessel lumina when: low shrinkage materials are used, pressures are carefully maintained at appropriate physiologic values, and vascular tone is maintained by casting quickly, perhaps even without preperfusion with saline or similar fluid as Potter and Groom (1983) have done. It would be worthwhile investigating the use of low shrinkage epoxies for casting.

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Discussion with reviewers

V.H. Gatton: The authors claim that perfusion fixation does not fix the dimension of blood vessels for their subsequent casting. Their conclusions are based on studies of aorta and carotid arteries, both of which are basically elastic vessels. However, they are extrapolating their findings to muscular arteries and arterioles which rely on cellular components for vessel stability rather than extracellular fibers. Wouldn't you expect that fixation prior to casting for quantitative measurements of muscular artery or arteriolar diameter would be useful or even necessary for obtaining accurate and reproducible data?

Authors: Perfusion fixation increases the stiffness of the vessel wall as we have shown for the dog carotid artery. The muscular arteries and arterioles are in general stiffer than the carotid before fixation but it is unknown how stiff they become after fixation. Prefixation would be an advantage when it is easy to maintain physiologic pressures, and thus physiologic dimensions, during fixation but difficult to do so during casting. Inappropriate filling pressures will have less effect on perfusion fixed vessels than on fresh vessels. There is insufficient data currently available to determine whether prefixation provides superior quantitative measurement from casts of muscular arteries and arterioles.

V.H. Gattone: Would you expect that shrinkage during polymerization to play a significant role in the quantitative measurements from small vessels?
Authors: This will depend on the experiment and on the choice of casting material. We would hope that quantitative data on shrinkage will be used by researchers in the design of future experiments.

A. Lametschwandtner: Would you please comment upon those factors in vascular corrosion casting which to your opinion up to now a) are optimized and b) deserve further studies in order to produce relevant quantitative data from corrosion casts of tissues and organs.
Authors: The viscosity of casting materials is probably the most optimizable factor with a large variety of resin mixtures of varying viscosity reported on in the literature. The setting rate of the resins is likely near optimal but not often discussed in the literature. How does setting rate affect cast quality? Do reports of incomplete filling imply that authors used a casting material with too rapid a rate of hardening? Corrosion resistance and hardness of casts are not much studied but are apparently quite adequate. Shrinkage of casting materials has been largely ignored until recently but is not large when compared to dimensional changes in other preparation methods. Possible vasoactive effects of the casting methods have not yet been studied. It would be difficult to measure active vascular diameter changes and separate these from passive pressure related diameter changes. However, some study in this area is needed.

A. Lametschwandtner: You consider it worthwhile to investigate the use of low shrinkage epoxies for casting. I think this task would be of interest in casting large dimensioned vessels only; capillaries and smaller calibers of arteries and veins would not get cast at all.
Authors: Hanstede and Gerritis (1982a) Have successfully cast the human liver microvasculature with the low viscosity epoxy, araldite. We are aware of no other authors who have used epoxy in vascular casting.

D.E. Schraufnagel: How would you estimate the capillary pressure during the casting of a whole organ in an intact animal?
Authors: We would measure the pressure in the afferent artery through which casting is done by cannulating a side branch and measure the pressure at the efferent vessel (vein) as well. If the entire organ is filled with casting resin, and the pressures at these two locations are maintained at physiologic values, then the capillaries will be near physiologic pressure as well. This method is only approximate since it assumes both fluids are Newtonian and flow is simple laminar flow following Poiseuille's law. The method only works while the resins are fluid. We have found as the material sets and flow stops these pressure measurements become unreliable.

E.D.F. Motti: Pigments (as far as I know they are particulate matter in a wide range of sizes) are commonly added to the casting mixtures. Did you investigate if the presence of pigments affects shrinkage on setting. I have had problems injecting the lowest cerebrovascular ramifications. When a particular mixture employed was adequate (being of the lowest viscosity as to obtain perfusion through the arterio-venous capillaries) it tended to produce "soft" "deformable" casts that tended to collapse. Later attempts to use the same mixtures with pigments produced more rigid casts. Could it be inferred that pigments affected differentially viscosity and the rigidity of the cast?
Authors: We have not studied the effect of pigments on viscosity shrinkage or hardness of casting materials. When you have difficulty filling part of the microvasculature, have you tried increasing the working time of the resin by reducing the amount of the setting agent in the mixture?