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Orbital and Ocular Micro-Vascular Corrosion Casting in Man

JANE M. OLVER[1,2] and ALISON C. E. McCARTNEY[2]
London

Summary

The methods of preparation and examination of complete orbital and ocular vascular casts, suitable for the study of anterior segment vasculature, are described from our experience of 20 casts. The use of low viscosity methylmethacrylate produced complete vascular filling with few artefacts when injected into isolated orbital preparations from human cadavers 36–48 hours post-mortem, despite suggestions by previous authors that injection should be within 12 hours.

Using scanning electron microscopy, arteries and veins are clearly distinguishable by their endothelial nuclear impressions. The vascular anatomy of the anterior segment and of other sites including the optic nerve and choroid in man can therefore be elucidated with this method.

Leonardo da Vinci[1] is credited as the originator of injection corrosion casting when he used wax to fill the cerebral ventricles. Subsequently in the eye, neoprene latex vascular casts of Schlemm's canal[2] and choroid[3,4] have been studied in man, and viewed with light microscopy. More recent technical advances include the introduction of low viscosity methylmethacrylate resins[5] which allow complete capillary filling particularly if thinned with mono-methylmethacrylate,[6] and scanning electron microscopy (S.E.M.) which enables specimens to be examined in fine detail at high magnification with recognition of characteristic morphological features from which a vessel cast can be identified specifically as either from an artery or a vein.

There is an extensive literature on ocular vasculature using casting in other species including cat,[7] dog,[8] duck,[9] pig,[10] monkey,[11] rabbit[12] and sheep,[13] but less in man.[14–20] This sparse literature presumably reflects the practical limitations in obtaining suitable human material.

We evolved our technique[13] specifically to study the human anterior segment vasculature in complete orbital and ocular vascular casts where the rectus muscle and episcleral vasculature remains intact. This paper describes the techniques involved in preparing casts of human cadaver orbits obtained 36–48 hours post-mortem and demonstrates some of the other ocular sites that can also be clearly visualised and studied using these techniques.

Materials and Methods

The orbital contents of adult human cadavers kept at 34° F for 36–48 hours post-mortem, were exenterated in a cosmetic lid-sparing procedure. The ophthalmic artery of the iso-

From: [1]Moorfields Eye Hospital, [2]Department of Pathology, Institute of Ophthalmology.
Presented at the Moorfields Alumni Meeting January 1989.
Correspondence to Jane M. Olver, Moorfields Eye Hospital, City Road, London EC1V 2PD.

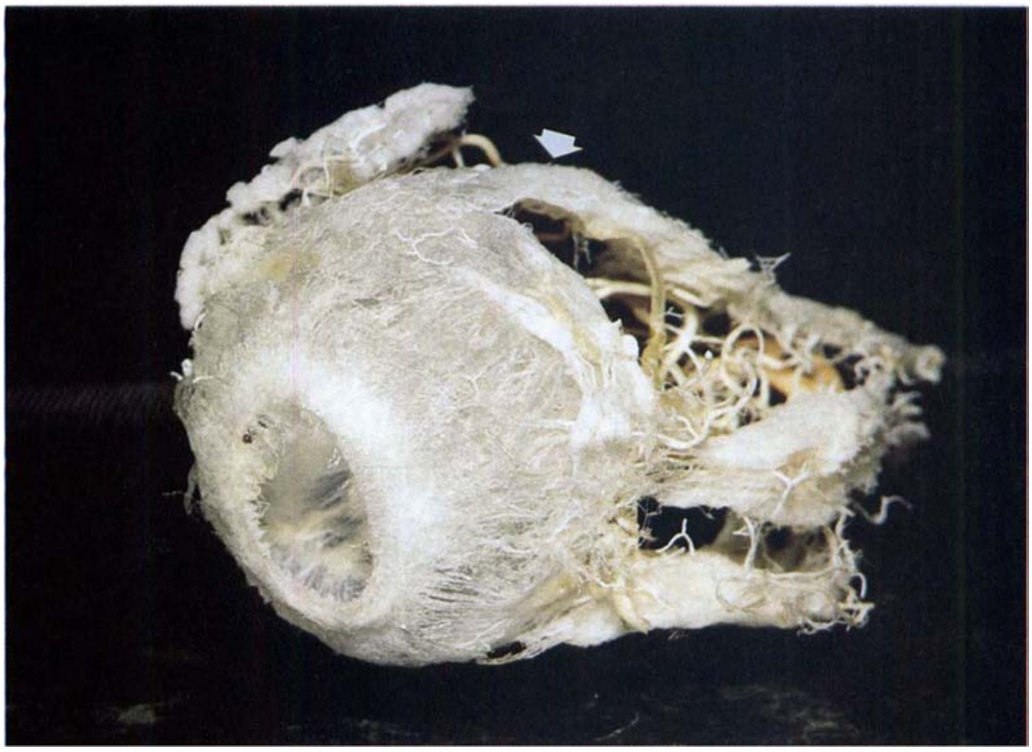

Fig. 1 *Macroscopic appearances of an uncoated complete methylmethacrylate cast of the ocular vasculature (left eye) in which the lacrimal gland, rectus muscle cone and inferior oblique muscle are attached. The orientation is such that the lateral rectus muscle is uppermost (see arrow).*

lated orbital preparation was cannulated. The residual lysed blood was irrigated with phosphate buffered saline and the endothelium was subsequently lightly fixed using gluteraldehyde 0.5% in Sorensen's buffered saline, both at 37° C and at perfusion pressures of 150–175 mmHg monitored manometrically. Anterior segment parancentesis was performed to lower intra-ocular resistance. Throughout perfusion, flow in the anterior ciliary arteries was observed under a Zeiss Opmi-6 operating microscope to confirm filling.

The resin mixture[6] consists of Batson's monomer 25 ml, catalyst 7.5 ml, promoter 0.5 ml (Polysciences) and thinner 12.5 ml (Sevriton). Thinned Batson's mixture (20–25 ml) was injected into each eye (150–175 mmHg) until polymerisation occurred. The isolated orbital preparation was placed in a water bath at 50° C for one hour to complete polymerisation. The surrounding tissue was then corroded in 6 Molar potassium hydroxide for 36–48 hours, changing the potassium hydroxide at 12 hourly intervals, until all the tissue had dissolved. The casts were washed in distilled water and air dried.

Following macroscopic examination the casts were gold sputter coated (50 μm gold) for examination under scanning electron microscopy at 20 kv and at magnifications ranging from ×12 to ×3000. The complete vascular cast was placed in the scanner for low power orientation and regional identification before micro-dissection. The superficial vasculature was sequentially removed to expose the deeper layers and the globe dissected systematically according to which vessel or vessel bed was being examined. After each dissection the cast was lightly re-sputter coated with gold to render the cut ends conductive for further examination in the scanning electron microscope.

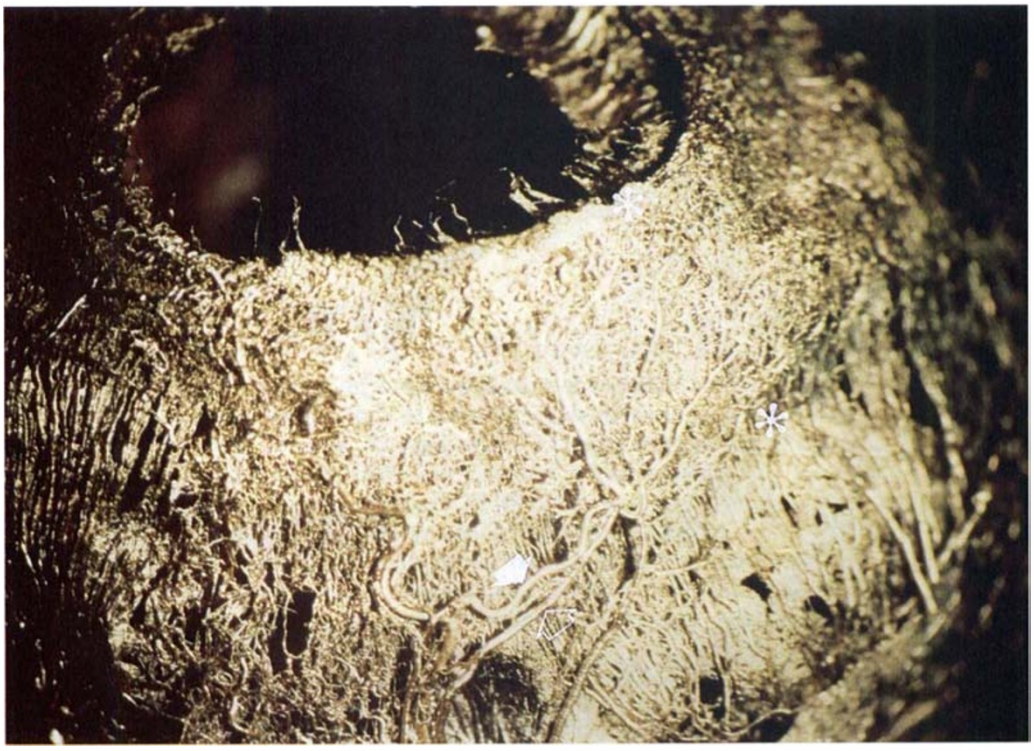

Fig. 2 *Slit lamp photograph of gold coated (50 μm) anterior segment showing inferior anterior ciliary artery (arrow), anterior vein (empty arrow), and dense anterior episcleral vasculature (between stars).*

Results

The ages of the patients at the time of death ranged from 21–83 years. The cause of death was known in each case from post-mortem examination. Intact orbital and ocular microvascular corrosion casts were reliably produced using the techniques as described and illustrated (Figs 1–11).

The macroscopic appearance of an uncoated orbital/ocular cast is seen in Figure 1. Casts of the anterior segment and part of the outer choroidal vasculature coated with gold for S.E.M. are shown in Figures 2 and 3 respectively. The same fragment of choroid is shown stabilised with a clamp for further micro-dissection in Figure 4.

When the casts were examined under scanning electron microscope, arteries and veins were clearly distinguishable. Arteries are recognisable by their endothelial nuclear impressions and longitudinal furrowing (Fig. 5a), whereas casts of veins have oval endothelial nuclear impressions and are generally wider, flatter and smoother surfaced (Fig. 5b). Endothelial nuclear impressions may be seen on capillaries but these impressions may also represent pericyte nuclei especially in retinal capillaries (Fig. 5c).

Examples of the completeness of vascular filling obtained are illustrated both by the episcleral vasculature (Fig. 6) and the peri-papillary capillaries (Fig. 7). The retrograde filling of Schlemm's canal from episcleral veins was a near constant finding (Fig. 8). The distinctive parallel arrangement of fine rectus muscle capillaries is illustrated in Figure 9. The vasculature of the choroid (Fig. 10a,b) and optic nerve (Fig. 11) were also clearly demonstrable from these casts.

Discussion

This method produces complete vascular casts of isolated human orbital preparations in which there is good capillary filling and pre-

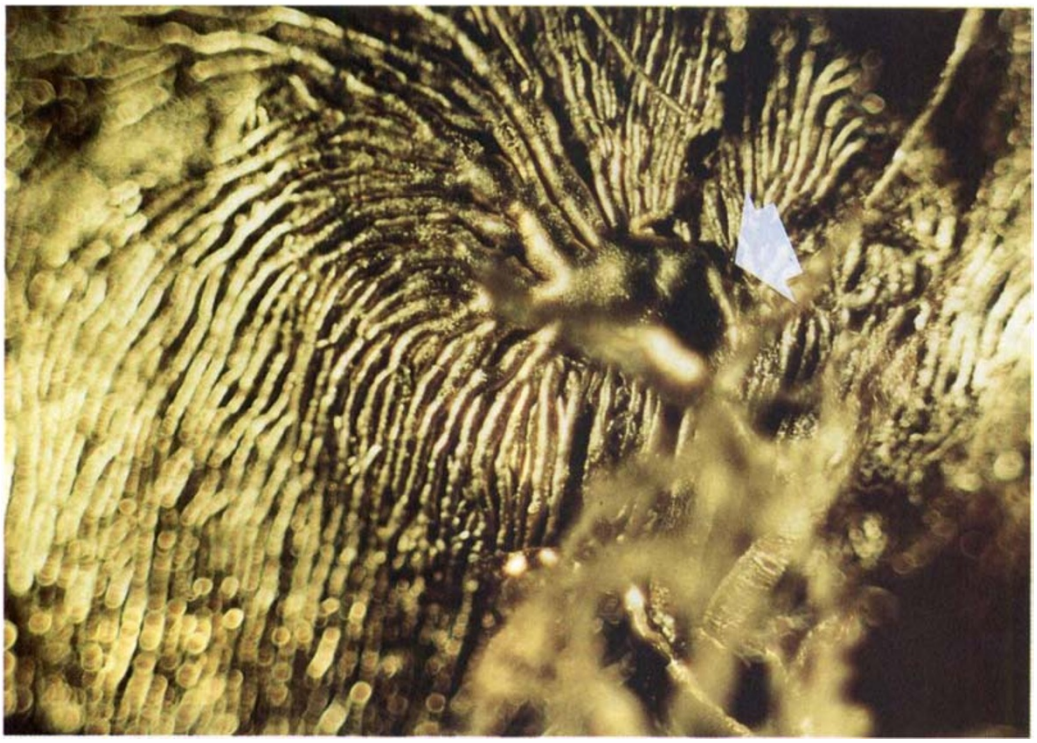

Fig. 3 *Slit lamp photograph of choroidal veins draining to vortex vein (arrow) in gold coated specimen.*

servation of vascular architecture. Intact episclera, rectus muscle microvasculature, deep anterior segment collaterals, as well as choroid and optic nerve can be studied from these casts.

Our results are comparable to those obtained within 6–12 hours post-mortem by other authors (see Table I) even though the procedure was not carried out until 36–48 hours post-mortem. Woodlief[14] injected methylmethacrylate via the carotid arteries of a small number of human infant cadavers within 24 hours of death and reported problems of cast distortion, 'waviness' and leakage. These problems led him[15] and later authors[16,19] to recommend that human ocular vascular casting be done within 12 hours of death. In contrast, in our larger series, done 36–48 hours post-mortem we rarely found leakage; such leakage as occurred was confined to episcleral and optic nerve capillaries and did not distract from identification of vessels and their routes. We initially encountered problems such as bacterial contamination and calcium complex deposition, both of which became avoidable. We also discovered that if the casts were originally incompletely cleaned, they were sufficiently robust to withstand further washings before gold sputter coating.

Ocular vascular casting in other species has

Fig. 4 *Choroid fragment held by bulldog on dissecting stand with two ball and socket joints allowing large range of movement for orientation of specimen under dissecting microscope.*

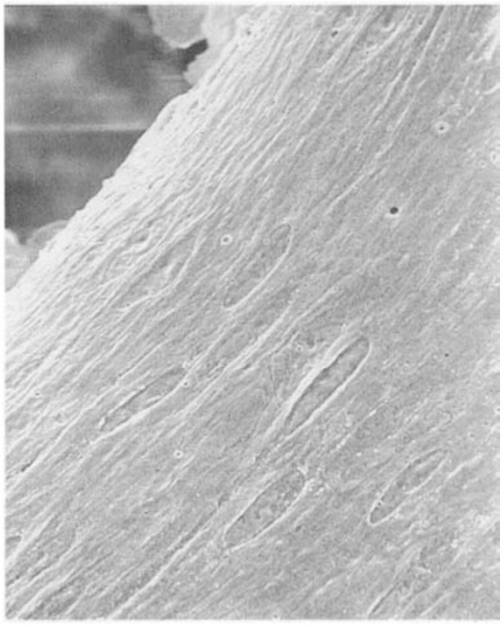

Fig. 5a

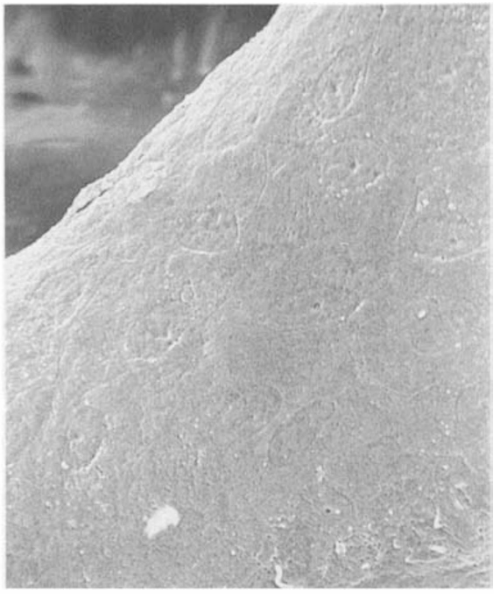

Fig. 5b

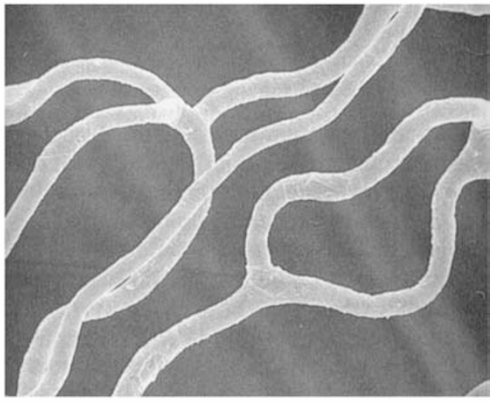

Fig. 5c

Fig. 5a *A scanning electron micrograph showing an artery with elongated endothelial nuclear impressions and deep furrowing. There is a suggestion of a nucleolar pit in the centre of the nuclear impressions. Magnification ×1,000.*

Fig. 5b *Scanning electron micrograph of part of vein showing rounder oval nuclear impressions with prominent nucleolar pits. Note that the surface of the venous cast is smooth and lacks elongated grooves. Magnification ×1,000.*

Fig. 5c *Scanning electron micrograph of retinal capillaries showing intermediate nuclear impressions (see arrows) which may either represent pericyte nuclei or endothelial nuclei. Magnification ×600.*

generally involved cannulation and perfusion of the carotid artery or great vessels, but Ashton's studies[2] of the aqueous veins (Part III) were after cannulation of the ophthalmic artery and anterior ciliary arteries. These results, and our own findings in sheep (that injection via the external ophthalmic artery produced consistently better results than through the carotid arteries), led us to choose an isolated orbital preparation with cannulation of the ophthalmic artery in man.

Several distinct advantages are offered by isolated orbital preparations compared to *in situ* perfusion via the carotid arteries. The episcleral, muscle and optic nerve vasculature remains intact in contrast to the unacceptable damage inflicted on the episcleral circulation during enucleation if the resin is allowed to polymerise *in situ*. The exenterated orbit (after lid-sparing) is easy to handle and requires only a small amount of resin injected via the ophthalmic artery. The proximity of the injection site to the capillary bed ensures filling since perfusion pressures are main-

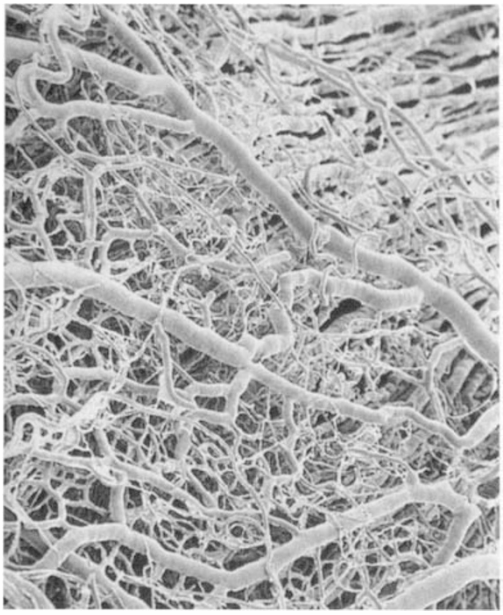

Fig. 6

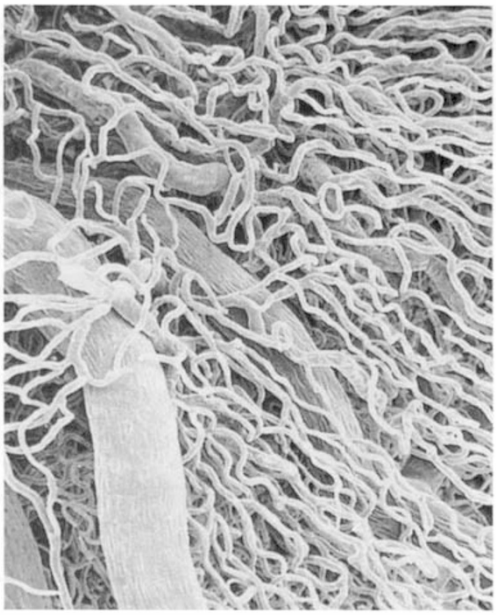

Fig. 7

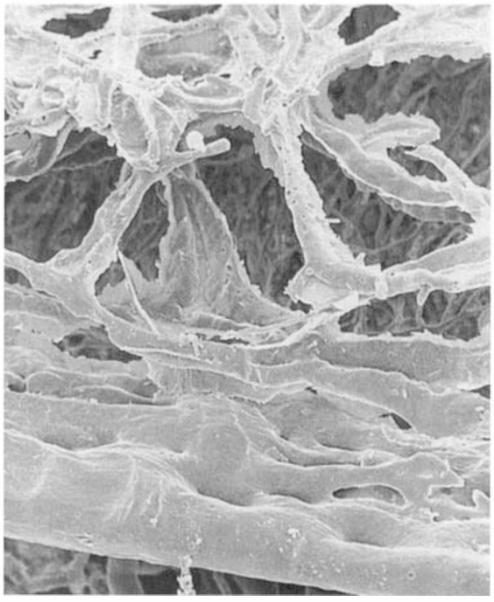

Fig. 8

Fig. 6 *Scanning electron micrograph of anterior episclera showing the predominantly venous vessels collecting blood both circumferentially and radially from the episclera to and from the anterior ciliary veins (out of picture on right). Part of an anterior ciliary artery only is seen in the lower right field. The episcleral vasculature thins posteriorly but is present as a loose net. The choroidal vessels are clearly seen since there is no intervening sclera. Magnification ×120.*

Fig. 7 *Scanning electron micrograph of part of prepapillary capillary network with a retinal arteriole in foreground emerging from disc. × 120.*

Fig. 8 *Scanning electron micrograph showing outer surface of Schlemm's canal with pits formed by fibrous trabeculi. The canal drains flattened collecting channels which are wider at their base on the canal. These numerous efferent channels drain into episcleral veins at the limbus around 360 degrees and rarely form true aqueous veins. Magnification ×120.*

tained. In contrast, *in situ* perfusion via the carotid arteries requires larger volumes of resin and it may be more difficult to flush out all the lysed blood.

Modern resins have sufficiently low viscosity when thinned with mono-methacrylate to ensure complete vascular filling. The cast produced is rigid in contrast to the earlier latex casts which were very flexible and had to be placed on the inside of a glass sphere or be suspended in fluid of similar specific gravity for examination. Another advantage of

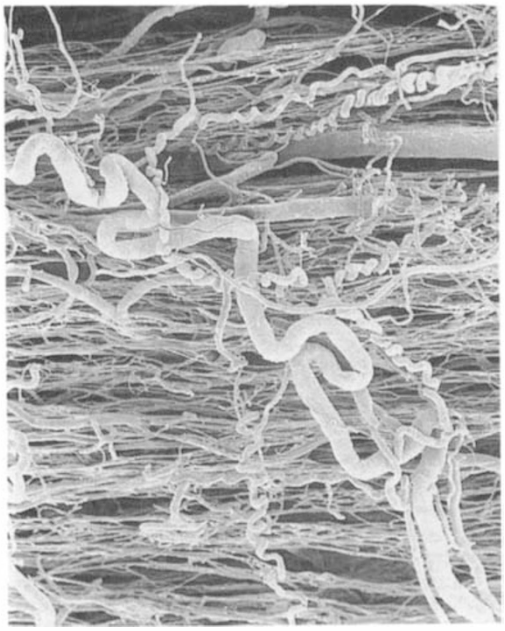

Fig. 9 *Scanning electron micrograph of outer surface of rectus muscle just behind the tendinous insertion, showing parallel capillaries and spiral shaped muscular artery and arterioles. Magnification ×40.*

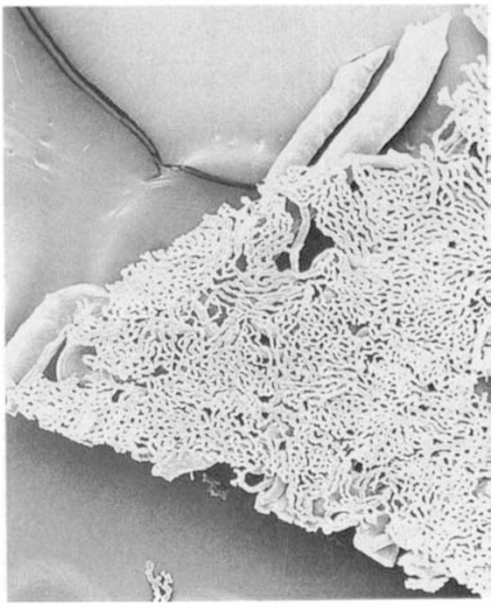

Fig. 10a *Scanning electron micrograph of fragment of choroid showing the inner choriocapillaris with outer larger choroidal vessels behind. Magnification ×40.*

Fig. 10b *Scanning electron micrograph showing cross section of choroid with outer layer choroidal vessels above and choriocapillaris below. Magnification ×1500.*

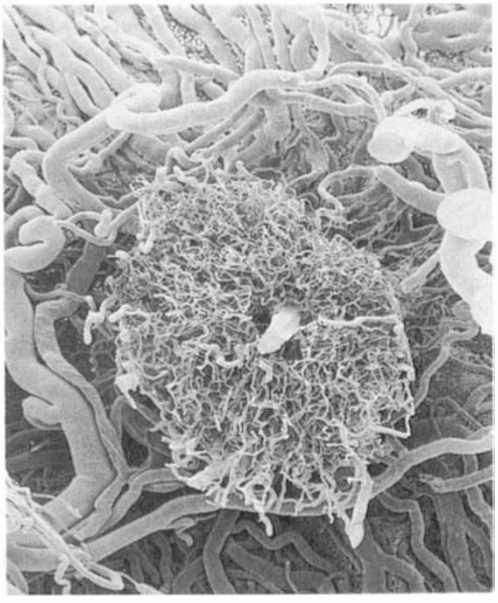

Fig. 11 *Scanning electron micrograph of cross section of retrobulbar optic nerve with central ophthalmic artery (arrow). Magnification ×25.*

Table I *Review of human ocular micro-vascular casting*

Authors	Resin	Post-mortem	No. of eyes	Injection route	Area under study
1. Ashton[2,3] 1951–3	a) Neoprene latex	<5 hours	5 (Melanoma)	Schlemm's canal	Aqueous veins
		Unspecified	200	Ophthalmic artery	Aqueous veins and anterior ciliary
1952	b) Neoprene latex	Unspecified	Unspecified	Ophthalmic artery	Choroid
2. Wybar[4] 1954	Neoprene latex	Unspecified	27	Ophthalmic artery	Choroid
3. Woodlief[14,15] 1980	a) Methylmethacrylate (Batsons)	<24 hrs	6 (Infants)	Common carotid artery	Anterior segment
	b) Methylmethacrylate (Batsons)	Few minutes	6 (Infants)	Internal carotid artery	Choroid
4. Yoneya[16,17] 1983	a) Methylmethacrylate (Mercox)	<12 hrs	9	Vortex vein } ? same eyes	Choroid
1987	b) Methylmethacrylate (Mercox)	<12 hrs	9	Vortex vein } ? same eyes	Choroid
5. Fryczkowski[18,19] 1984	a) Methylmethacrylate (Batsons)	4–6 hrs	3	Internal carotid artery	Optic nerve
1988	b) Methylmethacrylate (Batsons)	<12 hrs	10	Intra-cavernous part of Ophthalmic artery (excised)	Choroid
6. Vuillemy[20] 1984	Methylmethacrylate (Mercox)	<10 hrs	8	Ophthalmic artery (in situ)	Anterior uvea

methylmethacrylate casts is that they can be rendered conductive and examined in the scanning electron microscope where it is possible to recognise distinct morphological features of arteries and veins. These 'rigid' casts can also be dissected under an operating microscope, the dissection determined by the vessels under study, and subsequently be re-examined in the microscope, allowing good sequential documentation. The micro-anatomy of a wide range of ocular vasculature can be clearly viewed 'three-dimensionally' from two-dimensional scanning electron micrographs without the ecumbrance of surrounding tissue.

In summary, we have shown that satisfactory vascular casts, particularly of the muscular and episcleral vasculature, can be made from human cadaver orbits 36–48 hours post-mortem. Beyond 48 hours the results are poor. Ocular vascular casts serve to amplify and clarify the micro-vascular anatomy providing an important basis for the understanding of normal function and of ocular ischaemic syndromes.

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