A Monomeric Methyl and Hydroxypropyl Methacrylate Injection Medium and Its Utility in Casting Blood Capillaries and Liver Bile Canaliculi for Scanning Electron Microscopy

Takuro Murakami, Tatsuya Itoshima, Kusukuma Hitomi, Aiji Ohtsuka, and Albert L. Jones

Departments of Anatomy (Prof. T. Murakami), Internal Medicine (Prof. H. Nagashima), Okayama University Medical School, Okayama, Okayama Chuo Hospital Research Laboratory (Dr. T. Kaneshige), Okayama, Japan, and Cell Biology and Aging Section (Prof. A. L. Jones), Veterans Administration Medical Center, San Francisco, California, USA

Received September 12, 1983

Summary. A mixture of 50–60% monomeric methyl methacrylate and 40–50% monomeric 2-hydroxypropyl methacrylate was supplemented with 1.5% benzoyl peroxide (catalyst) and 1.5% N,N-dimethylaniline (accelerator) and injected into glutaraldehyde-perfusion fixed rat hypophyseal and other endocrine organ blood vessels and biliary tracts. This injection medium rapidly polymerized at room temperature and did not require partial polymerization prior to injection. Good casts of blood vessels, including the hypophyseal capillaries, were obtained for scanning electron microscopy. The monomeric methacrylate medium possesses a great advantage over previous ones, as its fluidity enables the casting of very fine vessels such as bile canaliculi. In the case of non-fixed tissues, the monomeric methacrylate medium should be injected carefully, as it is toxic and destructive to the vessels.

Scanning electron microscopy (SEM) of casted samples, now considered a standard method for studying microcirculation, has unraveled the microvascular organization of many tissues and organs which previously were only poorly visualized by conventional light or transmission electron microscopy (Murakami, 1971, 1972; Fujita and Murakami, 1973; Nowell and Lohse, 1974; Lametschwandtner and Simonsberger, 1975; Lee and Dempsey, 1976; Page et al., 1976; Gannon, 1978; Morris and Campbell, 1978; Hodde and Nowell, 1980; Bhalla et al., 1981; Ohtani et al., 1983; Olson, 1983; Rogers and Gannon, 1983). Many casting or injection media (polyester, latex, rubber, etc.) are commercially available (Schäfer et al., 1973; Nowell and Lohse, 1974; Frasca et al., 1978; Gannon, 1978; Nopanitaya et al., 1979; Hodde and Nowell, 1980; Northover et al., 1980). We, however, prefer using laboratory-prepared or partially polymerized methyl methacrylate mixtures (Murakami, 1971; Murakami et al., 1973) because they are less viscous and more easily injected into minute vessels. This is especially true of those diluted with monomeric hydroxypropyl methacrylate (Murakami, 1975). How-
ever, preparation of the mixtures is somewhat troublesome and tedious as partial polymerization requires heating prior to injection (Murakami, 1971; Murakami et al., 1973; Lametschwandtner and Simonsberger, 1975) or ultraviolet light treatment (Gannon, 1978). We have recently found that monomeric mixtures of methyl methacrylate and hydroxypropyl methacrylate can be directly injected without partial polymerization. This paper relates the details of this process and demonstrates its use in casting rat hypophyseal, thyroid, parathyroid and adrenal blood vascular beds and liver bile canaliculi.

**MATERIALS AND METHODS**

*Monomeric methacrylate injection medium*

A mixture of 50-60% methyl methacrylate monomer (v/v) (Katayama Chemicals Ltd., Osaka, Japan) and 40-50% 2-hydroxypropyl methacrylate monomer (v/v) (Nakarai Chemicals Ltd., Kyoto, Japan) was prepared. This was supplemented with 1.5% benzoyl peroxide (w/v) (catalyst) (Katayama Chemicals Ltd.) and 1.5% N,N-dimethyl-aniline (v/v) (accelerator) (Katayama Chemicals Ltd.) just prior to injection. This final mixture shall be referred to as the injection medium.

*Injection into the rat hypophyseal, thyroid, parathyroid and adrenal capillaries*

Adult rats weighing about 300 g were anesthetized by an intraperitoneal injection of sodium pentobarbiturate (2.5 mg per 100 g body weight). The right cardiac atrium was incised, and the ascending aorta cannulated after ligation of the thoracic aorta. Some of the anesthetized rats were decapitated, and their thoracic aorta cannulated for casting of the adrenal gland.

The animals were thoroughly perfused through the cannulated ascending or thoracic aorta with physiological saline followed by 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Immediately after the perfusion-fixation, the injection medium was infused via a syringe into the cannulated ascending or thoracic aorta at a pressure of 70-80 mmHg until the superior or inferior vena cava was filled (2-3 min). The injection pressure was decreased to 40-50 mmHg, and infusion continued until the injection medium in the syringe hardened.

*Injection into the rat liver bile canaliculi*

Adult rats weighing about 300 g each were anesthetized with pentobarbiturate (see above). The ductus choledochus and thoracic aorta were cannulated. The animals were thoroughly perfused with physiological saline and buffered 2% glutaraldehyde through the cannulated aorta to fix the liver and biliary tracts. Immediately after glutaraldehyde fixation, the injection medium was infused via the syringe into the cannulated ductus choledochus at a pressure of 70-80 mmHg. This injection continued, without any decrease in pressure, until the injection medium in the syringe hardened. In some cases, a small amount of commercially available Mercox injection medium (Japan Vilene Hospital Ltd., Tokyo, Japan) was infused into the portal vein after the methacrylate injection into the ductus choledochus.

*Digestion of tissue elements*

The injected animals or organs were heated in a water bath (50-60°C) for 3-6 hr, digested with 10% NaOH (50-60°C) overnight, and gently washed under a water tap.
for 8 hr to remove tissue elements. Undigested bone remnants surrounding the hypophysis were removed with forceps before freeze-cutting. Water-frozen casts were cut by razor blades into blocks suitable for SEM observation. The blocks were again immersed in hot 10% NaOH overnight and washed gently in running tap water (8 hr) to remove any remaining tissue elements. They were placed on filter paper and air-dried. The blocks containing bile canaliculi were frozen with water and freeze-dried (Model 2FS5, Hull Co., PA, USA).

**Scanning electron microscopy**

The dried blocks were mounted on metal stubs, and conductively stained by vaporized osmium tetroxide-hydrazine hydrate (MURAKAMI et al., 1982) or coated with gold in a vacuum evaporator (Model JE4-4C, JEOL Co., Tokyo, Japan) or an ion-coater (Model IB-3, Eiko Ltd., Ibaragi, Japan). Observations were performed with a scanning electron microscope (Model HHS-2R, Hitachi Naka Works, Ibaragi, Japan), using an acceleration voltage of 5–10 kV.

**Dissection or manipulation of casts**

The dried specimens occasionally were dissected in air or manipulated (extended or pressed) in an absolute ethanol bath (20–22°C) (MURAKAMI, 1972). Sharpened forceps, pincettes or needles were employed under a binocular light microscope (Model SMZ-10, Nikon Co., Tokyo, Japan). Dissection was performed both before and after the conductive treatment and scanning electron microscopy.

**RESULTS**

The monomeric methyl and 2-hydroxypropyl methacrylate medium was as viscous as water. It polymerized rapidly at room temperature (20–22°C), without heating or ultraviolet light treatment, and thus quickly increased its viscosity and temperature. In vitro it became as viscous as 10% glycerin aqueous solution (40°C) within 2 min, 50% glycerin aqueous solution (60°C) within 4 min, 100% glycerin (80°C) by 6 min, and completely polymerized by 8 min. Although the viscosity of the medium increased during injection, it remained very fluid and readily injectable at the early stages of polymerization. Initial infusion at 70–80 mmHg through the ascending aorta, followed by a decreased pressure of 40–50 mmHg as the medium polymerized, resulted in a near-complete filling of the glutaraldehyde perfusion-fixed capillaries and veins of the hypophysis and brain as well as the thyroid and parathyroid glands; there were few non-injected vessels and few leakages. The injected medium polymerized sufficiently within the vessels; hardening was aided by the supplementation of a catalyst and accelerator and by post-injection warming in a water bath. Thus the entire blood vascular beds of the hypophysis, thyroid gland and parathyroid gland were consistently casted along with those of the brain and other associated organs or tissues (Fig. 1–3, 7). Initial infusion at 70–80 mmHg through the thoracic aorta, followed by the decreased injection pressure, also allowed sufficient casting of the blood vascular bed in the adrenal gland (Fig. 8).

Retrograde, continuous injection of the monomeric methacrylate injection medium into the glutaraldehyde-fixed liver via the ductus choledochus casted the bile ducts, ductules, and canaliculi (Fig. 4–6). A constant pressure of 70–80 mmHg produced sufficient casting of ducts and ductules throughout the liver (Fig. 4–6). Canaliculi were
Fig. 1. Scanning electron micrograph of the cast of rat hypophyseal blood vascular beds (ventral view), casted by injection of monomeric methacrylate medium after perfusion-fixation. The cast was stained with vaporized osmium tetroxide. A internal carotid artery, a hypophyseal (hypothalamo-hypophyseal) arteries and their branches, AH adenohypophysis, M median eminence (anterior segment) (primary plexus of the hypophyseal portal system), p hypophyseal portal vessels, v hypothalamic veins, V basal vein. 5 kV acceleration voltage. ×32
Fig. 2. Dorsal view of rat hypophyseal blood vascular beds, casted by monomeric methacrylate injection medium after perfusion-fixation. Coated with gold. The anterior and posterior segments of the hypothalamic blood vascular beds were removed by dissection. The remaining middle segments (H) were bent and extended so that the median eminence (M) was exposed. a Hypophyseal (hypothalamic-hypophyseal) arteries and their branches, AH adenohypophysis, NH neurohypophysis, p hypophyseal portal vessels, PM posterior segment of the median eminence, s subependymal vessels in the median eminence, sp so-called short portal vessels, V and v hypophyseal veins and their branches, vv efferent convergence of adenohypophyseal capillaries (the vein or venule at this convergence was broken and lost during dissection). 5 kV × 34
Fig. 3. Legend on the opposite page.
not consistently filled. In poorly injected segments, only a few periportal bile canaliculi were reproduced (Fig. 5). In thoroughly injected segments, however, the peripheral bile canaliculi in the liver cell columns near the central vein were casted (Fig. 4). Medium leakages through the biliary tracts, including the canaliculi, were rarely noted (Fig. 5, 6). Additional casting of the portal vein branches after the biliary injection helped with positional identification of the casted biliary tree. By this method, for example, it could be clearly demonstrated that the biliary twigs form anastomosing networks around the portal vein branches (Fig. 5, 6).

The polymerized casts, including those of the bile canaliculi, were stable in the hot NaOH solution. The models were not damaged by the digestion procedure. Although casts of bile canaliculi required freeze-drying to avoid deformation of their ultrastructures, casts of blood vessels, bile ductules and thicker vessels were stiff and withstood air-drying without alterations.

Neither gold-coating (Fig. 2-8) nor staining with vaporized osmium tetroxide-hydrazine hydrate (Fig. 1) caused damage or deformation in any casts including those of bile canaliculi. Thus, both gold-coated and osmium-impregnated casts were fully suited for observation (without charging) in a scanning electron microscope at an acceleration voltage of 5-10 kV (Fig. 1-8). Beam damage of the casts was not noted during the scanning.

Although the casts were somewhat pliable, they were readily cut or broken with sharpened forceps, pincettes, or needles. This enabled exposure of regions of interest as the casts were dissected under a binocular light microscope (Fig. 2, 3, 6). Furthermore, the casts were softened in absolute ethanol and successfully manipulated (extended or pressed) without any marked breakage of the mold. This manipulation could be performed only within a few min; five min after immersion, the casts markedly diluted and began to dissolve.

The monomeric methacrylate injection medium shrunk to some extent (about 20%) during hardening. However, mural structures were sometimes imprinted on the casts. In such cases, vascular contractions caused by glutaraldehyde-fixation (or resistences of muscular elements in the vascular walls against the infusion pressure of the medium) were observed as circular ridges (Fig. 7), endothelial nuclear protrusions as oval impressions, and endothelial cell boundaries as fine linear impressions. The biliary endothelial microvilli were rarely imprinted.

DISCUSSION

Many laboratory-prepared or commercially available injection media have been used to cast minute vessels, including the hypophyseal capillaries, for scanning electron microscopy (Murakami, 1971, 1975; Nowell and Lohse, 1974; Frasca et al., 1978; Gannon, 1978; Nopanitaya et al., 1979; Hodde and Nowell, 1980; Northover et al.,...
Fig. 4. SEM view of the rat bile canaliculi, casted by monomeric methacrylate injection medium after perfusion-fixation (a well injected area). Coated with gold. B terminal twig of bile duct, b canal of Hering, c bile canaliculi. 5 kV. ×1,100
Selection criteria have also been discussed (Nowell and Lohse, 1974; Gannon, 1978; Hodde and Nowell, 1980), and can be summarized as follows: 1) the medium must have suitable physical and chemical properties to fill the vessels; 2) it must polymerize evenly; 3) the casts must withstand digestion, freezing, trimming, drying, and dissection; and 4) the casts must be capable of being made conductive and able to withstand bombardment by electrons during observation. Although its use is restricted to glutaraldehyde-perfusion fixed specimens, the monomeric methyl and 2-hydroxypropyl methacrylate medium sufficiently satisfies these criteria. The cast quality is com-

Fig. 5. SEM of rat bile canaliculi (c), ductules (b) and ducts (BB, Bl, Br), casted by monomeric methacrylate injection after perfusion fixation (a poorly injected area). In this case, portal vein branches (P) were reproduced by additional Mercox injection. Note the terminal twigs (Bl, Br) of the BB duct anastomosed with each other. L leakage of methacrylate medium, f filamentous remnants of tissue elements (probable collagen bundles). 5 kV. × 990
parable to that obtained by injection of partially polymerized methyl methacrylate or other low viscosity media into non-fixed specimens (Murakami, 1971, 1972, 1975; Fujita and Murakami, 1973; Kikuta and Murakami, 1982). This medium has the disadvantage of not being suitable for ethanol (or isoamyl acetate)–mediated critical point drying.

The medium should be used carefully in casting non-fixed vessels. It is toxic and destructive to tissues, especially minute vessels, and weakens the vessels making them intolerant of high perfusion pressure. For casting of non-fixed rat hypophyseal capillaries, the injection pressure should be lowered to 40–50 mmHg, in order to prepare adequate casts with minimal leakage (data not shown). Injection into non-fixed biliary tracts, via the ductus choledochus, always breaks the bile ductules, and does not cast the bile canaliculi, even when injection pressure is reduced to 20–30 mmHg. Formaldehyde, osmium tetroxide, or other strong fixatives are not suggested for perfusion-fixation prior to injection, as they harden the tissues or destroy the elasticity, thus rendering capillary and canaliculi filling difficult. The perfusion with saline which completely removes blood components prior to fixation is a necessary procedure as blood emboli hinder uniform fixation and filling of capillary beds.

Monomeric 2-hydroxypropyl methacrylate, when supplemented with benzoyl peroxide and N,N-dimethylaniline, polymerizes more rapidly than the monomeric methyl and 2-hydroxypropyl methacrylate medium. However, 2-hydroxypropyl methacrylate casts are weak and require freeze-drying to avoid deformation. Monomeric hydroxyethyl methacrylate and hydroxypropyl acrylate supplemented with the catalyst and

---

Fig. 6. SEM view of casted rat biliary (B, b) and portal vein (P) branches. Bile canaliculi were removed by dissection. L leakage of casting medium from biliary twigs. 5 kV. ×142
Fig. 7. SEM view of the blood vascular beds in rat thyroid (T) and parathyroid (P) demonstrated with the present cast medium after perfusion-fixation. A and a superior thyroid gland arteries, arrow: ridges probably representing vascular contraction during perfusion-fixation. 5 kV. ×115
accelerator polymerize as rapidly as monomeric 2-hydroxypropyl methacrylate does under the same conditions. Hydroxyethyl methacrylate and hydroxypropyl acrylate are soluble in water and readily dispersed into the tissues. At least three hours are necessary for monomeric methyl methacrylate supplemented with 1.5% benzoyl peroxide and 1.5% N,N-dimethylaniline to polymerize; thus, this last medium requires partial polymerization prior to injection (Murakami, 1971).

Increased concentrations of 2-hydroxypropyl methacrylate accelerate polymerization of the monomeric methyl and 2-hydroxypropyl methacrylate injection medium, but result in decreased stiffness of the casts. Decreased concentrations delay poly-
merization, but increase stiffness. Catalyst and accelerator concentrations also affect polymerization and stiffness. Supplementation with 2.0% benzoyl peroxide and 2.0% N,N-dimethylaniline allows complete polymerization within 6 min and increases pliability (or decreases stiffness). Lowering the concentrations to 0.5% each prolongs complete polymerization for 20 min or longer, and increases stiffness. Thus, the concentrations of 2-hydroxypropyl methacrylate, benzoyl peroxide and N,N-dimethylaniline can be adjusted for specific requirements.

The monomeric methacrylate injection medium can be injected into fixed biliary tracts to cast peripheral bile canaliculi located around the central vein. Preliminary experiments attempted to cast these vessels with some of the laboratory-prepared or commercially available injection media. Partially polymerized methyl methacrylate medium, when diluted with 30–50% 2-hydroxypropyl methacrylate (Murakami, 1975), is useful to cast a few periportal canaliculi connecting the Hering’s canal (Jones et al., 1980). It was difficult to cast bile canaliculi with commercially available Mercox injection medium (see above), even when diluted with monomeric methyl methacrylate (Ohtani and Murakami, 1978) or monomeric 2-hydroxypropyl methacrylate.

Scanning electron microscopic evaluation of the casted samples confirmed that the hypophyseal subependymal blood vessels in the median eminence are derived from the hypophyseal (hypothalamo-hypophyseal) arteries, and drain into the hypothalamic veins (Fig. 1–3). Furthermore, the subependymal vessels received hypothalamic capillaries, gave off capillaries anastomosing with the primary capillary plexus of the hypophyseal portal system, and presented additional drainages into the portal veins (Fig. 2, 3). The bile canaliculi were found to form anastomosing networks (Fig. 4, 5). The terminal or small twigs of the biliary ducts were also found to form anastomosing networks (Fig. 5, 6). Details of these findings of the hypophyseal vessels and biliary tree will be reported elsewhere. The blood vascular beds of the thyroid, parathyroid, and adrenal glands demonstrated marked differences which reflect tissue properties (Fig. 7, 8). These casts correspond in structure and quality to those prepared from non-fixed tissue injected specimens with partially polymerized methyl methacrylate or Mercox (Fujita and Murakami, 1974; Kikuta and Murakami, 1982).

This paper demonstrates that a monomeric methyl and 2-hydroxypropyl methacrylate mixture supplemented with benzoyl peroxide and N,N-dimethylaniline rapidly polymerizes at room temperature and does not require partial polymerization prior to injection. This monomeric methacrylate injection medium is useful in casting glutaraldehyde perfusion-fixed preparations. Hypophyseal capillaries and bile canaliculi were utilized as examples as they are complex in arrangement and structure and yet have been elucidated in detail; a medium which has been proved suitable for the demonstration of these vessels is believed to be widely applicable to the study of the minutest blood vessels and gland ductules.

REFERENCES

Bhalla, D. K., T. Murakami and R. L. Owen: Microcirculation of intestinal lymphoid follicles in rat Peyer’s patches. Gastroenterol. 81: 481–491 (1981).

Frasca, J. M., H. W. Carter and W. A. Schaffer: An improved latex injection media for replicating the pulmonary microvasculature. In: (ed. by) R. P. Becker and O. Johari: Scanning electron microscopy/1978/I. SEM Inc., AMF O’Hare, Illinois, 1978 (p. 485–489).
Fujita, H. and T. Murakami: Scanning electron microscopy on the distribution of the minute blood vessels in the thyroid gland of the dog, rat and rhesus monkey. Arch. histol. jap. 36: 181-188 (1974).

Fujita, T. and T. Murakami: Microcirculation of monkey pancreas with special reference to the insulo-acinar portal system. A scanning electron microscope study of vascular casts. Arch. histol. jap. 35: 255-263 (1973).

Gannon, B. J.: Vascular casting. In: (ed. by) M. A. Hayat: Principles and techniques of scanning electron microscopy, Vol. 6. Van Nostrand Reinhold Co., New York-Cincinnati-Atlanta-Dallas San Francisco, 1978 (p. 170–193).

Hodde, K. C. and J. A. Nowell: SEM of micro-corrosion casts. In: (ed. by) R. P. Becker and O. Johari: Scanning electron microscopy/1980/II. SEM Inc., AMF O’Hare, Illinois, 1980 (p. 88–106).

Jones, A. L., D. L. Schmucker, R. H. Renston and T. Murakami: The architecture of bile secretion. A morphological perspective of physiology. Digest. Dis. Sci. 25: 609-629 (1980).

Kikuta, A. and T. Murakami: Microcirculation of the rat adrenal gland: a scanning electron microscope study of vascular casts. Amer. J. Anat. 164: 19-28 (1982).

Lametschwandtner, A. and P. Simonsberger: Light- and scanning electron microscopical studies of the hypothalamo-adenohypophysial portal vessels of the toad Bufo bufo (L.). Cell Tiss. Res. 162: 131-139 (1975).

Lee, M. M. L. and E. W. Dempsey: Microcirculation of the rat placenta. Scanning and transmission electron microscopic observations on fetal blood vessels. Amer. J. Obstet. Gynecol. 126: 495-505 (1976).

Morris, J. L. and G. Campbell: Renal vascular anatomy of the toad (Bufo marinus). Cell Tiss. Res. 189: 501–514 (1978).

Murakami, T.: Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch. histol. jap. 32: 445-454 (1971).

———: Vascular arrangement of the rat renal glomerulus. A scanning electron microscope study of corrosion casts. Arch. histol. jap. 34: 87-107 (1972).

———: Pliable methacrylate casts of blood vessels: use in a scanning electron microscope study of the microcirculation in rat hypophys. Arch. histol. jap. 38: 151-168 (1975).

Murakami, T., M. Unehira, H. Kawakami and A. Kubotsu: Osmium impregnation of methyl methacrylate casts for scanning electron microscopy. Arch. histol. jap. 36: 119-124 (1973).

Murakami, T., A. Kubotsu, A. Ohtsuka, S. Akita, K. Yamamoto and A. L. Jones: Double staining by vaporized osmium tetroxide-hydrazine hydrate of biological specimens for non-coated scanning electron microscopy. In: (ed. by) R. P. Becker and O. Johari: Scanning electron microscopy/1982/I. SEM Inc., AMF O’Hare, Illinois, 1982 (p. 459–464).

Nopanitaya, W., J. G. Aghajanian and L. D. Gray: An improved plastic mixture for corrosion casting of the gastrointestinal microvascular system. In: (ed. by) R. P. Becker and O. Johari: Scanning electron microscopy/1979/III. SEM Inc., AMF O’Hare, Illinois, 1979 (p. 751–755).

Northover, J. M. A., E. D. F. Williams and J. Terblanche: The investigation of small vessel anatomy by scanning electron microscopy of resin casts. A description of the technique and examples of its use in the study of the microvasculature of the peritoneum and bile duct wall. J. Anat. 130: 43–54 (1980).

Nowell, J. A. and C. L. Lohse: Injection replication of the microvasculature for SEM. In: (ed. by) O. Johari and L. Corvin: Scanning electron microscopy/1974. IIT Research Institute, Chicago, 1974 (p. 267–274).

Ohtani, O. and T. Murakami: Peribiliary portal system in the rat liver as studied by the injection replica scanning electron microscope method. In: (ed. by) R. P. Becker and O. Johari: Scanning electron microscopy/1978/II. SEM Inc., AMF O’Hare, Illinois, 1978 (p. 241–244).

Ohtani, O., A. Kikuta, A. Ohtsuka, T. Taguchi and T. Murakami: Microvasculature as studied by the microvascular corrosion casting/scanning electron microscope method. I. Endocrine and digestive system. Arch. histol. jap. 46: 1–42 (1983).

Olson, K. R.: Effects of perfusion pressure on the morphology of the central sinus in the trout gill filament. Cell Tiss. Res. 232: 319-325 (1983).
Page, R. B., B. L. Munger and R. M. Bergland: Scanning microscopy of pituitary vascular casts. Amer. J. Anat. 146: 273–302 (1976).

Rogers, P. A. W. and B. J. Gannon: The microvascular cast as a three-dimensional tissue skeleton: visualization of rapid morphological changes in tissues of the rat uterus. J. Microsc. 131: 241-247 (1983).

Schäfer, D., E. Seidl, H. Acker, H. P. Keller and D. W. Lübbers: Arteriovenous anastomoses in the cat carotid body. Z. Zellforsch. 142: 515–525 (1973).

村上宅郎
〒700 岡山市鹿田町 2-5-1
岡山大学医学部
解剖学教室

Prof. Takuro Murakami, M. D.
Department of Anatomy
Okayama University Medical School
Okayama, 700 Japan