The vascular injection method and the functional geometry of the microcirculation

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The silicone elastomer microvascular casting method is readily adaptable for study of most microcirculatory beds. These perfusion materials are not acutely toxic to the microcirculation. The basic microvascular geometry can be profitably studied with this method and it demonstrates a pattern of right-angle branching in the lung, a manifold pattern at the inflow of the renal glomerulus, and a web or netlike capillary bed in the renal pelvis. Silicone rubber perfusion permits the retention of the in vivo physiological state of the blood vessels at the time of perfusion to make possible precise measurement of vessel diameters and examination of the blood vessel wall.

The vascular injection method is not new. Preparations for the demonstrations of blood vessels were first carried out systematically in 1670 by Swammerdam, in The Netherlands. The technique was refined and popularized by his student, Ruysch, during the following fifty years, but the interest in vascular casting was greatly diminished by the middle of the eighteenth century. Hyrtl in 1873 published a most elegant monograph on corrosion preparations. His illustrations clearly demonstrate injections of minute macroscopic blood vessels.

Injections designed to fill the microcirculation have been in use for approximately seventy-five years. With few exceptions the results were unsatisfactory: The capillaries were poorly filled and the techniques and materials available did not preserve the microcirculatory geometry in its antemortem state. Most of these techniques required the washout of blood and subsequent infusion of the opacifying materials, and, even today, these procedures incorporate the intravascular injection of a vasodilatory agent. Distortion of the vascular bed is almost inevitable. Prerequisites for preservation of living vascular geometry are that the circulation be undisturbed before tissue death, that agonal vascular alterations be prevented, and that the physical and chemical nature of the injection mass be compatible with the objective of filling the microcirculation under approximate physiological conditions. Perfusion with silicone elastomer results in the filling of open microvessels and the preservation of the functional microvascular bed.

Is there any need or justification for vascular injection methods today? Despite the great variety of in vivo methods for study

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of the microcirculation, some questions of size, pattern, and organization of these vessels have not been completely answered for a number of vascular beds. In fact, the very nature of blood flow in physiologically active channels in the living animal almost precludes the delineation of the full extent of the vascular bed. Krogh's plea for quantitative morphology is still being expressed.5

The silicone elastomer injection method must be considered as a supplementary investigative tool for the study of the microcirculation. It provides for the postmortem preservation of the vascular anatomy in a state approximating that immediately before tissue death. The various techniques and materials described below were developed and used in our laboratory during the past five years.6-10

Materials and methods

The phrase "microvascular casting" is descriptive of this method. A satisfactory preparation shows a faithful intraluminal cast of the microvessels. Subsequent processing of tissues may lead to cast shrinkage without concomitant change in blood vessel size or bed geometry.

Materials. The specific properties of silicone rubber perfusion materials are the basis of the developed techniques and procedures. Complete descriptions of both materials and methods have been published and will be given here only in summary form. The infusion material is a General Electric silicone rubber—RTV-201—especially prepared for vascular casting. It has a low surface tension and is immiscible with water; the viscosity can be adjusted to 20 centipoises with an appropriate diluent, so that perfusion may be carried out under physiological pressure; catalytic vulcanization occurs at room temperature without heat production or volume change and at a rate determined by the amount of catalyst. Preliminary washout of blood is unnecessary and even undesirable if retention of the physiological state of the blood vessels at the time of perfusion is to be achieved. The gum polymer is clear and colorless and the color of the compounded infusion material is imparted by an iron oxide filler with a maximum particle size of 0.8 μ in diameter.

In most instances it is desirable to maintain undisturbed blood flow until the beginning of the silicone rubber perfusion through the normal vascular channels. Accordingly, arterial perfusion is carried out by way of the aorta, perfusion of the pulmonary circulation by way of the pulmonary artery, and direct venous infusion limited to beds like the hepatic portal circulation. In all cases, since the silicone rubber readily traverses the capillary bed, a venous vent or runoff is necessary.

Procedure. The infusion system consists of a volume reservoir connected to a pressure reservoir and manometer on one side and the infusion cannula on the other. The silicone material is catalyzed immediately prior to infusion, which is carried out at mean arterial pressure. Specific additional information on the perfusion method, including the volumes infused and the time for adequate perfusion, have been detailed elsewhere.

Tissue processing. Microscopic sections are prepared from formalin-fixed tissue, sectioned on the freezing microtome to minimize evaporation of the silicone rubber diluent, stained with cresyl violet acetate, and mounted in glycerol-gel. Thick tissue slices to be examined by incident light are preserved and cleared in glycerin.

Results

I. Geometry of selected microvascular beds.

A. The lung. Studies were carried out on the cat, the dog, and the rabbit. The observations recorded below apply equally to all species, except for blood vessel size. Where measurements are given they apply only to the adult cat.

There is a right-angle branching pattern of pulmonary arteries, from vessels just visible to the unaided eye down to the final alveolar branch. In these three species lobular structure of the lung is so poorly developed that these minute vessels should be designated distribution arteries down to and including vessels of 60 to 120 μ in diameter, the smallest distribution arteries. A striking characteristic of these minute arteries is absence of significant tapering, despite considerable branching. From the smallest distribution arteries, precapillary alveolar vessels, 18 to 25 μ in diameter, arise at right angles, pass in the line of the intersecting planes of the adjacent alveolar walls, and send capillaries to a number of
alveolar walls from a single precapillary alveolar vessel. The precapillary alveolar vessels rapidly give rise to multiple alveolar capillaries and frequently are little more than orifice at the distribution vessel. Infrequently multiple capillaries to an alveolus originate directly from a small distributing artery (Fig. 1). This over-all pattern of capillary distribution supplies pulmonary artery blood to the margins of each alveolar surface. On occasion the alveolar capillary bed may be sufficiently well filled to demonstrate the real network, with small intercapillary spaces (3 to 4 μ) which are less than one half the width of the surrounding capillaries (8 to 10 μ). Venous drainage is characteristic: alveolar capillaries drain toward the center of the alveolar wall and away from the free edge of the alveolus. The confluence of minute venules joins larger trunks which show essentially a right-angle junctional pattern similar to that of the distribution arteries.

B. The renal pelvis. In all the species examined, the rat, the rabbit, the cat, the dog, and man, a separate and distinct microcirculation exists for the pelvic mucous membrane. Multiple arterial branches to the pelvis (and ureter) originate from the principal branches of the renal artery shortly after its division, follow the external wall of the pelvis for variable distances, and penetrate to immediately beneath the epithelium. The capillary bed exhibits a loose mesh and the capillaries measure 8 to 10 μ in diameter (Figs. 2 and 3). The venous drainage vessels closely parallel the arterial supply. Arteriovenous anastomoses could not be found.

C. Vasa vasorum. The basic patterns described for the pulmonary artery vasa in the rabbit have been extended to the cat and the dog. The most striking features of the microvasculature of the pulmonary

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Fig. 1. Pulmonary alveolar wall showing capillaries originating directly from small distributing artery. Diameter of artery, 65 μ. Diameter of capillaries, 10 μ average. Silicone rubber injection preparation. Cat. (Original magnification ×330.)

Fig. 2. Kidney pelvic mucosal capillary net. Glycerin cleared, silicone rubber injection preparation. Rabbit. (Original magnification ×60.)
artery are the helical course of these vessels and the specific circulatory beds characteristic of different parts of the pulmonary artery. At least three distinctive patterns are found: (1) on the anterior surface there is a three-dimensional capillary bed within the pulmonary artery wall which extends no deeper than one-half the wall thickness; (2) on the lateral surface, a two-dimensional capillary network is easily identified; (3) over the thin wall adjacent to the pulmonary valve sinuses, there is an unusual vascular pattern, with a central arteriole which divides via arborization, an external collecting venous basket, and an intervening fine capillary network.

D. The renal glomerulus. Perfusion of the renal arteries with silicone rubber often results in extensive filling of the renal microcirculation, especially the glomerular vessels. Serial sections of a well-filled kidney exhibit an unsuspected branching pattern of the continuation of the afferent arteriole within the glomerulus: a series of parallel branches characteristic of a manifold. The detailed wall structure of these vessels has not been investigated.

II. Vascular dimensions and the blood vessel wall. A consequence of the agonal state is diversion of blood into those vascular beds acutely critical for life from those whose blood supply can be acutely compromised. Consequently, it is not possible to determine the in vivo small artery, arteriolar, and venular dimensions in most vascular beds from usual histological preparations. Silicone rubber perfusion displaces blood from the functionally open vessels and, for reasons that are not yet apparent, nonperfused or "closed" vessels do not open during the subsequent period of increasingly severe tissue anoxia and metabolite accumulation. Thus it is possible reliably to visualize and quantify the dimensions of the microvascular bed as a faithful measure of the living state after silicone rubber perfusion and preparation of microscopic sections. A single set of examples is given. In the nonperfused normal cat kidney the lumen of the afferent glomerular arteriole measures 10 μ. In the silicone-perfused kidney the afferent glomerular arteriole measures 20 μ.

As a result of maintained patency of active capillary beds by silicone rubber perfusion, the blood vessel wall is especially accessible for examination. Under these circumstances the glomerular afferent and efferent arterioles show some remarkable differences in structure. In the afferent arteriole smooth muscle cell nuclei are arranged in a closely grouped palisade at right angles to the long axis of the vessel; they are infrequent in the juxtaglomerular region. The efferent arteriolar smooth muscle nuclei are sparse and placed either obliquely or in the direction of the long axis of the vessel. This striking difference in deposition of smooth muscle nuclei in these two vessels is highly indicative of a fundamental difference in the density and arrangement of smooth muscle cell fibrils.
The smooth muscle cell outline and its fibrils are rarely if ever seen in conventional histological preparations. Recently we have developed a polychrome staining technique with cresyl violet acetate and lissamine to stain differentially smooth muscle fibrils. The use of this staining method in combination with clear silicone gum perfusion of the vasculature should provide precise data on the geometric relationship of smooth muscle cells in the blood vessel wall.

III. Silicone perfusion and the retention of the physiological state of the blood vessels. Beyond the use of the silicone elastomers to demonstrate microvascular geometry is the curious persistence of the physiological state of the microvascular bed after catalysis and fixation of tissues. Vasoconstriction and vasodilatation of each of a pair of rabbit’s ears to heating and cooling and the response of a single ear to intra-arterially administered epinephrine were accurately retained by the silicone perfusion. Reactive hyperemia and the response to intra-arterial epinephrine in the lower extremity of the cat were likewise reproduced.

These observations suggested that either the perfusion materials chemically “froze” the microvascular bed or that the hydrophobic property of the injection mass played some role in the prevention of alteration of the vasoactive state of the blood vessels. To gain some information on the respective roles of these two factors acute toxicity studies were carried out on the cat kidney. The experimental kidney remained in situ and connected by way of appropriate tubing to the homolateral femoral artery and vein. Urine flow from both kidneys was recorded by drop counter. After a stable base-line interval an appropriately diluted clear gum elastomer was perfused at the animal’s mean pressure for a period of three minutes through the externalized arterial inlet while the venous outflow was voided. After termination of the silicone perfusion, blood was perfused for ten minutes and this perfused blood was also voided to prevent the entry of silicone rubber into the general circulation. The normal circulation was then re-established. Three experiments have been successfully concluded and clearly demonstrate that urine flow slows abruptly with silicone perfusion and resumes following washout of silicone and perfusion of blood. This can only indicate that uncatalyzed silicone material is not acutely toxic to the vascular endothelial structures involved in the complex process of urine formation.

Discussion
The purpose of this paper in this Symposium is the presentation of a different method of study of the microcirculation and some results of our investigations using it.

Knowledge of the different geometry of microvascular beds from various tissues and organs is as old as the study of the microcirculation, but the precise geometry of many beds is not established. The few examples given demonstrate close correlation between the organization of the capillary bed and the purpose it serves: right-angle branching of the pulmonary microcirculation may well be pressure conserving in a low-pressure system. This is also suggested by hydraulic flow in manifolds. In addition, the difference between right-angle and dichotomous branching at the alveolar level, associated with the orifice-like structure of the alveolar capillary vessel, suggests pressure conservation even at the alveolus (Fig. 4). The peculiar manifold construction at the inlet of the glomerulus of the kidney may be correlated with the small pressure drop known to occur across the glomerulus.

The extensive pelvic mucosal network inevitably would escape detection in routine preparations and its position within the kidney is such as to escape direct observation except in clinical surgery. The density of the capillary bed, relative to tissue surface, is low and recalls the similar sparse distribution of capillaries within the tracheal mucous membrane. The meta-
Fig. 4. Diagram illustrating two types of origin of precapillary alveolar vessels from distributing arteries. On left, right-angle manifold pattern; on right, dichotomous branching. Cross-hatched area indicates pulmonary alveoli.

bolic tissue requirements of the pelvic mucosa are obviously low in comparison to those of a parenchymatous organ.

The vasa vasorum of the pulmonary artery serves the nutritional needs of the wall structure, and the differing geometry in the microcirculation may be correlated with the different location of each bed and possible wall structure of the pulmonary artery at the site.

Vascular dimensions are necessary for meaningful hydraulic modeling of any microvascular bed. It is difficult to understand how such data may be obtained from three-dimensional solid tissues which do not lend themselves to translumination. The silicone rubber injection technique seems admirably suited for this purpose.

The difference between the wall structure of the afferent and efferent glomeralar arterioles may indicate some mechanisms for control of glomerular circulation that differ from those usually stated. It is difficult to understand an interplay between these two vessels to regulate flow where there are anatomically such remarkably different smooth muscle structures in these two blood vessels.

The apparent ability of these silicone materials to capture or retain the physiological state of the blood vessels at the moment of infusion may be an important attribute of this method. Its positive demonstration could make available an unusual tool for investigation of physiological and pharmacological events. The absence of acute toxicity of these perfusion materials, at least as indicated by the ability of the kidney to form urine after temporary perfusion of silicone rubber, suggests that a complete block in the transfer of aqueous soluble materials across the blood vessel wall in the microcirculation may be involved in the capture of the antemortem state by silicone rubber perfusion.

REFERENCES