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Three-Dimensional Vascular Architecture of the Dog Heart as Revealed by Injection Replica Scanning Electron Microscopy

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ABSTRACT

The three-dimensional vascular architecture of the dog myocardium was investigated by means of injection replica scanning electron microscopy.

Coronary arteries entered into myocardial wall at almost right angle. They repeated bicornal divisions and run toward endocardium. The arterioles and precapillary arterioles branched in bicornal, tricornal or multicornal fashions. These branches were arranged usually in a plane in short distances. Capillaries were arranged parallel to the myocardial cells and had many anastomotic channels. The branching of the capillaries was usually Y-, T-, H- or K-shape, and Y-shape was the most common. Many anastomotic channels between precapillary arterioles were recognized just under the endocardium, while same connections were very difficult to identify in the myocardial wall. Numerous venous capillaries joined together with postcapillary venules and collecting venules almost exclusively in a plane parallel to the capillary sheets. Junctional architecture of these capillaries and venules was usually fan-shaped, finger-shaped or feather-shaped in appearance. The postcapillary venules and collecting venules were oriented usually perpendicular to the muscle fiber or capillary direction. The orifices of Thebesian veins, arterio-luminal vessels and arterio-sinusoidal vessels were observed between ventricular trabeculae as small masses of injected resin. The number of these orifices were more abundant in the right ventricular wall than in the left.

It is well known that cardiac microcirculation is intimately related to the metabolism of cardiac muscle cells and the cardiac function. However, less attention has been payed on the architecture of the coronary vessels. Several investigators previously studied on the three dimensional architecture of the coronary vessels, using following technic: (1) India ink injections, followed by formalin fixation and serial sectioning for light microscopic examination[5], (2) Microfil perfusion, clearing of tissue and slicing for photography or radiography[3,7], and (3) Colorpaque perfusion, followed by formalin fixation, clearing and slicing for microangiographic examination[8]. Precise architecture of the coronary microvasculature, however, is still unknown. Recently, a method of injection replica scanning electron microscopy was developed and three-dimensional architecture of blood vessels in many organs was intensively investigated by many investigatos[2,9,10]. The present study was carried out to investigate the precise architecture of the coronary microvasculature of the dog heart, using the method of injection replica scanning electron microscopy.

MATERIALS AND METHODS

Five adult mongrel dogs, weighing 10 to 15 kg, were used in this study. The dogs were anesthetized with an intramuscular injection of Ketaral (2-o-methylaminocyclohexanone-hydrochloride) and heparinized. After sacrifice, the hearts were quickly removed and perfused with heparinized Ringer's solution through a polyethylene tube inserted into both coronary arteries. Thereafter, a dilute mixture of comercially available methacrylate medium (Mercox CL-2B-5, Dainippon ink Co., Ltd.) was injected through the tube until the coronary vein was filled with the injected medium. The injected resin was polymerized at room temperature for 1 hr and then placed in a warm bath for 24 hr for further polymerization.

The myocardial tissue of the experimental animals was then removed by hydrolysis in repeated changes of 20 to 25% NaOH aqueous solution for 24 hr. Vascular samples were washed in tap water to remove remaining tissue, cut with razor blades into suitable blocks and washed again. The blocks of vascular replicas were dried in air and fixed on metal stubs. They were coated with gold in an ion coater (Eiko IB-3) and observed in a scanning electron microscope (Hitachi S-430) with an accelerating voltage of 20 kV.

RESULTS

1) Coronary arteries

Right and left coronary arteries repeated bicornal divisions in the pericardium and ran parallel to the myocardial surface. The barnches of the coronary arteries entered into myocardial wall at almost right angle. They also repeated bicornal divisions in the myocardial wall and ran toward the endocardium. The division of these arteries showed usually an acute angle, while some of them were obtuse. The branches of these coronary arteries ran not only toward endocardium but also in any direction. Some of them formed recurrent branches toward pericardium. The peripheral branches of these coronary arteries became arterioles and further precapillary arterioles (Figs. 1, 2 and 3). The arterioles and precapillary arterioles showed bicornal, tricornal or multicornal divisions and transformed to capillary vessels (Figs. 3 and 4). The multiple or fork-like branches of precapillary arterioles were arranged almost exclusively in a plane. Anastomosis of the coronary arteries was very difficult to recognize in the myocardial wall, because of a limitation of visual fields. In the subendocardial wall, however, anastomosis between precapillary arterioles was frequently observed (Figs. 5 and 6). The arterioles and precapillary arterioles located under the endocardium usually showed wavy or spiral arrangement (Figs. 5 and 6).

2) Capillary vessels

Capillary vessels originated from precapillary arterioles and were parallel to the cardiac muscle cells (Figs. 1, 2, 3 and 4). They were 4 to 8 μm in diameter and connected with each other with anastomotic channels (Fig. 4). The branchings of these capillary vessels showed usually Y-, T-, H- or K-shape, and Y-shape was the most common. The capillaries distributed in the subendocardial layer were usually wavy or spiral (Figs. 5 and 6)

3) Coronary veins

Numerous venous capillaries were collected and transformed together into postcapillary venules and further collecting venules (Figs. 7, 8, 9 and 10). These venous capillaries joined to the post capillary venules and collecting venules almost exclusively in a plane parallel to the capillary sheets. The junctional architecture of these vessels were fan-shaped, finger-shaped or feather-shaped in appearance (Figs. 7, 8 and 10). Some of them showed "turnip root" appearance as previously reported by Brown[5] (Fig. 9). The collecting venules were usually perpendicular to the muscle fiber or capillary direction (Figs. 7 and 10). These venules were collected and transformed to coronary veins, which ran together with coronary arteries and came out of the myocardium.

4) Thebesian vein and other channels between ventricular cavities and coronary vessels

There are some kinds of channels between ventricular cavities and coronary vessels[11,12]. The Thebesian vein is well known as the channels between coronary vein and ventricular cavity. In addition, there are channels between ventricular cavity and coronary artery (arterioluminal vessels), or myocardial sinusoid (arteriosinusoidal vessels). Numerous orifices of these channels could be observed between ventricular trabecles as small masses of injected resin which

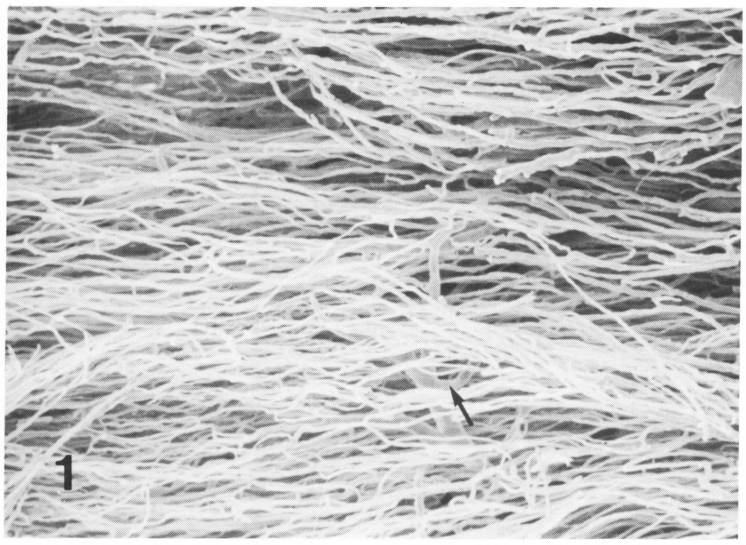

Fig. 1. A scanning electron micrograph of vascular casts in the left ventricular myocardium of the dog. Capillaries are usually arranged parallel to the myocardial cells. Arterioles occasionally branched at an obtuse angle (arrow). × 174

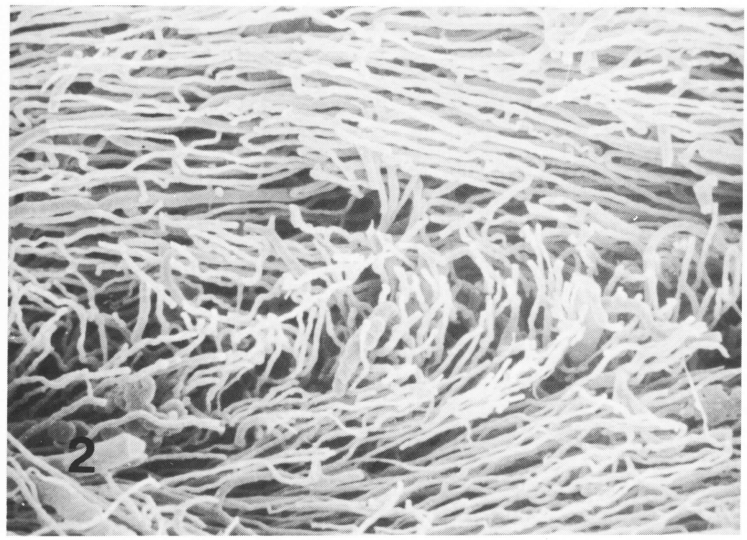

Fig. 2. Vascular casts of the right ventricular myocardium. Capillaries run together with muscle bundles. × 260

flowed out from these bypass channels. The number of these channels per unit wquare were more numerous in the right ventricular wall than in the left.

DISCUSSION

In the present study, three-dimensional architecture of the coronary vessels, especially of the arterioles, capillaries and venules, was observed by injection replica scanning electron microscopy. The coronary arteries principally showed bicornal division in the epicardium and myocardial wall. These coronary arteries were previously considered as end-arteries[6]. However, it is now well known that there are a number of anastomoses between branches of main coronary arteries[4,7,11]. In the present study, anastomoses between intramyocardial coronary arteries were very difficult to identify, because of a limitation of visual fields. Anderson and Anderson[2] previously investigated myocardial and intracranial microvasculature by injection replica scanning electron microscopy and reported that interarteriolar anastomoses were numerous only among the intracranial vessels, and that intercapillary anastomoses were

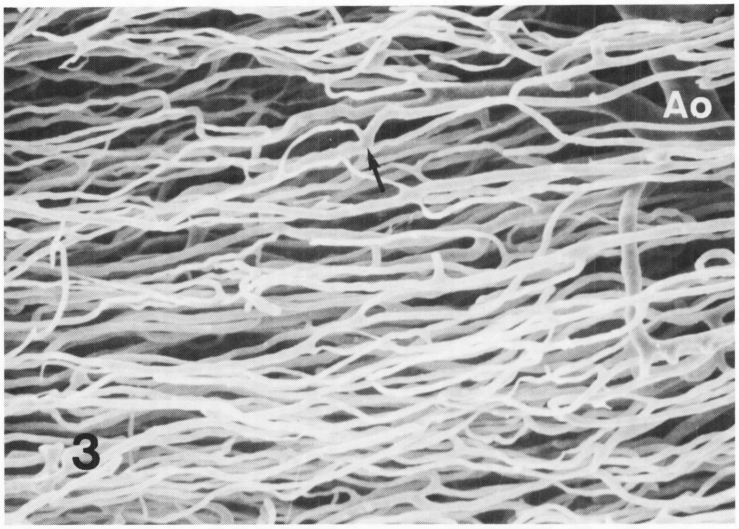

Fig. 3. Left ventricular myocardium. Transition of arterioles (Ao) to precapillary arterioles. Ramification of precapillary arterioles (arrow) is well observed. × 320

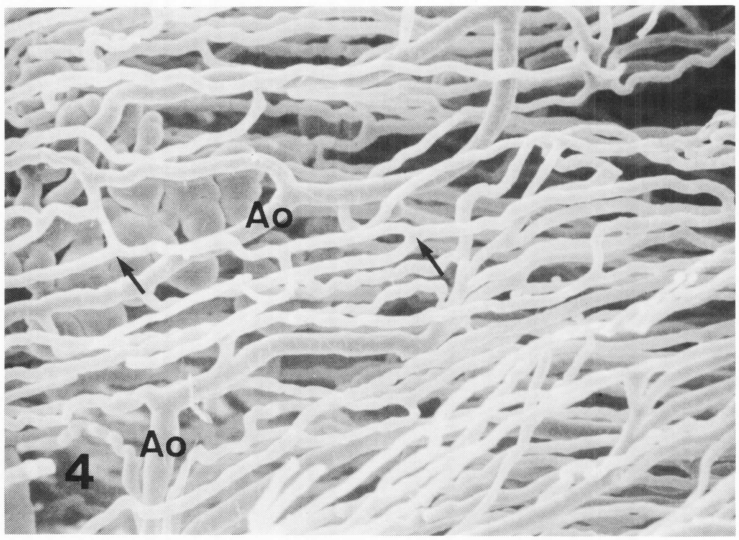

Fig. 4. Large magnification of vascular casts of right ventricular wall. Y- and T-shaped branchings (arrows) and anastomotic channels of the capillaries are frequently observed. Ao: arterioles. × 440

common in the myocardium. In the subendocardial layer, however, a number of anastomotic channels were observed between arterioles. In ischemic heart diseases, especially myocardial infarction, thin layer of cardiac muscle cells were well preserved beneath endocardial connective tissue. The subendocardial anastomoses of the coronary arterioles might contribute to the preservation of muscle cells in the ischemic heart diseases. Precapillary arterioles supplied many capillary vessels around the muscle cells. These capillary vessels ran parallel to the muscle cells and frequently branched off anastomotic channels to neighboring capillaries. The branching of anastomotic channels was Y-, T-, H- or K-shape. Bassingwaighte et al[3] reported that Y- and T-shape was 66%, H-shape 28% and others 6%. Our results supported those of Bassingthwaighte, et al[3], and Y-shape was the most common. Capillary densities in the myocardial wall were very high compared with other organs. It was previously reported that capillary densities within cardiac muscle bundles were 3100 — 3800/cm, giving intercapillary distances of 17.5 — 19 um[3]. In the present study, capillary vessels located in subendocardial layer

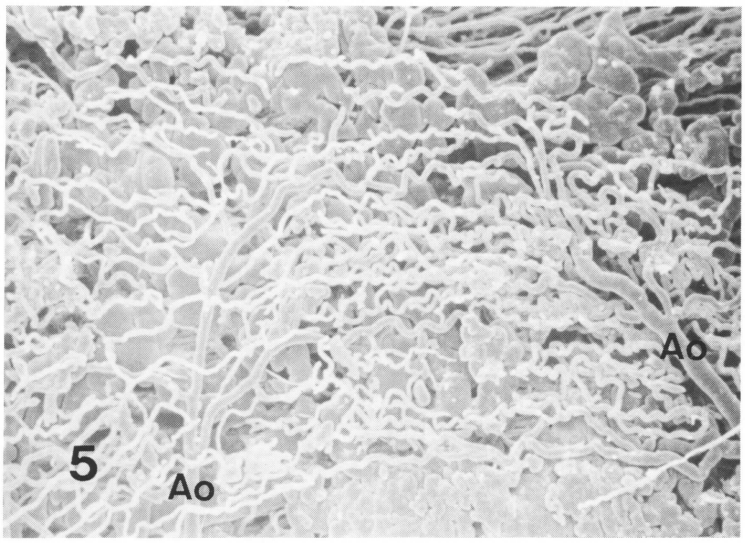

Fig. 5. Vascular arrangement in the subendocardial layer of the left ventricular papillary muscle. Two arterioles (Ao) and capillary network are observed. × 220

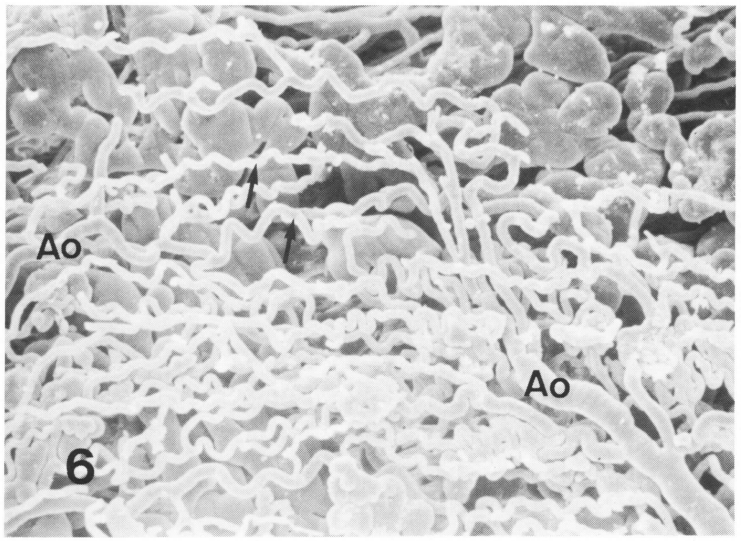

Fig. 6. Higher magnification of Fig. 5. Connections (arrows) between two arterioles are identifiable. × 340

were wavy or spiral, while those of subepicardial layer were usually straight. These changes might have resulted from postmortem status of the heart which usually remained in the systolic state. Anderson and Anderson[1,2] reported that precapillary arterioles were usually herical or undulating in arrangement. In our present study, however, this herical or undulating arrangement of precapillary arterioles was uncommon. Numerous venous capillaries and postcapillary venules joined together in short distance and transformed in collecting venules. Brown[5] previously reported that the venous capillaries came from all directions to join the "turnip root" venules. Anderson and Anderson[1], however, pointed out the fact that the capillaries joined the venules almost exclusively in a plane parallel to the capillary sheets and to the perpendiculary oriented venules. Our results were same as those of Anderson and Anderson[1]. The figure of these collecting venules was finger-, feather- or fan-shape appearance. These venules were oriented perpendicular to the muscular fiber or capillary direction. This arrangement of the capillaries and venules might aid in rapid venous return during myocardial contraction.

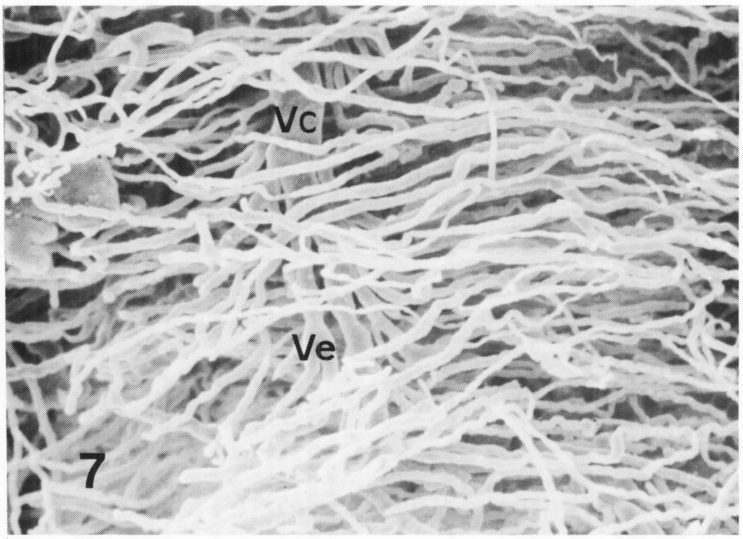

Fig. 7. Left ventricular myocardium. Fan-shaped or finger-shaped pattern of venules (ve) and collecting venules (Vc) is seen int he center of the photograph. × 320

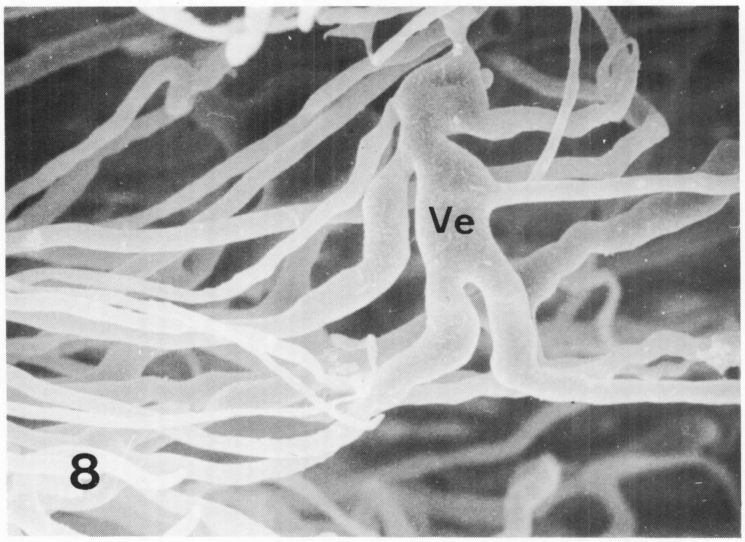

Fig. 8. Left ventricular myocardium. Many capillaries join to the venule (ve) at a right angle. × 1000

There were numerous channels between ventricular cavities and cononary vessels, i.e., Thebesian veins, arterio-luminal vessels and arterio-sinusoidal vessels. The number of these channels per unit square were more numerous in the right ventricular wall than in the left. Moreover, these channels were also observed in both atrial walls.

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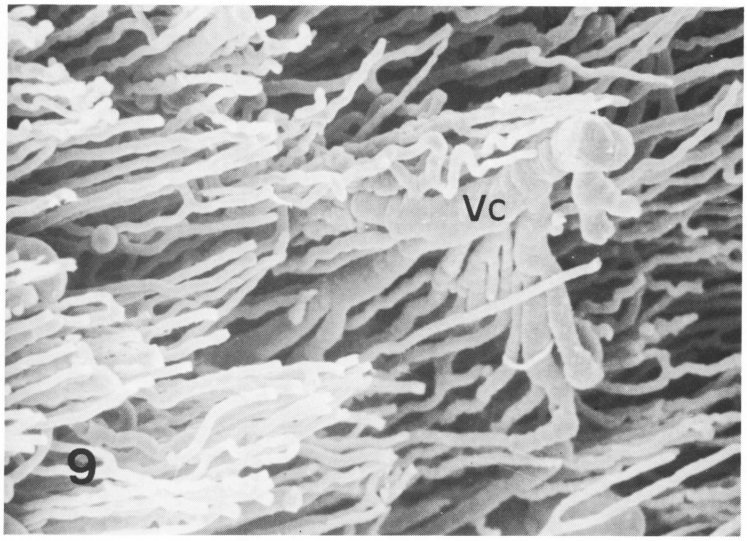

Fig. 9. Right ventricular myocardium. Numerous postcapillary venules are connected with collecting venules (Vc) and show "turnip root" appearance. × 400

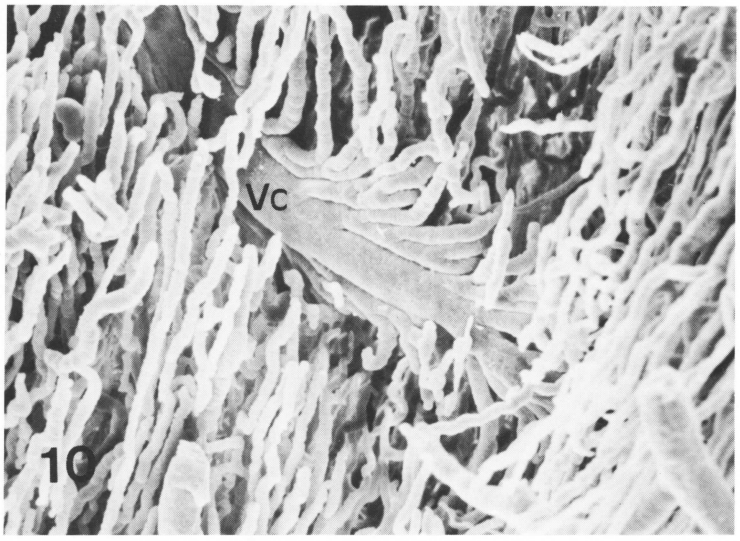

Fig. 10. Left ventricular myocardium. Numerous venous capillaries and postcapillary venules are connected together with collecting venules and show feather-like appearance. × 400

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