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SCANNING ELECTRON MICROSCOPIC STUDIES ON MICROVASCULAR ARCHITECTURE OF HUMAN CORONARY VESSELS BY CORROSION CASTS: NORMAL AND FOCAL NECROSIS

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Abstract

Microvascular architecture of the normal human heart and myocardial focal necrosis were studied by scanning electron microscopy of corrosion casts. Casts macroscopically identical in form to the left ventricular posterior wall were prepared.

The following results were obtained in the normal human heart. (1) Most of the arterioles communicated with capillary plexuses smoothly and straightforwardly in the left ventricular posterior free wall. (2) Arterioles which branched from the arteries ran in various directions and continued into capillaries either at right angles or obliquely in the trabeculae carneae. (3) capillaries running parallel with the cardiac muscle fibers ran in different directions to cross over with each other in different layers of myocardium. Capillaries in the myocardium formed a continuous and coarse net-like architecture with many bifurcations and anastomoses. Capillaries were about 5–7 μm in diameter. (4) Some veins gathering capillaries in the epicardium ran into the myocardium and the others ran in the epicardium. Veins connecting with capillaries in the myocardium ran in the myocardial layer and communicated with larger veins. (5)An arterio-venous anastomosis and two different types of venous-venous anastomoses were observed in the left ventricular posterior wall.

At the site of focal necrosis, cross sections of dilated vessels were observed in large numbers by light microscopy and scanning electron microscopy. (1) At the site of focal necrosis, dilated capillaries running with tortuosity were seen in large numbers by scanning electron microscopy of corrosion casts. (2) When compared with vessels in the normal myocardium, small arterial branches were dilated and run tortuously. (3) These dilated capillary plexuses were observed in the area which communicated with twigs branching off at the right angle from the arterial branch.

Key Words: Corrosion casts, scanning electron microscopy, human coronary vessels, microvascular architecture, focal necrosis.

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Introduction

The morphology of the coronary blood vessels of the heart has been extensively investigated by various methods, including dissection, dye injection, serial dissection, roentgenography, injection corrosion, plastic cast and their combinations. (2,3,6,7,10, 12,16,18–20) These studies have been primarily focussed on the arrangement and appearance of relatively large branches and on the general distribution pattern of myocardial capillaries, but hardly any reports have been made on the detailed arrangement or distribution of the capillaries in the normal and abnormal heart.

This paper describes our findings on the stereomicrovascular architecture in the normal left ventricular posterior wall of a 24 year old Japanese female and focal necrosis in the heart of a 60 year old Japanese male obtained by scanning electron microscopy with the use of corrosion casts.

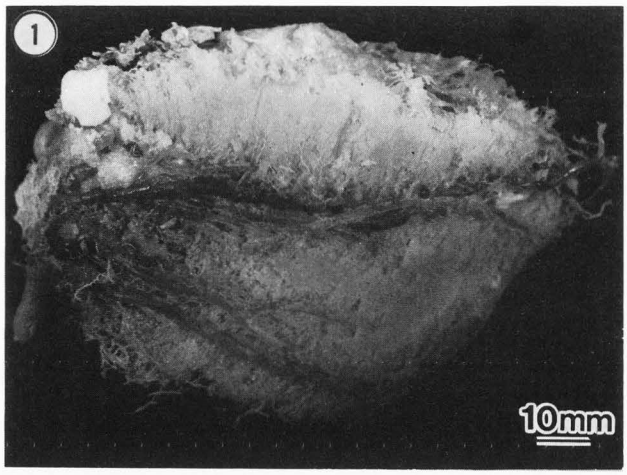
Materials and Methods

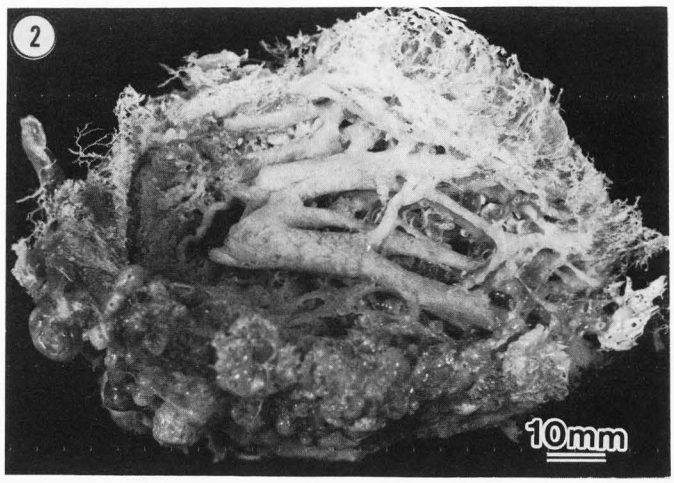
The posterior free wall of the left ventricle of seven autopsy cases was removed together with a part of the ventricular septum and right ventricular free wall at time of autopsy.

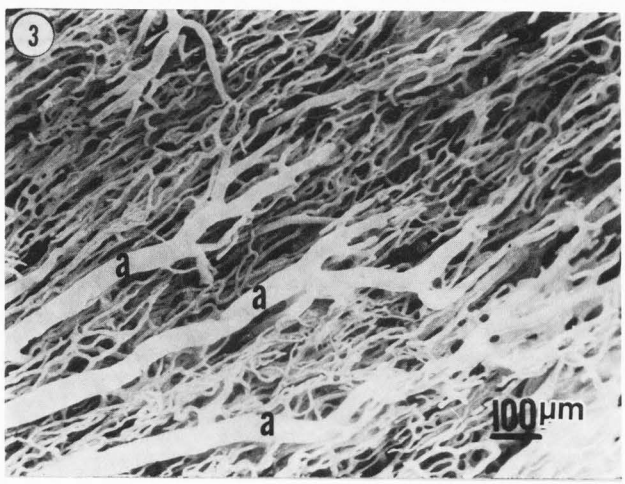
A teflon sheath needle was inserted into the left circumflex branch of the coronary artery, which was irrigated thoroughly with saline solution (1000ml). The cut ends of the relatively large coronary vessels in the myocardium were closed with ties. Then, half polymerized methylmethacrylate resin prepared by Murakami's method (13) was injected under a pressure of 100–150 cmH_2O until the coronary vessels were filled with the injected resin.

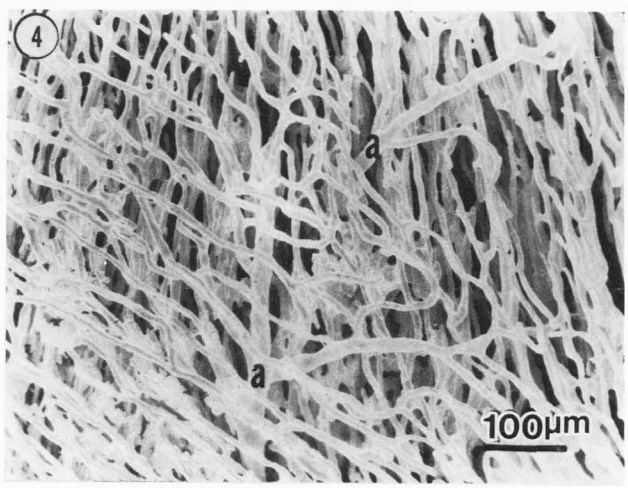
The resin injected heart was immersed in hot water of 70°C for 12–24 h, macerated for 10 h in hot bath (50°C) of concentrated sodium hydroxide solution, washed for 2 h in running water, and then dried in air.

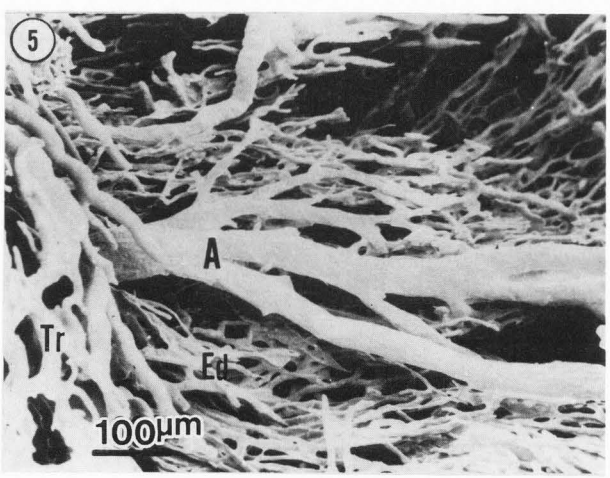
The corrosion casts of the left ventricular posterior wall thus prepared were trimmed into appropriate blocks suitable for scanning electron microscopy (SEM), mounted on metal-stubs with silver paint, coated by vaporized gold, and observed with a JSM-24 type scanning electron microscope at an accelerating voltage of 15kV.

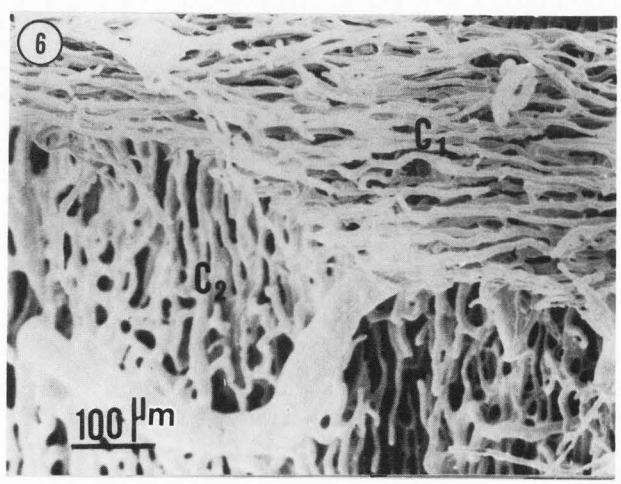












Results

Vascular beds of the left ventricular posterior wall were sufficiently casted by injection of half polymerized methylmethacrylate resin (Figs. 1,2). It was macroscopically observed that coronary arterial branches ran in the epicardium, giving off a number of twigs, and that the myocardium of the ventricular wall and trabeculae carneae were filled with a capillary network.

The following findings were observed by scanning electron microscopy of the corrosion casts.

Arteries

Comparatively large arteries with a caliber of 100–150 μm

Fig. 1. The myocardium of the ventricular wall was filled with capillary network, and coronary arterial branches ran in the epicardium.

Fig. 2. Trabeculae carneae were also filled with capillary networks in the endocardium.

Fig. 3. In the left ventricular wall, arterial branches (a) divided into several arterioles which communicated with capillary plexuses smoothly and straightforwardly. (Epicardium of the left ventricular posterior wall).

Fig. 4. In the trabeculae carneae, the arterial branches (a) ran to the endocardium from the deep zone of the ventricular wall and divided into several arterioles which continued into capillaries at right angles or obliquely.

Fig. 5. Comparatively large arteries (A) penetrated between the trabeculae carneae and the endocardium and gave off twigs, communicating with capillary plexuses. (Tr: trabeculae carneae, Ed: endocardium)

Fig. 6. Capillaries in different layers (C_1, C_2) of the myocardium ran in different directions, crossing over each other. (The capillary plexus of deep zone is too deep to bring into focus).

ran among the capillary plexuses in almost a straight line, divided into several finer branches while running almost parallel with the capillary network. Arterioles with a caliber of 14–20 μm communicated with capillary plexuses smoothly and straightforwardly in the left ventricular free wall (Fig. 3).

The diameter of the vessels was almost the same as that seen in the normal myocardium in all cases. In the endocardial surface of the trabeculae carneae, arterial branches with a caliber of about 20 μm ran to the endocardium from the deep zone of the left ventricular free wall and divided into several twigs. These arterial branches continued into capillaries either at right angles or obliquely (Fig. 4). Comparatively large arterial branches, which were about 70 μm in diameter, penetrated between the trabeculae carneae and the endocardium of the left ventricular wall and gave off twigs which communicated with capillary plexuses (Fig. 5).

Capillary plexuses

It appeared that capillaries in the myocardium ran parallel with the cardiac muscle fiber. Thus, capillaries in different layers of the myocardium ran in different directions, crossing over with each other (Fig. 6).

Comparatively large vessels with a caliber of about 70 μm ran between these different layers. Capillaries in the myocardium formed a coarse net-like architecture with many bifurcations and anastomoses (Fig. 7). The diameter of each capillary was measured to be about 5–7 μm, and the distance between the capillaries was about 17–30 μm. Capillary plexuses in the trabeculae carneae had the same architecture as the left ventricular free wall.

Veins

The connections between capillary plexuses and veins were observed to be much more numerous than those between capillaries and arterioles. Veins, gathering capillaries like branches of a tree, were observed in the epicardium and myocardium (Fig. 8). A relatively large number of veins, connecting with capillaries in the epicardium, ran and joined with a large vein in the epicardium (Fig. 9), while others ran into the myocardium from the epicardium and connected with a larger vein having a caliber of about 85–100 μm (Figs. 10, 11). Almost all veins, communicating with capillary plexuses in the myocardium, ran and joined with a larger vein in the myocardium (Fig. 12).

Anastomosed vessels

The arterio-venous anastomosis in the subepicardium of the left ventricular posterior wall was about 150 μm in diameter and connected also with capillary plexuses (Fig. 13). A type of venous-venous anastomoses with a caliber of about 30–75 μm formed a coarse net-like architecture with some anastomoses in the epicardium. Some of these anastomotic vessels connected with capillary plexuses in the epicardium and ran into the myocardium (Fig. 14). Another type of venous-venous anastomosis was observed in the myocardium of the left ventricular posterior wall. This anastomosis formed a loop and connected with veins running into the myocardium from the epicardium (Fig. 15).

Focal necrosis

Many cross sections of dilated vessels were observed in large numbers in focal necrosis areas by light microscopy (Fig. 16). The normal myocardium has many muscle bundles, while few muscle bundles and dilated vessels were observed in areas of focal necrosis by scanning electron microscopy (Fig. 17). Dilated capillaries and arterial branches, running with tortuosity, were observed in a site of focal necrosis of the left ventricular wall by scanning electron microscopy of the corrosion casts (Figs. 18, 19, 20). These dilated capillary plexuses were observed in the area communicating with twigs which divided at a right angle from the arterial branch (Figs. 21, 22).

Discussion

The morphology of the coronary blood vessels of the heart has been studied extensively by various methods. Recently, the microvascular architecture of coronary blood vessels of the heart has been observed by scanning electron microscopy (8, 13, 15–17, 22), but little study has been made on the detailed arrangement of the microvascular architecture in the ventricular wall of the human heart. This paper describes the well defined findings of the microvascular architecture in the ventricular wall of the human heart observed by scanning electron microscopy of casted sample with methylmethacrylate.

Arterial branches divided into arterioles, communicating with capillary plexuses smoothly and straightforwardly. Yokota et al. (24) have reported in their scanning electron microscopic study of casted sample with Mercox that the distance from the point connecting with capillaries to the point dividing the arterioles is short. In this study, various lengths of arterioles, which divided from arterial branches and connected with capillary plexuses, were observed. Arterial branches divided into arterioles and connected with capillaries either at right angles or obliquely in the trabeculae carneae.

Comparatively large arterial branches, which have been reported by Yokota et al. (24), were also observed in this study and these arterial branches penetrated between the trabeculae carneae and the endocardium, and divided into twigs communicating with capillaries in the endocardium. Vessels opening into the ventricular cavity were not observed by scanning electron microscopy with corrosion casts, but leakage of the fluid, when the coronary vessels were irrigated, was observed.

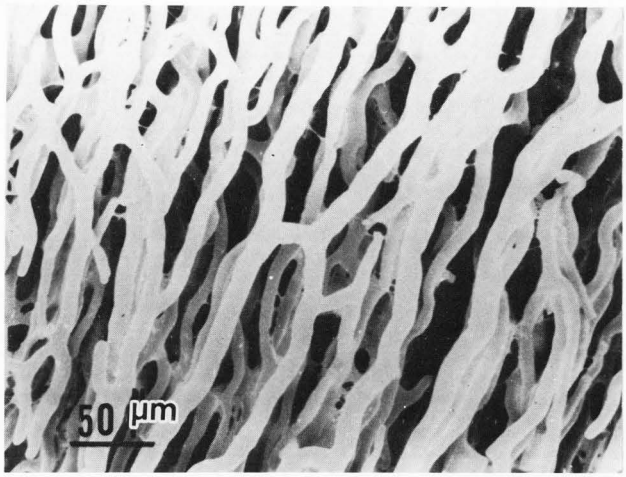


Fig. 7. The capillary plexuses with many bifurcations and anastomoses formed a coarse net-like architecture in the myocardium.

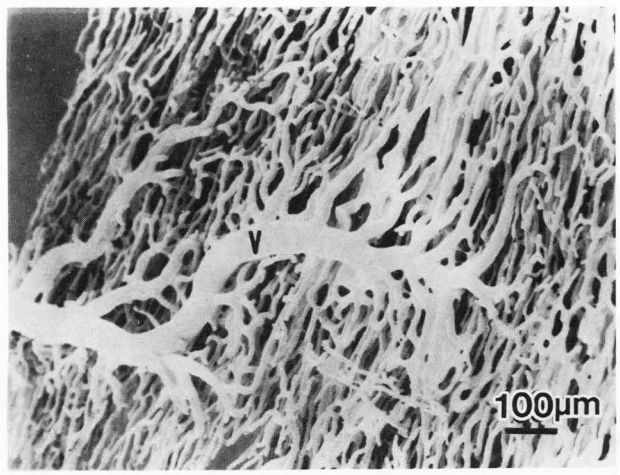


Fig. 8. This vein (V), gathering capillaries, ran tortuously in the myocardium.

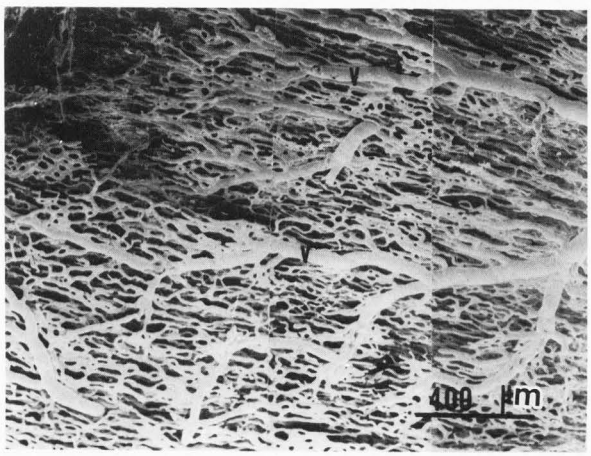


Fig. 9. Relatively large number of veins (V), gathering capillaries, ran in the epicardium and joined with larger veins.

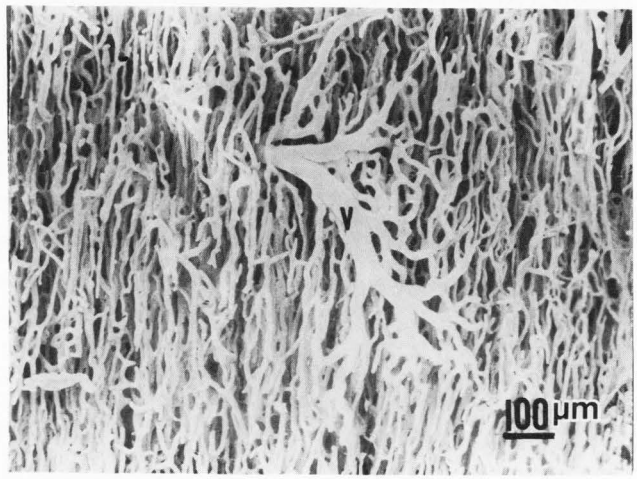
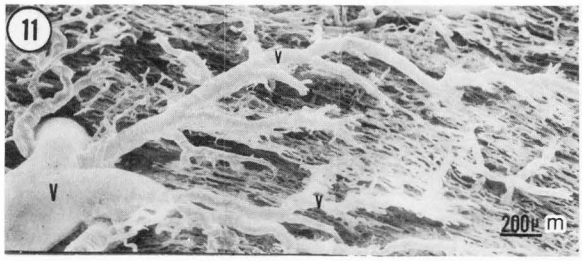


Fig. 10. Some veins (V) ran into the myocardium from the epicardium.

Capillaries ran parallel with cardiac muscle fibers (8,9,14,15,16) forming a coarse net-like architecture with many bifurcations and anastomoses. Thus, capillaries in different layers of the myocardium ran in different directions, crossing over with each other. These findings are similar to those observed in the rabbit (8,16). According to Shozawa et al. (22,23) and Kaneko et al. (11), "the intramyocardial microvascular network can be subdivided into three parts, arterial, capillary and venous sites," but in this study a continuous capillary network, communicating with arterioles and venules in various parts, was observed.

Some veins gathering capillaries in the epicardium ran into the myocardium and communicated with larger veins. To our knowledge, findings of this nature have not yet been reported. Considerable variations in pattern of anastomosed vessels have been reported by several researchers (1,4,21). These variations seem to be due to a difference in techniques and species used. Several patterns of anastomosed vessels of the animal heart have been demonstrated by the scanning electron microscopic method. An arterio-venous anastomosis and two types of venous-venous anastomoses, forming the coarse net-like architecture and loop, were observed in this study.

Focal myocardial necrosis is said to occur in the hearts of various diseases. It was reported by Eng et al. (5) that a greater number of microspheres were present in the subendocardial layer, while necrotic lesions were more frequent in the midwall and epicardial layers by experimental microsphere embolization. Shozawa et al. (22) have reported that the intramyocardial microvascular network is subdivided into three parts, arterial, capillary and venous sites, and focal necrosis has occurred only in the arterial site of the capillary network. In our observation, focal necrosis in the myocardium of aged human heart was observed in the areas connecting with twigs divided from arterial branches at right angles. Dilated capillaries and arterial branches with tortuosity were observed in sites of focal necrosis.



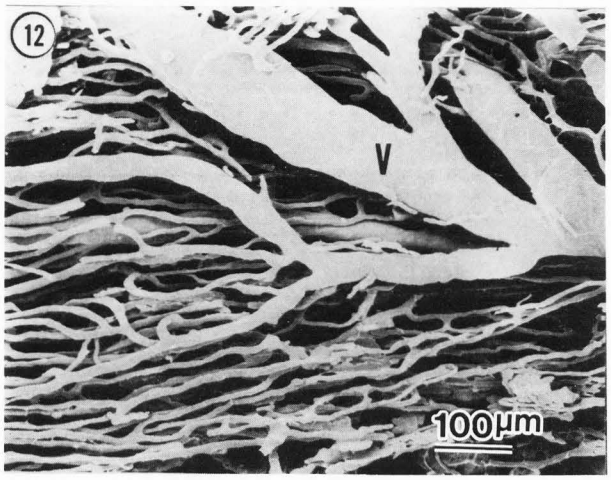


Fig. 11. The veins, running into the myocardium from the epicardium, connected with larger veins (v) which joined with the largest vein (V) in the myocardium. (Capillary plexuses in the epicardium were removed. Some of cut ends of the veins connected with the veins which ran into the myocardium from the epicardium.)

Fig. 12. The veins, connecting with capillaries in the myocardium, joined with larger veins (V) in the myocardium.

Fig. 13. An arterio-venous anastomosis in the subepicardium of the left ventricular posterior wall was about 150 μm in diameter. (A: artery, V: veins. The capillary plexuses around the anastomoses were removed.)

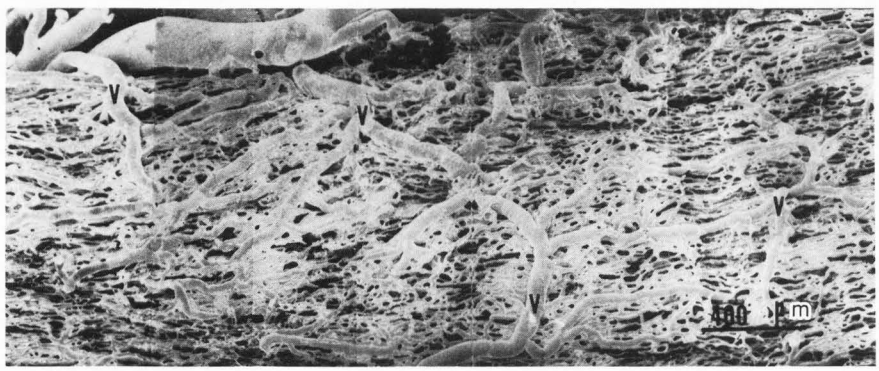


Fig. 14. Venous-venous anastomoses formed a coarse net-like architecture with some anastomoses in the left ventricular posterior wall.

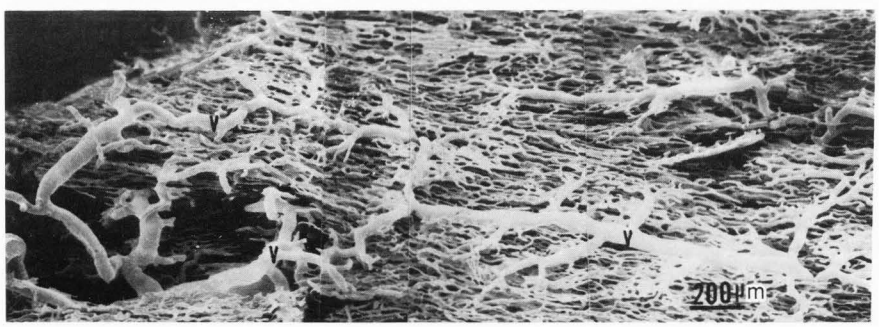
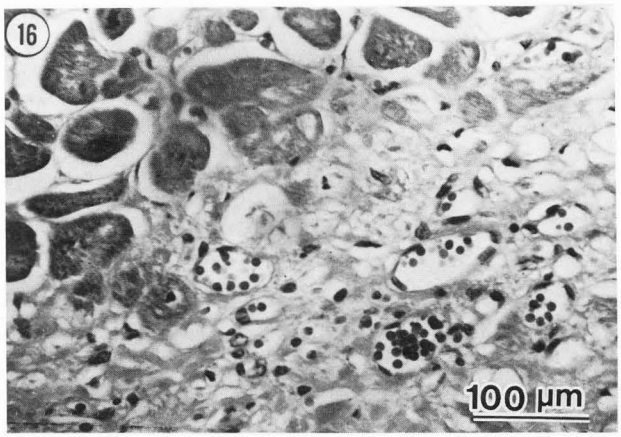
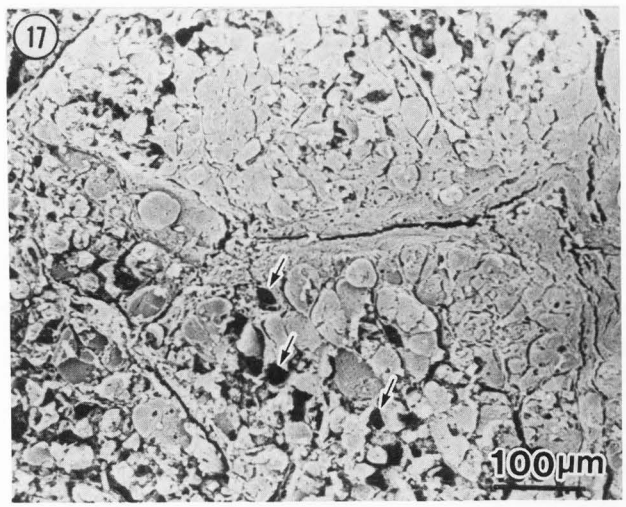
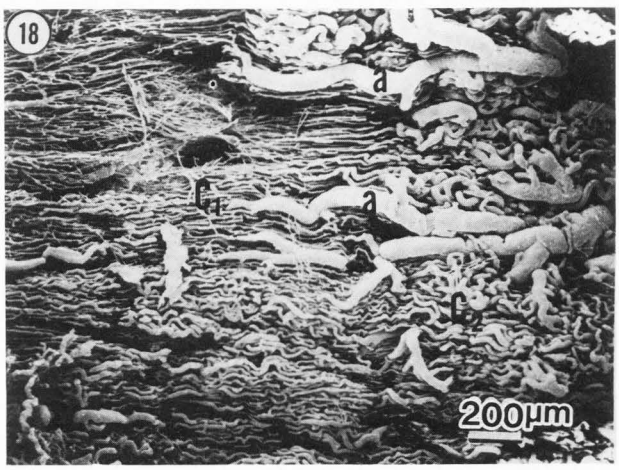
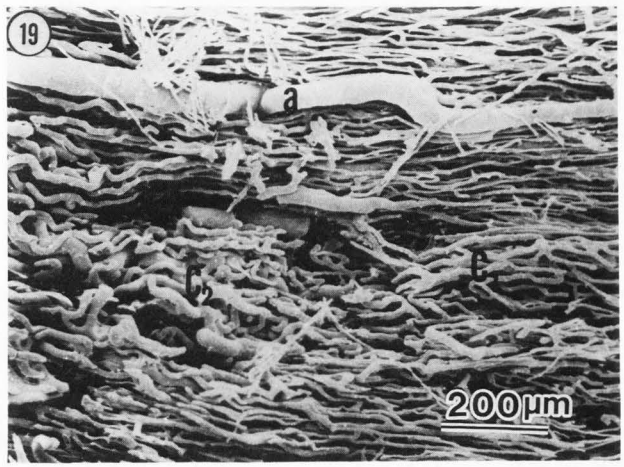


Fig. 15. In the myocardium of the left ventricular posterior wall, a venous-venous anastomosis formed a loop, and connected with capillary plexuses and veins which ran into the myocardium from the epicardium. (V: veins, Capillary plexuses in the epicardium were removed.)









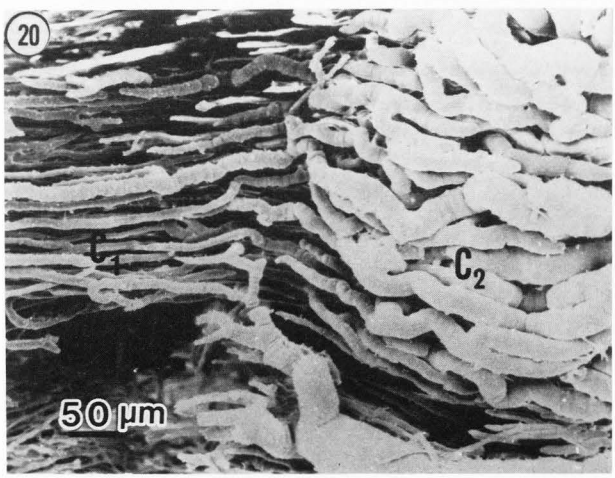


Fig. 20. Dilated capillaries (C_2), communicating with normal capillary plexuses (C_1) were observed in the site of focal necrosis.

Fig. 21. These dilated capillary plexuses were observed in the area communicating with twig (arrow) which divided at right angles from the arterial branch (a).

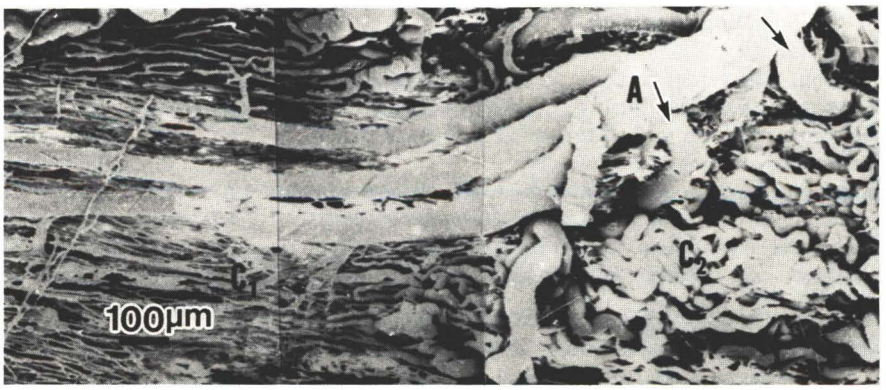


Fig. 22. The twigs (arrows), dividing at right angles from the artery (A), connected into the dilated abnormal capillary plexuses (C_2) with tortuosity. (C_1: normal capillary plexuses)

Fig. 16. Many dilated vessels were observed in areas of focal necrosis, while a few capillaries were observed in the normal myocardial layer. (light microscopy).

Fig. 17. In areas of focal necrosis fewer muscle bundles and many dilated vessels were observed (arrows).

Fig. 18. Dilated capillaries (C_2) and arterial branches (a) were running with tortuosity in the site of focal necrosis (C_1: normal capillary plexuses)

Fig. 19. In the site of focal necrosis, dilated capillaries (C_2) ran tortuously and formed coarse net-like architecture. (a: arterial branch, C_1: normal capillary plexuses)

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Discussion with Reviewers

A.C. Nelson: Apparently, the tissue preparation did not involve fixation nor was the tissue likely to be fresh. Were there any problems in casting that may have resulted from tissue distortion or rupture or from clotted vessels?
Authors: The heart was contracted by heating for polymerization, therefore the corrosion casts, in the dilated states of vessels in the contracted myocardium, were observed in this study. Rupture of vessels was observed in some spots.

A.C. Nelson: The authors have observed dilated capillaries in the region of necrosis. What is the origin of these dilated vessels? Since necrosis results from reduced vascular perfusion which leads to tissue death, why does the necrosis appear to be heavily vascularized with dilated vessels? This seems counter intuitive.
Authors: The origin of the dilated capillaries was unknown. It was obscure that the dilated capillaries were made with the decrease of myocardium after necrosis or with the view of hyperemia for restoration. These dilated capillary plexuses were observed in the area which communicated with the twigs branched at right angles from the arteries, therefore it was supposed, anoxia was repeated under various states in life.

A. Lametschwandtner: Could you please comment on the perfusion pressure you used to clean the circulatory system of the excised left posterior wall of the autopsy-heart free of blood? Did you use thrombolytic agents in the rinsing solution?
Authors: Thrombolytic agents were not used. Saline solution was injected under pressure of about 100–200 cmH_2O to clean the circulatory system, and saline solution (10–20ml) sometimes injected manually within 5–10 sec.

A. Lametschwandtner: How did you trim the casts to blocks of appropriate sizes?
Authors: The casts were cut and trimmed with a knife and a pair of scissors.

A. Lametschwandtner: When referring to the veins draining the capillaries of the epi-and myocardial layers of the heart you referred to them as, "gathering capillaries like branches of a tree." Do you mean that these veins are the so-called turnip-root-veins described by Brown (1965)? What is the situation in the endocardium?
Authors: The veins observed in this study were probably the same as the so-called turnip-root-veins, but the veins were not as thick as turnip-root and like branches of a tree in the human heart. In this study, the vein gathering capillaries in the endocardium was not observed.

A. Lametschwandtner: There is a still lasting debate on the nature of Thebesian veins of the heart. Would you please comment upon your failure to demonstrate these veins in your material?
Authors: In the macroscopical observation, a leakage of the saline solution was observed behind the trabeculae carneae. Thebesian veins probably extend into the left ventricular cavity behind the trabeculae carneae, therefore it was very difficult that the Thebesian veins were observed by scanning electron microscopy with the use of corrosion casts.

A. Lametschwandtner: Interestingly in the area of focal necrosis you convincingly could demonstrate dilated capillaries. The same observation is reported to occur in the capillary bed of skeletal muscle neighboring a malignant skin tumor in laboratory mice (Grunt et al., 1986). Could you comment upon the cause of capillary dilatation?
Authors: The cause of capillary dilatation was unknown. In our studies, the dilated capillaries were found in the peri-infarction area of the human and the rabbit heart. Probably, the dilated capillaries were observed in the damaged myocardium or skeletal muscle which resulted from anoxia and others.

A. Castenholz: Did you also examine stained sections in the light microscope which can give information about the structural situation of the vascular wall so that a more exact classification of the vascular corrosion casts with regard to capillaries, arteriolar and venous vessels as well as small arteries and veins was possible?
Authors: The light microscopical observation was not done. In the SEM observation of corrosion casts and tissues, arteries ran in straight lines and veins showed slight tortuosity. On the surface of artery, longitudinal groove-like indentations were observed. The surface of vein showed round to oval depressions probably made by the lining endothelial cell nuclei.

A. Castenholz: In the discussion you mention that, "a leakage of the fluid, when the coronary vessels were irrigated, was observed." Has this phenomenon preferably been observed on the venous side of the capillary bed in the normal myocardium or in the dilated vessels appearing in the necrotic region? It might then indicate an increased permeability or fragility respectively in these vascular areas.
Authors: In the macroscopical observation, a leakage of the fluid was observed behind the trabeculae carneae of the normal heart and also the pathological heart with focal necrosis. In this SEM observation, a leakage of the resin was not found.

S.J. Phillips: The authors show a SEM viewed area of focal necrosis. Where in the wall thickness was this lesion seen?
Authors: Focal necrosis area was seen in the endocardial side of the myocardium.

S.J. Phillips: Figure 19 shows in places a state similar to that we showed in the contracture-state cat heart. Would the authors comment on this similarity?
Authors: The abnormal arterial branches and the dilated capillary plexuses were also observed around the myocard infarct of the rabbit and the human heart. Probably, these abnormal vessels could be found in the damaged myocardium which result from anoxia.

Additional Reference

25. Grunt TW, Lametschwandtner A, Karver K, Staindl O. (1986). The angioarchitecture of the Lewis lung carcinoma in laboratory mice: A LM and SEM study. Scanning Electron Microsc. 1986; in press.