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THE MORPHOLOGY OF THE LYMPHATICS OF THE CORONARY ARTERIES IN THE DOG

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ABSTRACT

On the supposition that pericoronary lymphatics play an important role in the efflux of interstitial fluid from the blood vessel wall, we examined the morphology of pericoronary arterial lymphatics in the dog. After ligation of the principal epicardial drainage lymphatics, after ligation of the left anterior descending coronary artery, after induced pericoronary inflammation and after instillation of India Ink into the pericardial sac using light, dissecting, and electron microscopy. The findings were compared with non-operated (control) dogs.

Lymphatic drainage of the coronary arteries is via adventitial lymphatics, which do not penetrate to the media and via periadventitial lymphatics consisting of a subepicardial lymphatic plexus overlying the coronary arteries. The smaller arterioles in the ventricular muscle have many more accompanying lymphatics than do epicardial coronary arteries. In the latter arteries, prelymphatic channels formed by collagen fibers in the media likely transport interstitial fluid to the adventitial and periadventitial lymphatics. Arterial contraction also likely plays a role in propulsion of coronary arterial interstitial fluid towards adventitial lymphatics.

The vasa vasorum and the lymphatics are part of an influx-efflux fluid transport mechanism in the arterial wall. Accordingly, an understanding of their morphology is pertinent to an understanding of the metabolism of the arterial wall and certain diseases such as atherosclerosis. The vasa vasorum of the coronaries have received considerable attention (1-6), and it has been generally agreed that they are consistently limited to the adventitia and the outer layer of the media in normal arteries. The morphology of the lymphatics draining the coronary arteries, by comparison, has received scant attention probably in part because they are so difficult to study. Previously, Johnson (7) reported in the dog that there were no direct connections between the cardiac lymphatics and the coronary arteries but that lymphatic capillaries in the subpericardium overlay the coronary arteries and were in the superficial adventitia. In humans he noted that the subepicardial lymphatics overlying the main coronary arteries penetrated the adventitia as far as the junction with the media. He concluded that there was an abundant supply of vasa vasorum in lymphatics and the adventitia of the arteries.

A number of authors have proposed that periarterial lymphatics play an important role in the removal of lipoproteins from the arterial wall (8-13) and hence may be critical to the pathogenesis of atherosclerosis. Accordingly, we decided to study initially the coronary artery lymphatics in the normal dog, an animal with a low incidence of spontaneous atherosclerosis.

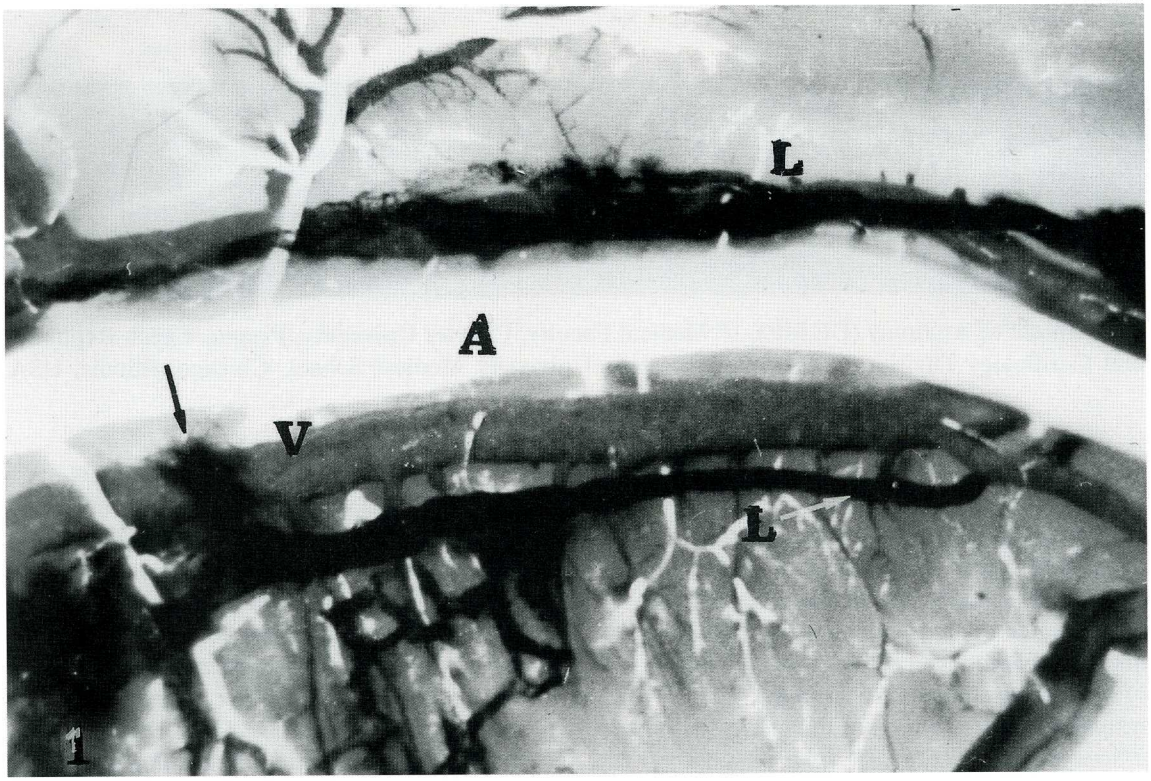

Fig. 1. Lymphatic vessels (L) accompanying the anterior interventricular artery (A) and vein (V) and approaching the artery (arrow). Cleared specimen, artery and vein injected with distinguishing markers. Original magnification x3.8.

MATERIALS AND METHODS

Collections of Specimens

Material was collected from dogs of both sexes weighing between 13 and 20 Kg. To determine the best method of visualizing pericoronary lymphatics, we initially studied five groups of dogs. Anesthesia was used in all operative preparations with sodium pentobarbital (25 mg/Kg body weight). Euthanasia was done by an overdose of intravenous sodium pentobarbital before harvesting the heart. In each instance, specimens were taken from the left anterior descending coronary artery, the left circumflex artery and surrounding tissue.

Group 1 (14 dogs)

This group (control) had no intervention. In 9 dogs, immediately postmortem the subepicardial lymphatic vessels of the heart were injected from the apex to the base of the aorta with a mixture of 2% gelatin and India ink through a 27 gauge needle (14,15). In these dogs and in all other dogs where we injected lymphatics, supplemental injections of the gelatin/India ink mixture were instilled at various sites in the adventitia of the coronary arteries. In 7 dogs, the main trunks of the coronary arteries and veins were injected with 5% gelatin colored with blue, green and violet pigments. The hearts were fixed in 10% formalin, and then the epicardial vessels with adjacent muscle were removed and dehydrated with increasing concentrations of alcohol and cleared in methysalicylate. In 5 dogs, the left anterior

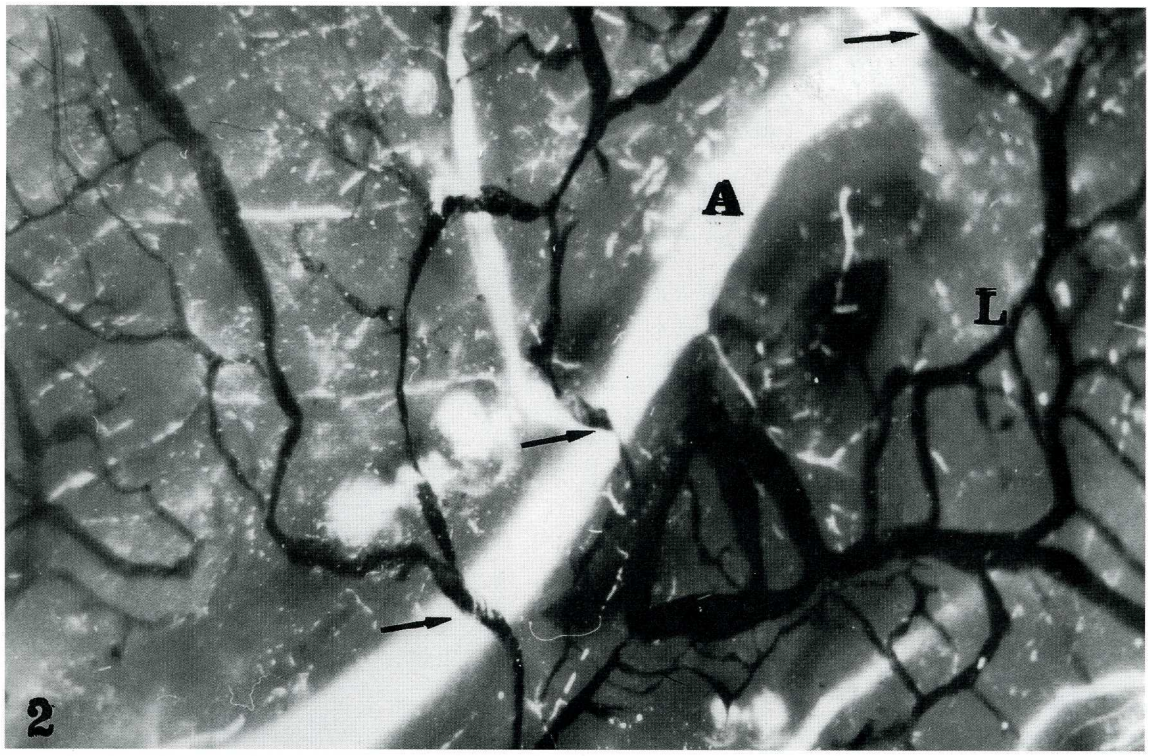

Fig. 2. Middle segment of the anterior interventricular artery (A) crossed by lymphatics (arrows). Cleared specimen, injected artery and vein. Original magnification x4.5.

descending coronary and the left circumflex arteries were removed with their adjacent tissue for light and electron microscopy.

Group 2 (14 dogs)

Using aseptic precautions and under general anesthesia and via a left-sided thoracotomy, both major ascending cardiac lymphatics were ligated at the level of the base of the aorta (14). The dogs were allowed to recover and survived from four hours to two months. After sacrifice, in 9 dogs, the subepicardial lymphatics adjacent to the coronary arteries were injected with a 2% gelatin/India ink mixture. Tissue specimens were then cleared with methysalicylate after dehydration. In 5 dogs, samples were taken of the left coronary and adjacent tissue on days 1, 3, 7 and 14 after the initial operative procedure for electron microscopy.

Group 3 (7 dogs)

In these dogs, a small amount of mercury (0.3-0.5 ml) was injected into the area of the adventitia around the left anterior descending coronary artery to produce an aseptic pericoronary inflammation. The dogs were sacrificed after 1 to 14 days, and the subepicardial lymphatics in 4 were injected with a 2% gelatin/India ink mixture. The removed tissue was then dehydrated and cleared with methysalicylate. In the remaining 3 dogs, the arterial samples were prepared for electron microscopy.

Group 4 (4 dogs)

A thoracotomy was performed and 0.4 ml India ink injected into the intact pericardial sac. Between 1 and 7 days thereafter, the dogs were sacrificed and tissue samples

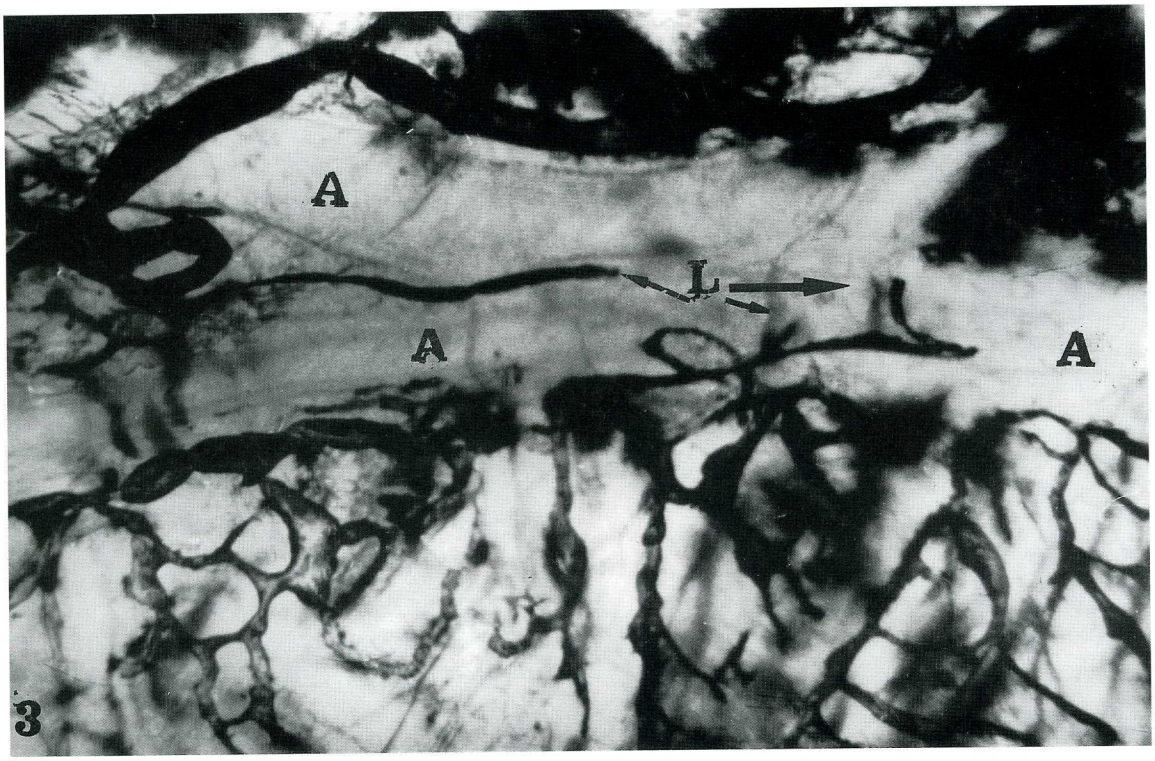

Fig. 3. A segment of the coronary artery paralleled by a lymphatic (L) on its surface. Cleared specimen. Original magnification x7.

including the epicardial coronary arteries were prepared for electron microscopy.

Group 5 (5 dogs)

In each dog, the chest was opened with a left-sided thoracotomy and the anterior descending coronary artery was ligated in its more proximal portion. The dogs were allowed to survive 1 to 6 weeks, after which specimens of the anterior descending coronary artery and surrounding tissue were taken from above and below the coronary arterial ligature.

Handling of Specimens

Light microscopy

These specimens were fixed in 10% formol saline. Transverse sections were stained with hematoxylin eosin, blue Masson trichrome and resorcin fuchsin (modification of Weigert method for elastic staining).

Electron microscopy

Each sample was taken from the proximal and middle portions of the left anterior descending and left circumflex coronary arteries. Transverse sections were made from eight sites from each artery. In half of the hearts in each group, an intravital perfusion of the coronary arteries was performed using Karnovsky solution (a mixture of paraformaldehyde and glutaraldehyde) under a pressure of 100 mmHg before removal of the specimens. In the remaining hearts, samples of the coronary arteries were taken without prior perfusion. All sections for electron microscopy were fixed in Karnovsky fixative and postfixed in

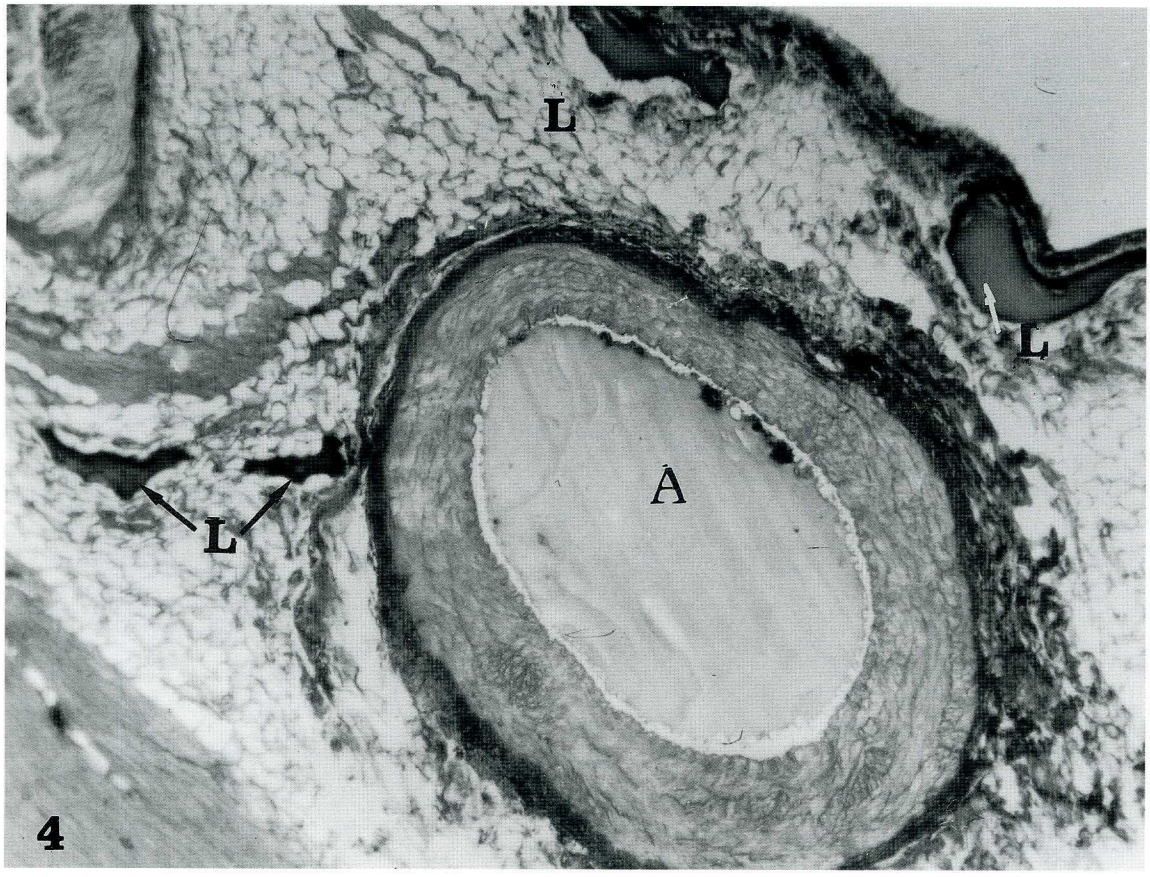

Fig. 4. Transverse section from the proximal anterior interventricular artery (A). India ink injected lymphatics (L) pass beneath the epicardium, and also branch at the lateral wall of the artery (arrows). Masson trichrome stain. Original magnification x15.

2% osmium tetroxide solution and embedded in araldite. Semithin sections were stained with toluidine blue, thin sections were contrasted with uranyl acetate and lead citrate. Sections were studied with the Philips electron microscope.

RESULTS

Observations Under the Dissecting Microscope

Study of the specimens from the dogs in Group 1 showed that injected principal epicardial and subepicardial lymphatics accompanied the epicardial coronary veins and coursed from the cardiac apex towards the base on both sides of the left anterior descending coronary artery *(Fig. 1)*. These principal lymphatics ascended along the coronary artery at a distance of from 0.5 to 4.0 mm from the artery. In areas where the lymphatic was closer to the artery *(Fig. 2)*, fine lymphatic branches traversed above or below the artery to join lymphatics surrounding the coronary vein. In these areas, the lymphatics formed eyelets around the artery. In cleared specimens of successful gelatin/India ink injections, fine lymphatics were seen near the adventitia of the arterial trunk *(Fig. 3)* which then opened into larger ascending lymphatics. These collections of

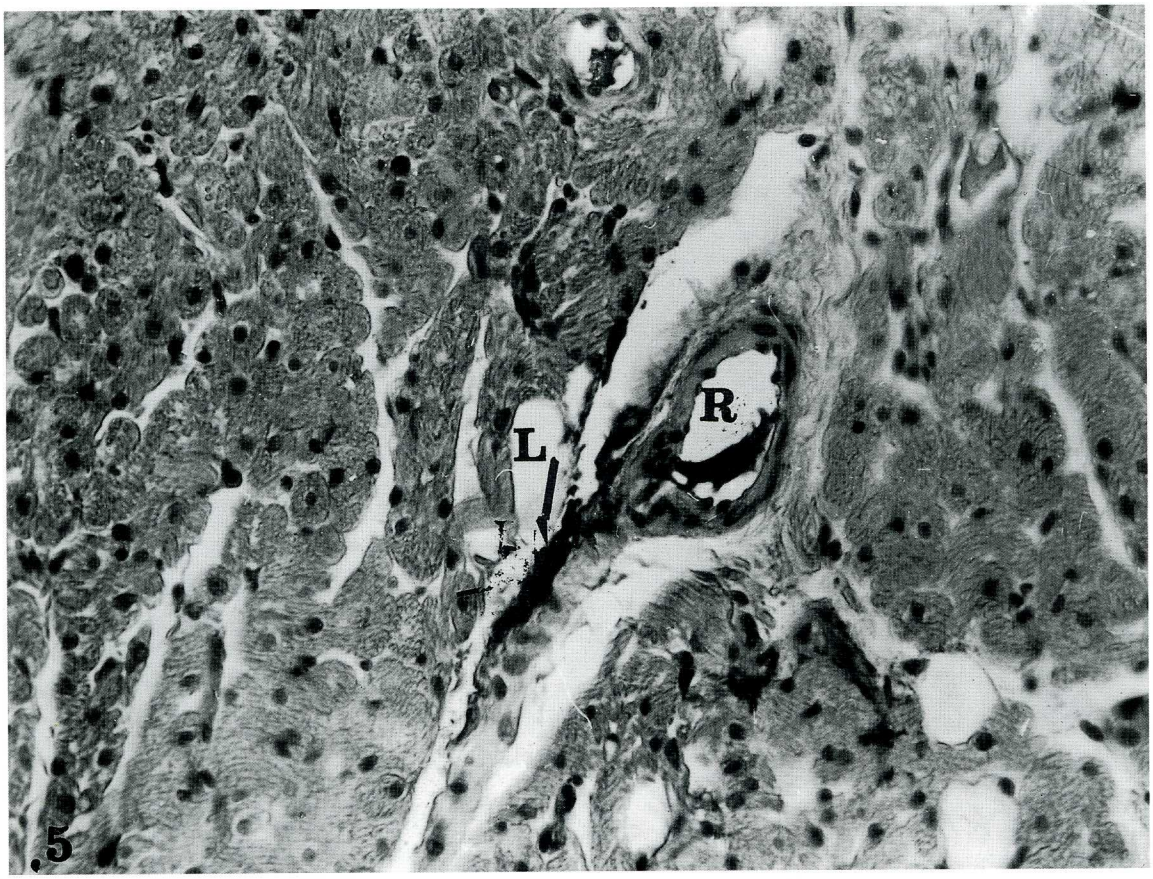

Fig. 5. Arteriole (R) is accompanied by a lymphatic (L). Hematoxylin eosin stain. Original magnification x41.

adventitial lymphatics in the coronary arteries were seen at a distance of 5 to 15 mm from each other; their relative sparseness was in distinct contrast to the rich plexi of vasa vasorum. A number of transverse sections showed injected lymphatics under the epicardium and adjacent to the lateral wall of the coronary artery *(Fig. 4)*. At times lymphatics were seen accompanying smaller coronary artery branches, but they were less frequent than the lymphatics seen in the adjacent muscle *(Fig. 5)*. In dogs that had ligation of the proximal lymphatic trunks (Group 2), the periarterial lymphatics were very similar to the findings in dogs without obstruction to cardiac lymph flow (Group 1). In both groups, the intramural coronary arterioles were accompanied by more lymphatics than the major epicardial coronary arteries.

Pericoronary injections of mercury (Group 3) produced localized edema in which lymphatics were dilated and more visible. Morphologically they looked similar to lymphatics in Groups 1 and 2.

Histological and Electron Microscopic Studies

Lymphatics were less abundant than vasa vasorum in each of the specimens studied and were present only in the outer layer of the adventitia *(Figs. 6,7)*. At no time were lymphatics seen to penetrate to the media. Both lymphatics and vasa vasorum

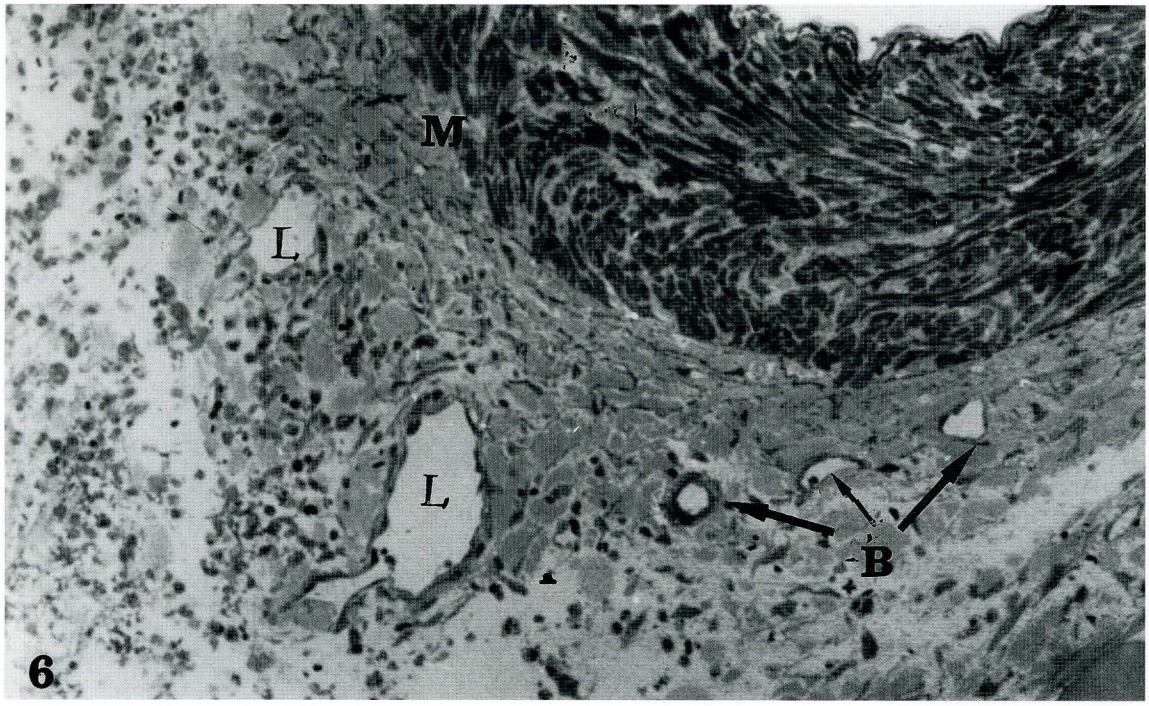

Fig. 6. Lymphatics (L) and vasa vasorum (B) in the outer layer of the adventitia of the anterior interventricular coronary artery. Smooth muscle cells of the media (M). Semithin section. Toluidine blue stain. Original magnification x80.

were more frequent in areas of thickened adventitia and were more prevalent on the lateral sides of the epicardial arteries. These adventitial lymphatics were more readily identified in dogs with lymphatic obstruction or pericoronary inflammation. In some areas, the walls of the lymphatics showed abundant vacuolization with opening of interendothelial junctions *(Figs. 8,9)*.

Lymphatics were not found in the muscular layer of an artery. However, between the smooth muscle cells collagen fibers were present which appeared to form interstitial tissue channels *(Fig. 10)*. In specimens taken from dogs with pericoronary inflammation and myocardial infarction, these tissue channels were substantially enlarged. Of note, too, was considerable coronary subintimal edema and the proliferation of smooth muscle cells. In the coronary endothelium, interendothelial gaps were widely patent and interendothelial channels were well formed *(Fig. 11)*. At several sites, the adventitial collagen and elastic fibers entering the muscle layer formed wide tissue channels but no lymphatics were seen.

DISCUSSION

Our findings support those of Johnson (7), who in the dog found lymphatics only in the periadventitial areas of the epicardial coronary arteries. He also did not find a direct anatomical relationship between the cardiac lymphatics and the coronary arteries, noting that the lymphatic capillaries in the subepicardium overlie the coronary arteries and are in the superficial adventitia. We identified lymphatics in the outer layer of the adventitia in the left anterior descending and left circumflex coronary arteries but did not detect adventitial or periadventitial lym-

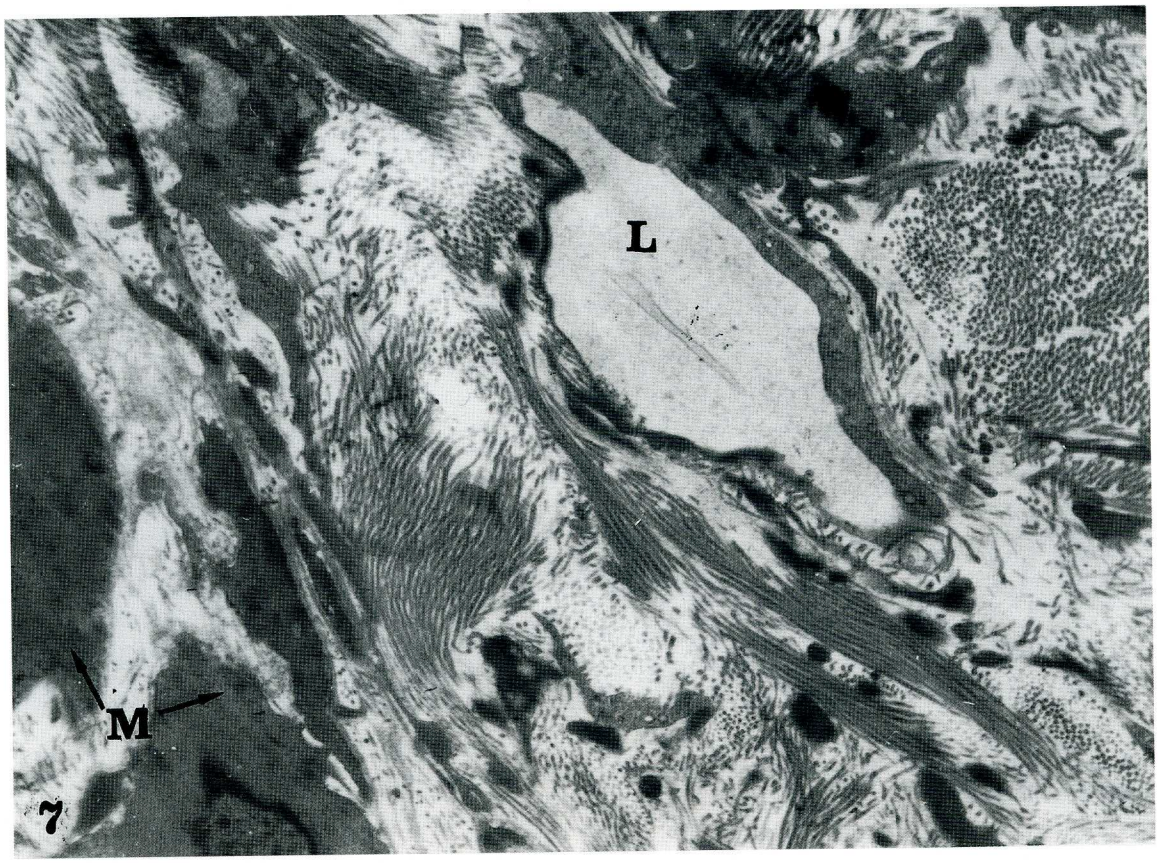

Fig. 7. Lymphatic (L) in the adventitia of the circumflex coronary artery. Smooth muscle cells (M). Original magnification x4,500.

phatics penetrating to the media in control or cardiac lymphatic obstructed dogs. The limited number of adventitial lymphatics we identified were irregularly spaced and the periadventitial lymphatics were sometimes so far removed from the coronary artery as to cast doubt on their having a significant role in fluid absorption from the arterial wall.

Fluid, proteins and macromolecules (e.g., lipoproteins) probably pass from the bloodstream through the arterial intima and across fenestrations into the internal elastic membrane. Once beyond the barrier of the internal elastic membrane, interstitial fluid and its contents may pass between smooth muscle cells along collagen fibers that form a network of "prelymphatics" (16,17). The numbers of such prelymphatics increase in the more peripheral aspects of the circular muscle in the media. In this scenario, arterial wall muscle contraction may facilitate the propulsion of interstitial fluid and its contents toward the adventitia.

Johnson (7) noted that macromolecular substances that are not capable of re-entering the bloodstream directly from the interstitium via venous capillaries because of molecular size, weight, and stoichiometric structure return to the blood circulation via lymphatics. He reasoned that the forceful pulsatile action in the arterial wall serves to push interstitial fluid substances through intercellular cleavage planes towards the adventitia, where they can then be reabsorbed by lymphatics.

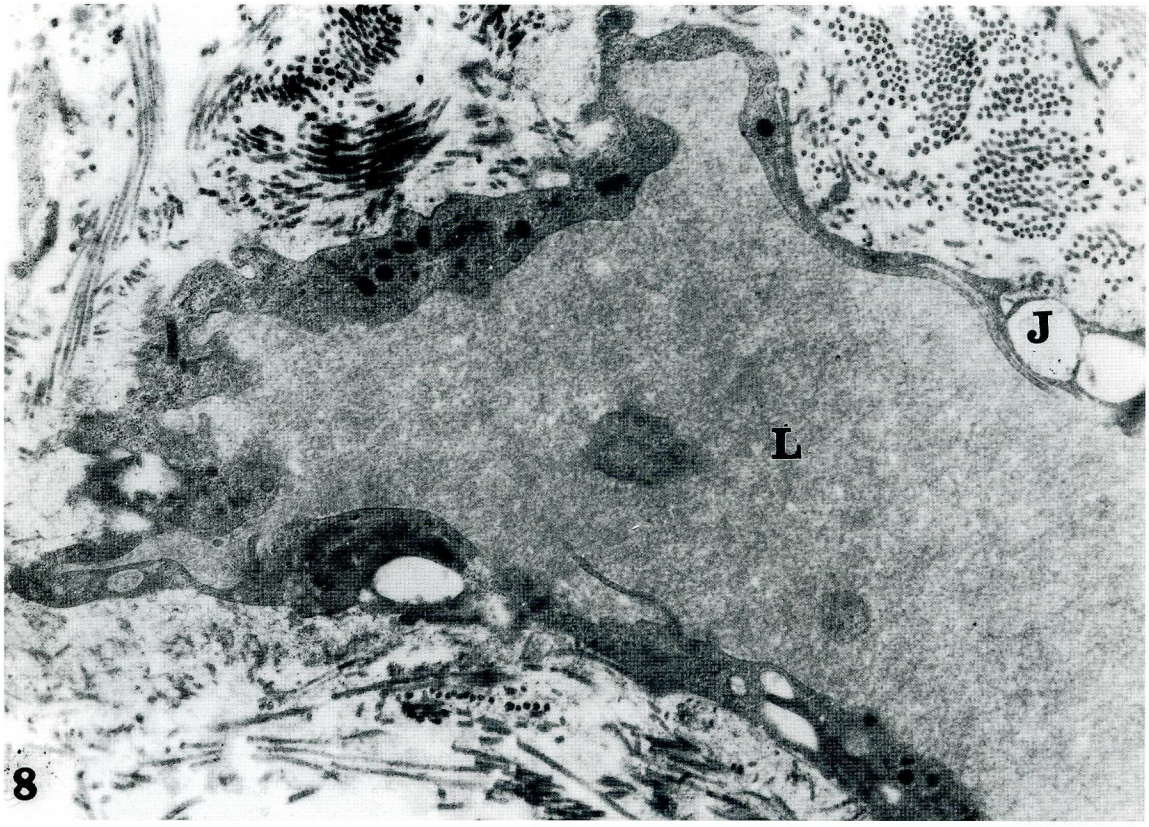

Fig. 8. Lymphatic (L) in the adventitia of the anterior interventricular artery from a dog with pericoronary inflammation. Vacuoles and open interendothelial junctions (J) form interendothelial channels. Original magnification x3,900.

The intercellular cleavage planes that Johnson described are comparable to the collagen created channels that are considered to be prelymphatics. In any case, it remains likely that a failure of the fluid efflux mechanism from an intramural region of a coronary artery is associated with entrapment of lipoproteins and predisposition to the development of atherosclerosis. Our studies in the dog complement the previous data and support some of the previous hypotheses but clear demonstration that lymphatic dysfunction plays a prominent role in the development of coronary atherosclerosis is still lacking.

It should also be emphasized that the technique of injecting the epicardial lymphatics is laborious and relatively crude. Visualization of the lymphatics that is attained is spotty, so that only certain sections of an artery can be meaningfully studied and assessed. The injection of 2% gelatin/India ink mixture into the adventitia is blind and rather haphazard, as is also the injection of mercury to incite a local inflammatory reaction. Accordingly, the conclusions drawn are necessarily based on relatively few anatomical sites at which there has been a fortuitous revelation of the morphology under study. At this stage, we know, however, of no other available techniques to make these studies easier and more productive.

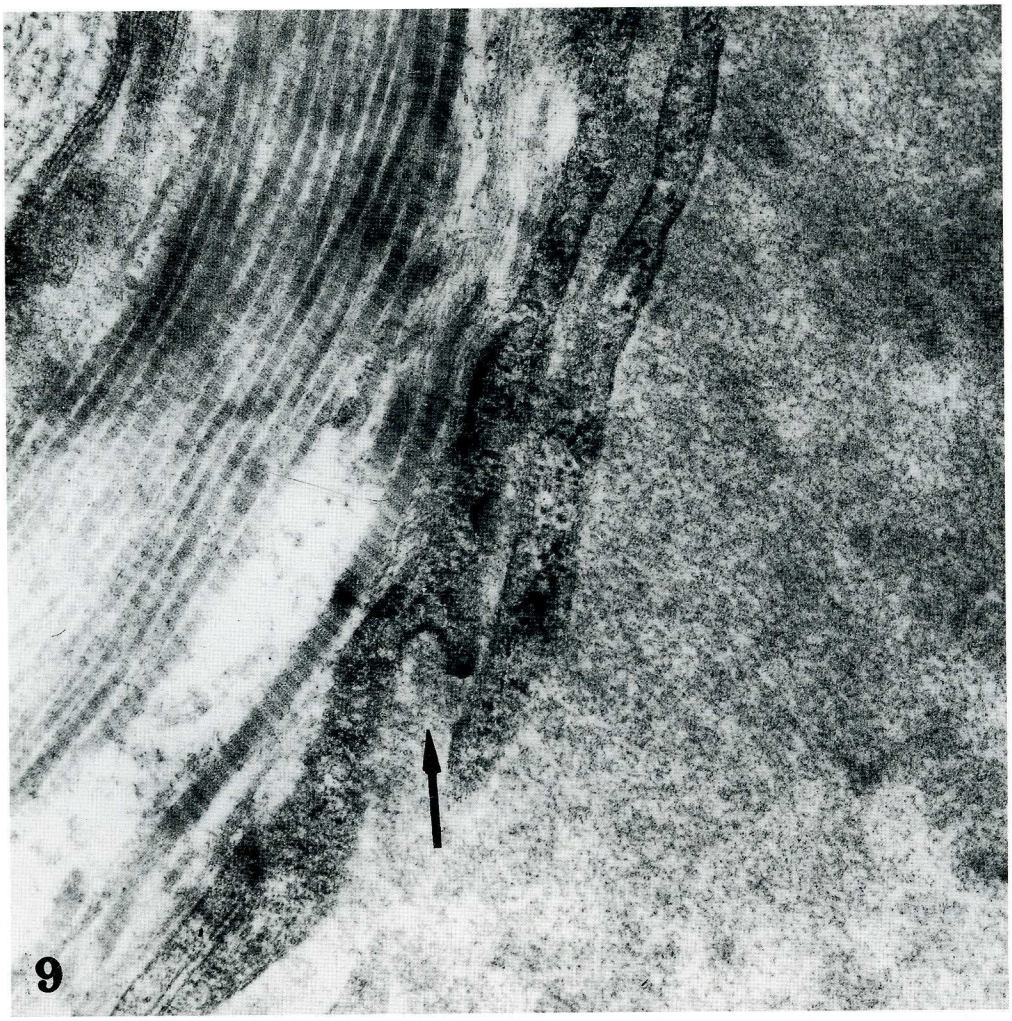

Fig. 9. Partially opened interendothelial junction (arrow) of a lymphatic capillary in the adventitia of the anterior interventricular coronary artery. Original magnification 12,500.

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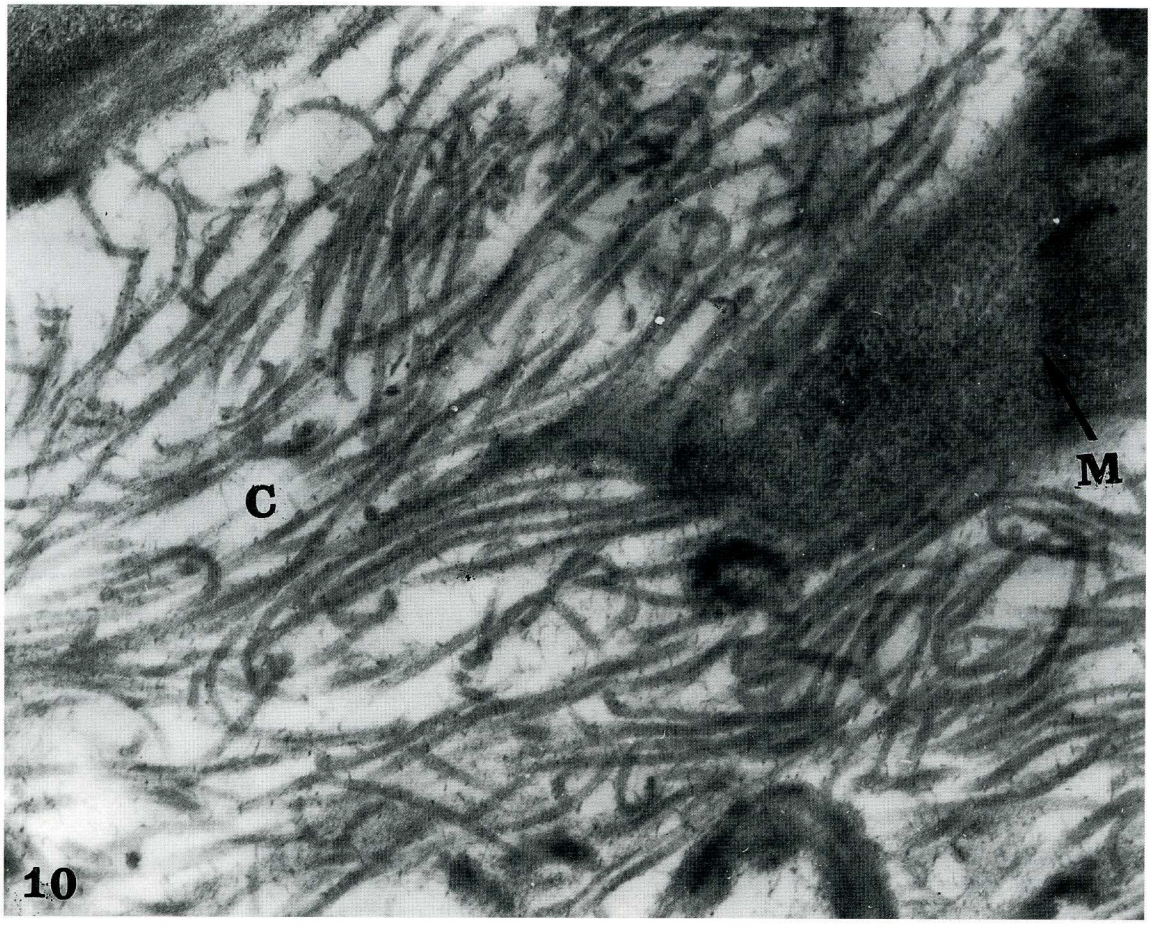

Fig. 10. A network of collagen fibers (C) between smooth muscle cells of the media (M) of the anterior interventricular artery. Original magnification 16,000.

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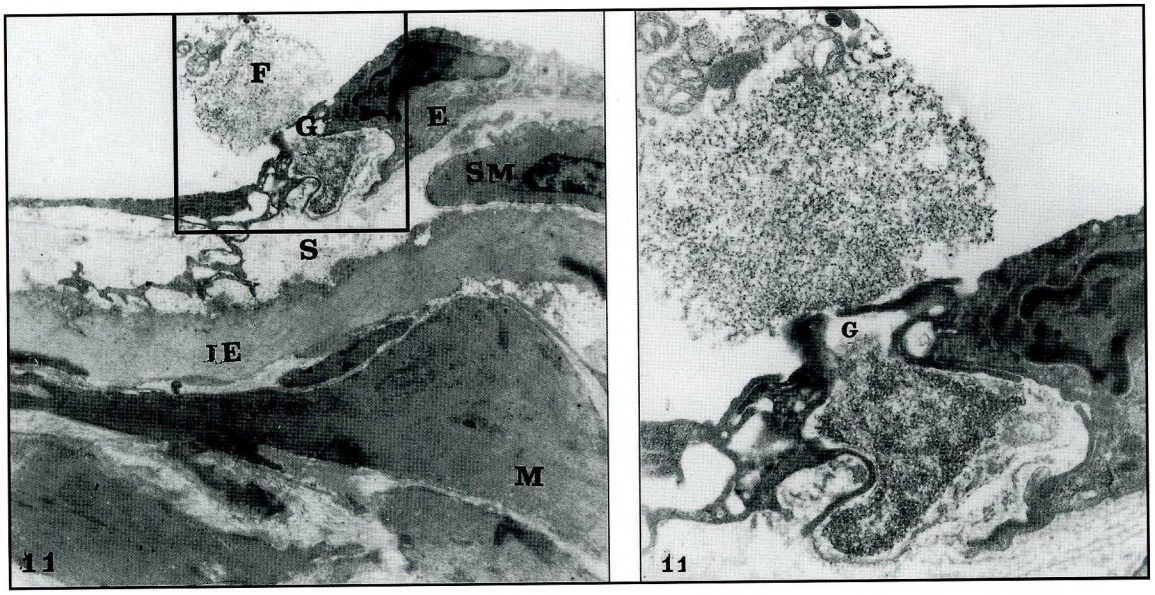

Fig. 11. (Left) Wall of the anterior interventricular artery 10 days after the periadventitial injection of mercury. Gaps (G) between endothelial cells of artery (E) allow communication between the arterial lumen and the subendothelial space (S). Smooth muscle cell (SM) is seen in the subendothelial space. Internal elastic lamina (IE), and smooth muscle cells of media (M) are also depicted. A partially cytolytic cell (F) traverses the endothelial gap. Original magnification x3,900. (Right) Close up of inset shown in Fig. 11. Original magnification 12,500.

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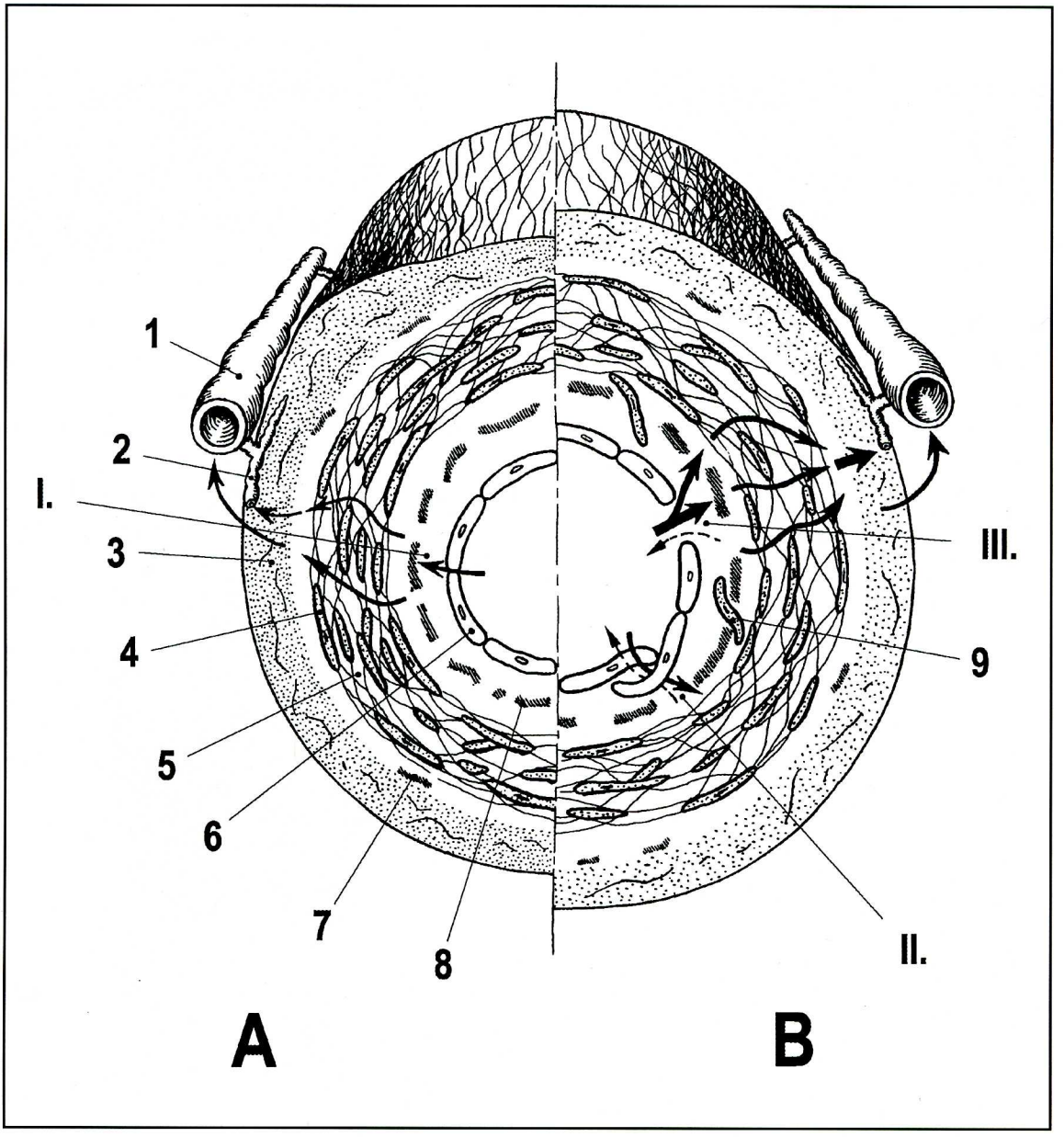

Fig. 12. Diagram of possible morphological drainage routes for interstitial fluid from the wall of the coronary artery. I—Arrows indicate the transendothelial passage of interstitial fluid from the lumen of the coronary artery to the subintimal area under normal circumstances. II—Arrow shows possible route of interstitial fluid through interendothelial channels. III—Arrows show possible route of interstitial fluid through gaps from the lumen of the coronary artery to the subendothelial space. The thick arrows show the drainage of the interstitial fluid through the whole wall to adjacent lymphatics. Section A—The wall of the coronary artery under normal conditions. Section B—An inflammatory reaction in the wall of the coronary artery, in which the wall is distended by edema. 1—Periadventitial lymphatic; 2—adventitial lymphatic; 3—adventitia; 4—media smooth muscle; 5—collagen fibers between smooth muscle cells forming interstitial tissue spaces (?prelymphatics); 6—coronary endothelium; 7—incomplete external elastic lamina; 8—fenestrated internal elastic lamina; 9—smooth muscle cells in subendothelial space.