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CORONARY ARTERIOPATHY AFTER LYMPHATIC BLOCKADE: AN EXPERIMENTAL STUDY IN DOGS

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ABSTRACT

The effects of lymph stasis on the histological and biochemical properties of the coronary arterial wall and on the coronary circulation were studied in 72 dogs. Cardiac lymph stasis was produced in 52 dogs by cardiac lymphatic blockade whereas in 20 dogs only a sham operation was performed. Blockade of cardiac lymph drainage promoted characteristic injury to the coronary arteries including subendothelial edema with plasma imbibition, interstitial and intracellular edema in the tunica media with degeneration in the smooth muscle layer, swelling of the adventitial space with dilated lymph vessels and, later, fibrosis. The biochemical properties of the coronary arterial wall also were adversely affected by cardiac lymph stasis. Thus, the collagen and hexosamine content of the coronary arteries increased and the metabolism of the coronary wall shifted in an anaerobic direction. Whereas coronary blood flow was slightly decreased with lymph blockade, the coronary circulatory reserve capacity and the adaptability of the coronary vascular system was markedly reduced. The histological changes were most apparent in the smaller coronary arteries. The coronary microvasculature was also pathologically altered with the development of numerous coronary arteriovenous microshunts. These findings in conjunction with other experimental and clinical information suggest that impaired cardiac lymph drainage contributes to the pathogenesis and progression of coronary artery disease.

Arteries are an organ system constantly bathed in plasma constituents with ongoing intramural metabolic processes. Interstitial substances within the arterial wall that are unable to re-enter the circulation or adventitial blood capillaries because of molecular weight, size and stoichiometric structure are transported back to the bloodstream by the lymphatic system within the vasa vasorum. With lymph stasis, the clearance of macromolecules such as lipoproteins slows, and, as previously shown promotes arteriopathy in a variety of vascular beds (1-8). In this study we examined alterations in the histologic and biochemical properties of the coronary wall after cardiac lymphatic blockade and the effect of lymph stasis on coronary blood flow.

MATERIALS AND METHODS

Experimental Design

Experiments were carried out on 72 mongrel dogs of both sexes (weight 13-21kg). Under sodium pentobarbital anesthesia and controlled endotracheal ventilation, lymph drainage from the coronary arteries in the heart was blocked in 52 dogs after an anterolateral thoracotomy through the third intercostal space. To facilitate visualization of lymphatics and nodes, Evans blue dye (10ml)

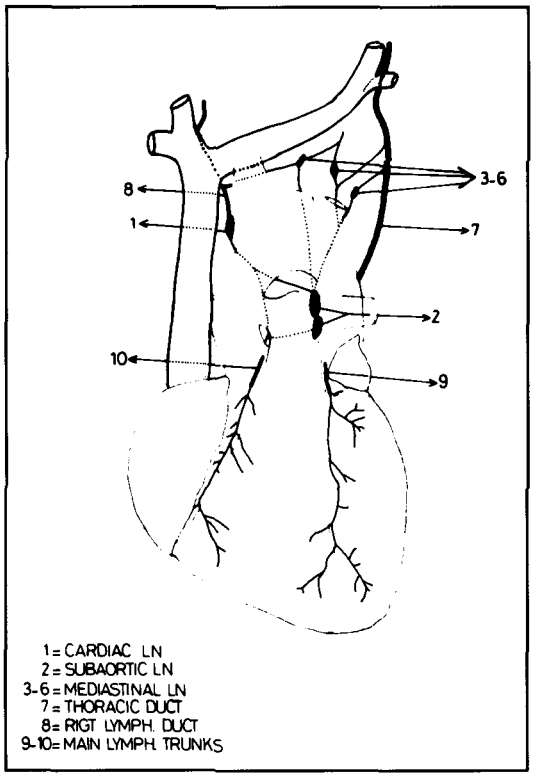

Fig. 1. Anatomy of the cardiac lymphatic system in the dog. In these experiments, the main cardiac lymphatics (9,10) were ligated and regional lymph nodes (1-6) were resected to produce lymph stasis.

was administered intravenously one hour before operation. The two major cardiac lymph trunks were ligated in the mediastinum and the cardiac lymph node, the subaortic lymph node and the mediastinal lymph nodes (2-4 present in the anterior and posterior mediastinum) were resected. In 20 dogs, only a sham thoracotomy was performed (control group). Twenty dogs were killed at two to four days following thoracotomy (acute lymph stasis); 18 at 7-13 days and 14 at 2 weeks (chronic lymph stasis).

Light and Electron Microscopy

For light microscopy, the specimens were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin, Van Gieson, PAS alcian blue, Mallory, azan and toluidine blue. For electron microscopy, the specimens were fixed promptly in cold 4.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.4. Durcupan ACM embedded ultrathin sections were subject to transmission electron microscopy.

Coronary Circulation

In 20 dogs (12 experimental and 8 controls), the coronary microcirculation was assessed (capillaries, precapillaries, and postcapillary venules) using benzidine reaction and gelatin India ink injection techniques. 2% India ink was injected into the left ventricle of the anesthetized dog just before killing with a concentrated solution of potassium chloride. Specimens were taken from various areas of the heart, fixed in 10% formalin and processed as frozen sections. After 24 hours in formalin fixative, sections of the heart were made in various thicknesses (20, 40, and 100μm) in three directions — parallel to the surface of the left ventricle, vertical to the base of the heart, and perpendicular to both directions of intersections. The blood supply of capillaries, precapillaries, and postcapillary venules was determined by benzidine reaction according to a technique described by Romeis (9). Gelatin India ink indirectly depicted coronary microshunts as "empty vessel spaces" usually with dilated postcapillary venules instead of capillaries filled with red blood cells. A polyvinylchloride (PVC) injection preparation was used to detect coronary arteriovenous shunts.

Biochemical Analysis

10mm cylinder segments were cut from the left and right coronary arteries. These segments were freed from loose adjacent periadventitial tissue and 1.0g was tested for cytochrome C oxidase, succinyl dehydrogenase and uridine diphosphate dehydrogenase levels using enzymatic Farb test as described by Brautigan et al (10) and Burchell (11). Arterial wall O_2 consumption and O_2

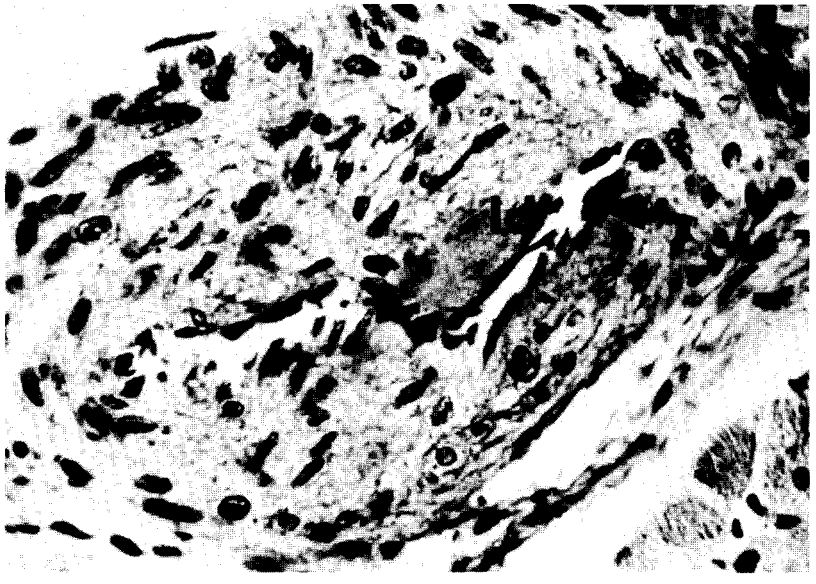

Fig. 2. Histological changes in a small coronary artery 4 days after lymphatic blockade. The arterial wall is thickened and the lumen is narrowed. Subendothelial pad is swollen (arrow) with plasma imbibition (Pi). Note homogenization and hyalinization of the media. Hematoxylin and eosin (x400).

production were measured by manometric changes in the O_2 and CO_2 pressure (12). The lactate content of the coronary arterial wall was determined by LDH UV-monotest and hexosamine level by colorimetry (13). A colorimetric method (14) was also used to measure arterial collagen content. In 18 dogs (10 experimental and 8 intact or controls), total coronary blood flow was determined using a ^{86}Rb isotope technique (15). Left coronary blood flow was measured with an electromagnetic flowmeter on the descending coronary artery in a baseline state during thoracotomy before cardiac lymphatic blockade and later during rethoracotomy.

For statistical analyses of the experimental data, the paired (flow meter studies) and unpaired Student's t tests (all other studies) were applied.

RESULTS

Light and Electron Microscopy

With lymph stasis, there was coronary arterial subendothelial edema with plasma imbibition within the intima (accumulation of PAS positive substance under the endothelial cells). Subendothelial edema was evident in the small coronary arteries producing an intimal cushion with narrowing of the vessel lumen. The media displayed interstitial edema, degenerative changes (fibrinoid necrosis) in the smooth muscle. Electron microscopy showed patchy necrosis and swelling of the mitochondria with ruptured cristae in the muscular layer. The adventitia demonstrated prominent interstitial edema with markedly dilated lymph vessels filled with granular material. Subendothelial edema with plasma infiltration, interstitial edema, and intracellular edema of smooth muscle with mitochondrial damage appeared early (i.e., two days after lymph blockade). Muscle damage of the media (patchy necrosis, fibrinoid necrosis) was seen one week after cardiac lymphatic ligation and nodal resection. Fibrosis in the coronary wall was apparent by ten days *(Figs. 1-4)*.

Coronary Microvasculature

With lymph stasis, there were regularly longer, more saccular dilated pre and postcapillary microvessels. Although coronary blood flow with lymphatic blockade was only

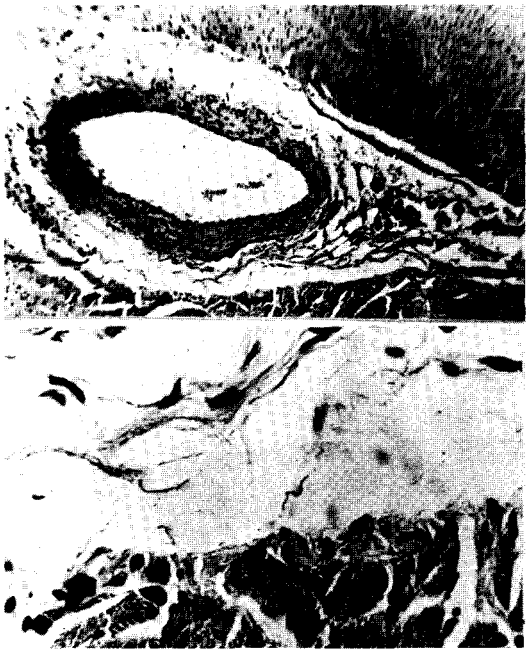

Fig. 3. Photomicrograph of coronary artery 12 days after cardiac lymphatic blockade. Note that the arterial wall is thickened with hyalinization and fibrosis of the media. The adventitial layer is also hypertrophied with evidence of fibrosis, dilated lymph vessels and infiltration with mononuclear cells. Hematoxylin and eosin x300 (upper); x500 (lower).

slightly diminished (~15%), coronary autoregulation was notably diminished as shown by decreased reactive hyperemia (after adenosine administration) and decreased peak flow following myocardial hypoxia (temporary coronary occlusion).

Biochemical Analyses (16 experimental at 7-14 days; 12 sham-operated controls)

With lymphatic blockade, there was greater lactic acid, sialic acid, collagen and hexosamine content in the coronary arterial wall *(Table 1)*.

Metabolism (Coronary Artery)

With lymph stasis, O_2 consumption fell, CO_2 production rose, succinyl dehydrogenase activity and cytochrome oxidase activity decreased whereas uridine diphosphate dehydrogenase activity rose *(Table 2)*.

DISCUSSION

Lymph flow potentially plays a pivotal role in the removal of macromolecules and cells that pass from plasma into the wall of an artery. Whereas transmural egress of plasma constituents into the arterial wall ensures nourishment and vascular integrity, the removal of proteins, lipoproteins, cells and other particulates is likely cleared solely by lymphatics within the vasa vasorum. With lymphatic insufficiency, accumulation of these constituents and especially lipoproteins may not only damage the arterial wall but also facilitate the development and progression of atherosclerosis (16,17). Plasma imbibition into the subendothelial space associated with lymph stasis probably increases the permeability of the endothelial cells which, in turn, likely aggravate arterial wall injury (18,19). The present study demonstrates that readily identifiable macroscopic changes occur in the coronary arteries with lymph stasis. The biochemical properties of the coronary arteries are also modified. Arterial metabolism shifts toward anaerobic glycolysis as the lactic acid, collagen content, and the hexosamine concentration each rise during lymph stasis. Of note, an increase in collagen and hexosamine content of the arterial wall is often observed with injury to the artery and in atherosclerosis (20-24). Moreover, the biochemical changes in the coronary artery with lymph stasis resemble those in the femoral artery after lymphatic blockage (8). It is also noteworthy that the arterial changes with lymph stasis were most pronounced in the small coronary arteries. As reserve coronary blood flow diminishes including the formation of microvascular arteriovenous shunts, the ability of the coronary circulation to respond to metabolic or hypoxic demands decreases (4). Along these lines, von Hausaman observed cardiac lymph stasis or lymphangitis in patients with

TABLE 1
Biochemical Properties (Mean ± SEM) of the Canine Coronary Wall Without (Control) and With Lymphatic Blockade (Experimental)

	Control (n=12)	Experimental (n=16)
Lactic acid content (mM/g protein)	38.20 ± 1.00	62.50 ± 7.80*
Sialic acid content (mg/g protein)	2.82 ± 0.50	6.05 ± 0.60**
Collagen content (mg/g protein)	42.50 ± 8.20	56.50 ± 0.75*
Hexosamine content (mg/g protein)	360.70 ± 44.10	570.20 ± 50.00**

*p<0.05, **p<0.01 (one tailed t test)

coronary sclerosis (25). Moreover, coronary sclerosis can be induced in experimental animals by artificial infection with a lymphotrophic type virus in which mediastinal lymphadenitis often accompanies intramural vascular change to the coronary arteries (26-30). Coronary atherosclerosis also has been observed after irradiation of the mediastinal cardiac lymph nodes (31-37). Impaired cardiac lymph drainage probably explains the genesis of such coronary arterial lesions. Furthermore, coronary atherosclerosis can synergistically be produced by combining high cholesterol diet with mediastinal irradiation (38). Severe coronary arterial lesions also are associated with Kawasaki disease in children (39,40), a mucocutaneous lymph nodal disorder in which viral mediastinal lymphadenitis typically precedes the manifestation of coronary arterial lesions. A variety of other clinical and experimental data also favor that cardiac lymph stasis contributes to the development and progression of cardiac

TABLE 2
Metabolism of the Coronary Wall After Lymphatic Blockade*

O_2 consumption	↓ 55.0 ± 8.1**
CO_2 production	↑ 35.0 ± 4.5**
Enzyme activity	
Succinyl dehydrogenase	↓ 60.5 ± 8.0†
Cytochrome oxidase	↓ 70.6 ± 9.2†
Uridine diphosphate dehydrogenase	↑ 90.5 ± 10.5**

*Data (Mean ± SEM) are expressed as the Percentage change from Sham-Operated (Control) Values
**p<0.05
†p<0.01 (one tailed t test)

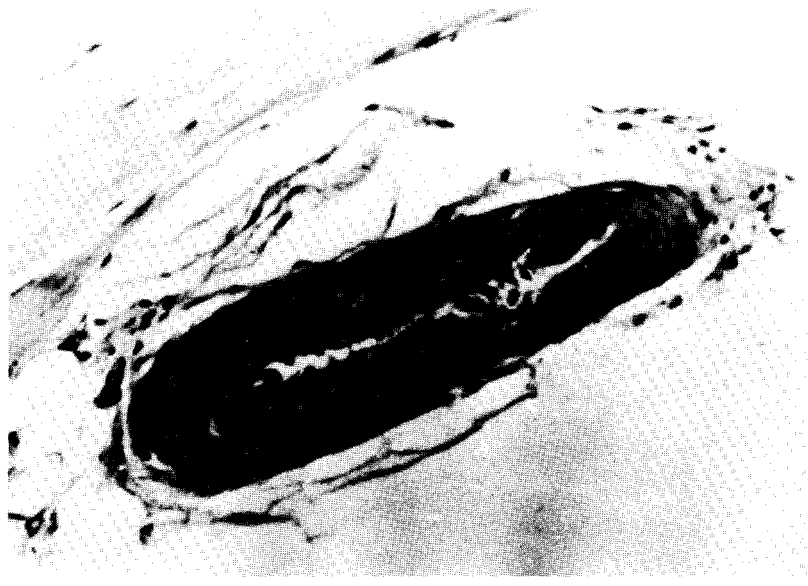

Fig. 4. Photomicrograph of coronary artery 14 days after cardiac lymph blockade. The lumen is markedly narrowed, the wall homogeneously thickened with hyalinization, necrosis of the media with fibrosis of the media and adventitia. Mallory's phosphotungstic acid-hematoxylin (x400).

atherosclerosis (41-43). Finally, in four patients with extensive mediastinal lymph nodal disease (two with Hodgkin disease and two with sarcoidosis), we noted the sudden onset of angina pectoris. In 2 of these patients, coronary arteriography confirmed highly stenotic coronary arterial lesions.

In conclusion, extensive blockage of cardiac lymphatic drainage promotes damage to the coronary arterial vascular wall with greater anaerobic glycolysis, light microscopic and ultrastructural changes consistent with subendothelial edema, altered microvascular permeability and abnormal microvascular flow dynamics to the heart musculature. In support of earlier studies of other arteriopathies, it is reasonable to conclude that lymph stasis contributes to the pathogenesis of coronary atherosclerosis.

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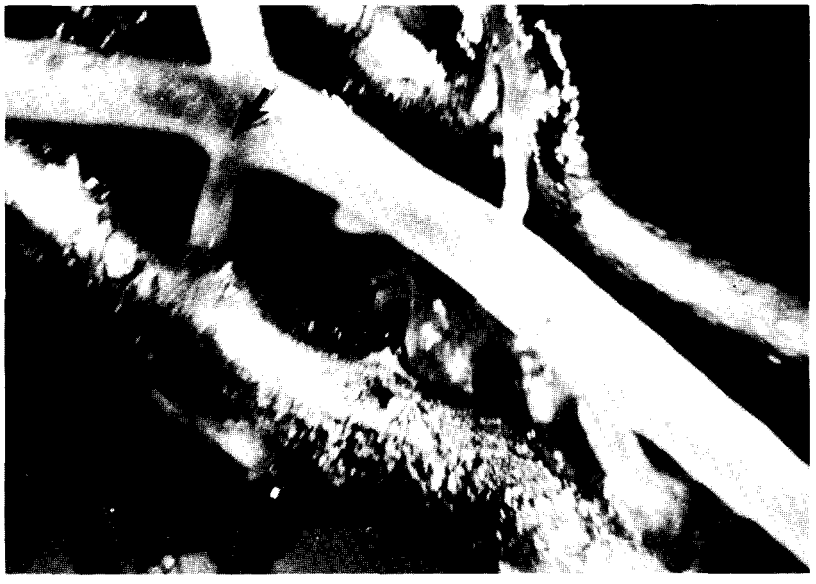

Fig. 5. PVC corrosion cast preparation of the heart 72 hours after cardiac lymph blockade. Note a major arteriovenous shunt (↓) between a coronary arterial branch and a collecting cardiac vein.

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