not resulted in any microscopic pathological alteration. These characteristics combined with the ease of preparation and administration suggest that the technique may be useful for acute as well as long term studies in experimental animals and possibly in man.

Summary

This report describes a method for obtaining x-ray visualization of hepatic lymph nodes following intravenous injection of contrast material. The method depends upon the uptake of particles of tantalum by the liver and subsequent transport of tantalum in hepatic lymph. Three to four days prior to x-ray visualization of these nodes, the liver and spleen developed a high degree of radiopacity. Because tantalum is chemically and physiologically inert, the technic may be useful in experiments in animals and possibly in man.

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The Lymphatic System of the Heart*

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In recent years lymphatics of the heart have been studied with renewed interest. There have been investigations of the drainage and composition of cardiac lymph (1, 2, 3, 4, 5) as well as studies on the effects of impeding the flow of lymph from the heart through major drainage channels (6, 7). However, many facets of the anatomical details of the intrinsic lymphatic vasculature of the heart itself are still lacking. The more recent anatomical studies (8, 9, 10, 11) have been with injection techniques to acquire information concerning lymphatics in the mammalian heart. In addition, investigations have added further to the knowledge of the relationship of lymphatics to the valves of the heart (9, 12) and have implied also a pathogenic relationship of impaired myocardial lymph drainage to endocardial fibroelastosis (13). We have observed in our laboratory that masses injected into either the coronary artery or vein in pig and dog

* Supported in part by the Mississippi Heart Association, and Grants HE-07159 and 2-F2-HE-19, 626-02 from the National Heart Institute, U. S. Public Health Service.

hearts appear readily in the lymphatic system suggesting presence of lymphatic-blood vascular communications (11).

In general, the major factors interfering with obtaining adequate information about the anatomical features of cardiac lymphatics are the limitations of the available methods of study. The small size and delicate structure of peripheral lymphatics and the presence of valves practically preclude their demonstration by direct injection. Often lymphatics are visualized with vital dyes which are injected into living specimens. In 1922 *Magnus* and *Stübel* (14) described a method of demonstrating lymphatics on serous surfaces in nonviable tissue by the direct application of hydrogen peroxide. This method in conjunction with injection techniques was applied in our laboratory to delineate some of the anatomical relationships of subepicardial and subendocardial lymphatics of the heart (15).

Methods

Hydrogen peroxide initiates an oxidoreduction reaction with catalase and peroxidase in tissue or lymph, or both, producing oxygen and water (16, 17, 18). The released oxygen causes distention of lymphatics and sometimes blood vessels; the two may be differentiated by their morphological characteristics. In regard to serous surfaces such as the epicardium and endocardium, lymphatics are superficial, transparent, and generally have a bulbous, irregular contour due to presence of valves (3, 4, 8, 19). Blood capillaries are narrower, uniform in shape, and have a general arrangement more orderly than lymphatics. Artifacts produced by interstitial dissection of released oxygen beneath the epicardium or endocardium form inconsistent patterns unlike lymphatics or blood vessels and are recognized easily.

Hearts of 25 pigs, 13 dogs, and 20 humans were examined. Pig hearts were obtained at a local abbatoir from healthy animals approximately six months of age. The dog hearts, obtained from animals approximately two years of age, were furnished by the experimental surgical laboratory. Human heart specimens were acquired at random from autopsies. Observation of subepicardial and subendocardial lymphatics is facilitated by cutting open the hearts according to a method described by *Schlesinger* (20), modified by leaving the ventricular septum intact. A 1% solution of hydrogen peroxide applied topically with a cotton-tipped applicator results in distention of lymphatics in the region of application. The reaction is more effective in some hearts than in others but seems to be improved in specimens refrigerated 24 hours or longer before study, and still occurs in those refrigerated for more than a week. Frothing produced by the peroxide reaction is a complication that obscured some vessels but can be diminished by placing the specimens in 10% formalin for 30 to 60 minutes prior to the use of peroxide. Whole specimens can be immersed effectively in peroxide, but in general, results are best in unfixed tissue with local application.

Specimens are observed through a stereomicroscope or with the naked eye. As they become distended, lymphatics extend beyond the frothy area where peroxide is applied and are seen clearly. They remain distended for several minutes allowing time for photography and measurement with an ocular micrometer. The channels can be distended repeatedly by reapplication of peroxide.

Photographs are made with Kodachrome II, professional type A film, and a 35 mm Exakta camera back attached through one ocular of the stereomicroscope with magnification ranging from 7 to 30 times. Exposure times are determined by means of a photometer inserted into the other ocular of the stereomicroscope. Highlights of the vessels are brought out by varying the angle and intensity of the light from a standard 35 mm slide projector equipped with a 500-watt bulb.

Injection of India ink or lead chromate (11) and clearing by the *Spalteholz* method (21) were used in a number of specimens included in this study.

Results

Observations in Dog and Pig Hearts

A dense network of subepicardial lymphatic capillaries (15–20 microns in width) surrounds the heart. These join channels of intermediate size which converge toward larger channels (2–3 mm in width) accompanying the anterior and posterior coronary blood vessels. The channels accompanying the major coronary vessels arise from a capillary plexus at the apex of the heart and progress with increasing caliber toward the base of the heart. The general arrangement of the apical plexus of lymphatics and the paracoronary ducts is illustrated (Fig. 1) in a pig heart specimen in which lead chromate was injected into the left anterior descending coronary artery. (This phenomenon has been discussed in a previous report (11) and suggested that lymphatic-blood vascular anastomoses were present.) Continuation of the anterior and posterior paracoronary lymphatic ducts form a large duct in the atrio-ventricular sulcus from which the main cardiac lymph duct arises (4). Other intermediate sized channels over the myocardium course toward the base of the heart and join the channel in the atrio-ventricular sulcus. The subepicardial capillary network over the atria is similar to that over the ventricles, and ducts of similar magnitude join the duct in the atrio-ventricular sulcus.

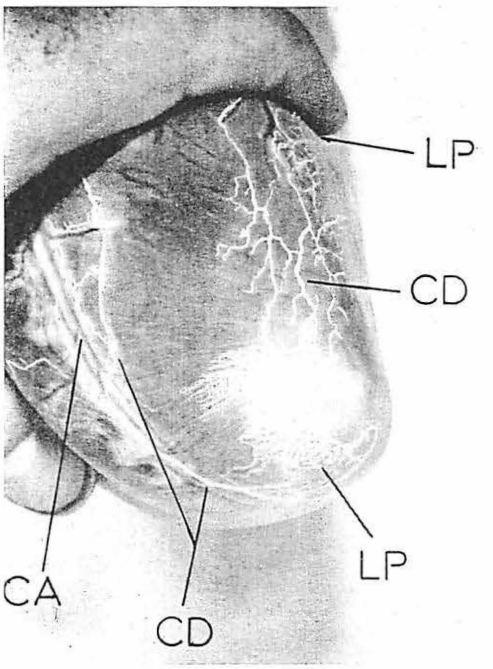

Fig. 1 Pig heart in which lead chromate was injected into the left anterior descending coronary artery with concomitant filling of the lymphatic system (see text). CA-left anterior descending coronary artery; CD-lymphatic collecting duct; LP-lymphatic capillary plexus near the apex of the heart.

Beneath the endocardium of both ventricles, including the septum, small lymphatic vessels of relatively uniform caliber (15–20 microns in width) form

a dense network oriented transversely to the subjacent muscle fibers. These vessels are regular in contour and do not appear to contain valves (Fig. 2). Re-approximating the cut edges of the heart and viewing the ventricular chambers from above, the network appears in a spiral arrangement.

Over the apices of papillary muscles the channels become larger, with a bulbous appearance suggesting the presence of valves (Fig. 3), and in dog hearts these channels form a denser network than in the pig heart (Fig. 4). In both species, blood vessels course from the apices of the papillary muscles along the chordae tendineae and con-

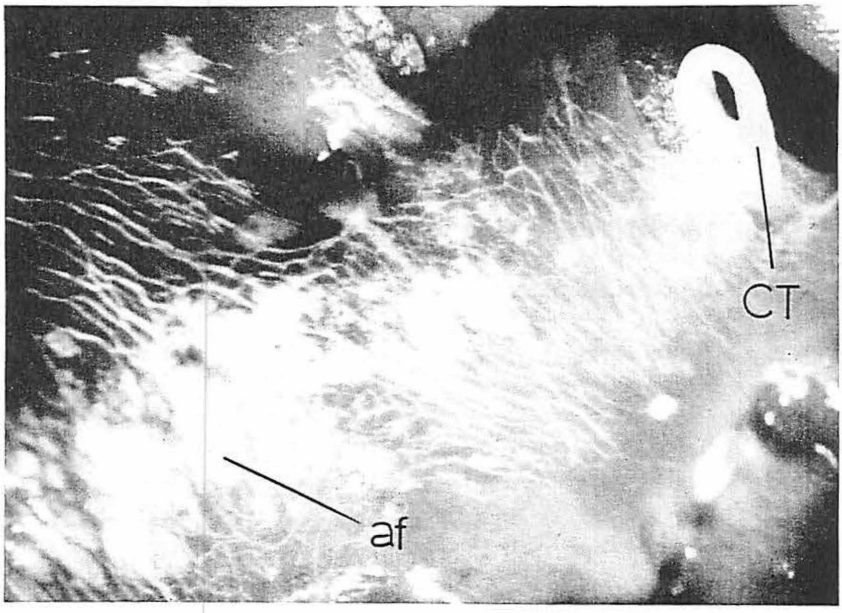

Fig. 2 Subendocardial lymphatic capillaries (15–20 microns in width) overlying a papillary muscle and adjacent myocardium in the left ventricle of a pig heart. The longitudinal axis of the papillary muscle extends from left lower toward the right upper portion of the photograph. A fragment of chorda tendinea (CT) is near the apex of the papillary muscle which has been cut. The fluffy areas (af) are artifacts produced by the peroxide reaction. Magnification 8×.

tinue into the atrioventricular valve cusps (Fig. 5). Lymphatics extend from the apices of the papillary muscles for only short distances along the chordae and communications with the lymphatics in the atrio-ventricular valves are not demonstrated.

In the superior portions of the interventricular septum, channels (60–100 microns in width) are directed longitudinally toward the atrioventricular junction, but their termination has not been identified.

Beneath the endocardium of the atrial surfaces of the tricuspid and mitral valves lymphatics extend from the free margins of the cusps to the annulus of each valve, where they join a larger channel ranging in width between 110 and 225 microns (Fig. 6). Networks on the valves are not as dense as those beneath the ventricular endocardium.

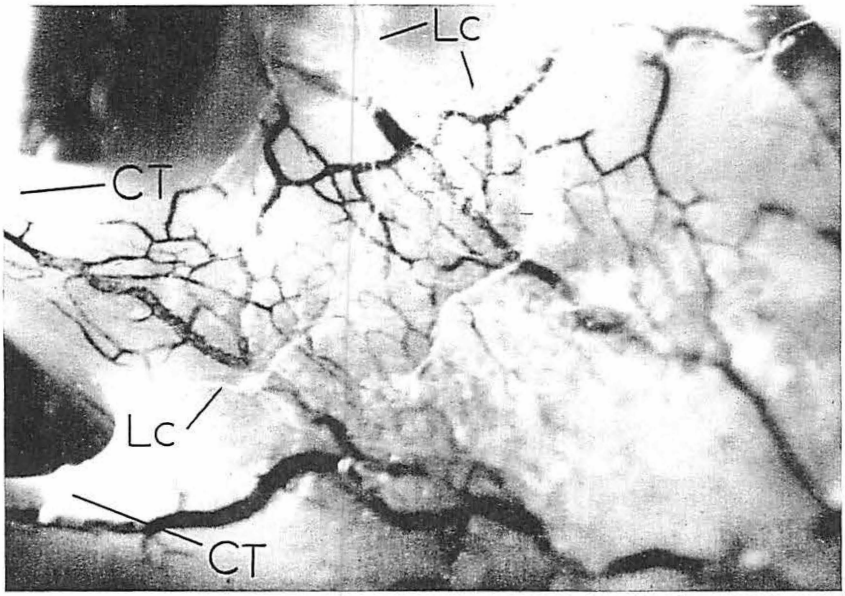

Fig. 3 Lymphatic channels overlying the apex of a papillary muscle in a pig heart. Blood vessels have been injected with India ink and are seen extending along the chordae tendinea (CT). Lymphatic channels (Lc) 30–60 microns in width are superficial to the blood vessels. Frothing artifact due to the peroxide reaction is in the right lower portion of the illustration. Magnification 18×.

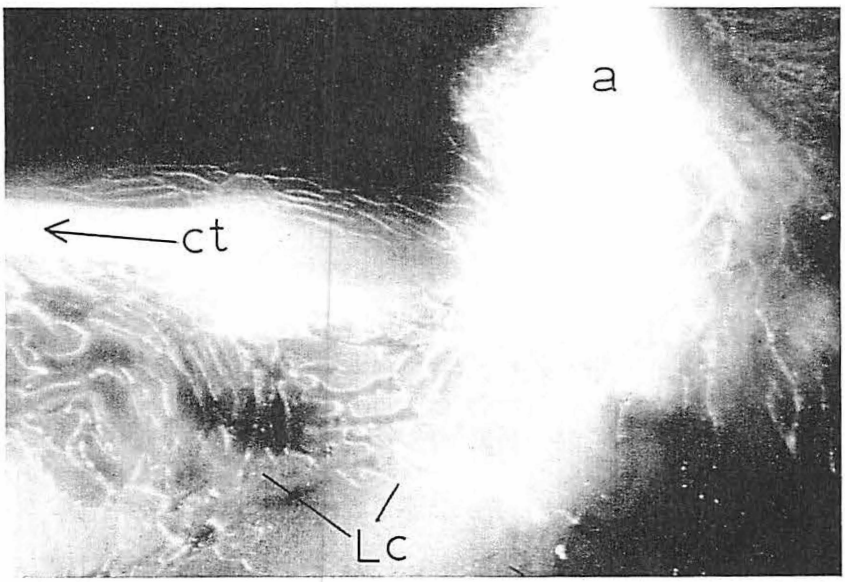

Fig. 4 Lymphatic channels (Lc) 40–60 microns in width overlying apex of a papillary muscle in the left ventricle of a dog. A chorda tendinea (ct) extends from the center of the illustration toward the left and lymphatics are seen in the initial portion of the chorda near its attachment to the apex (a-frothing artifact produced by the peroxide reaction). Magnification 10×.

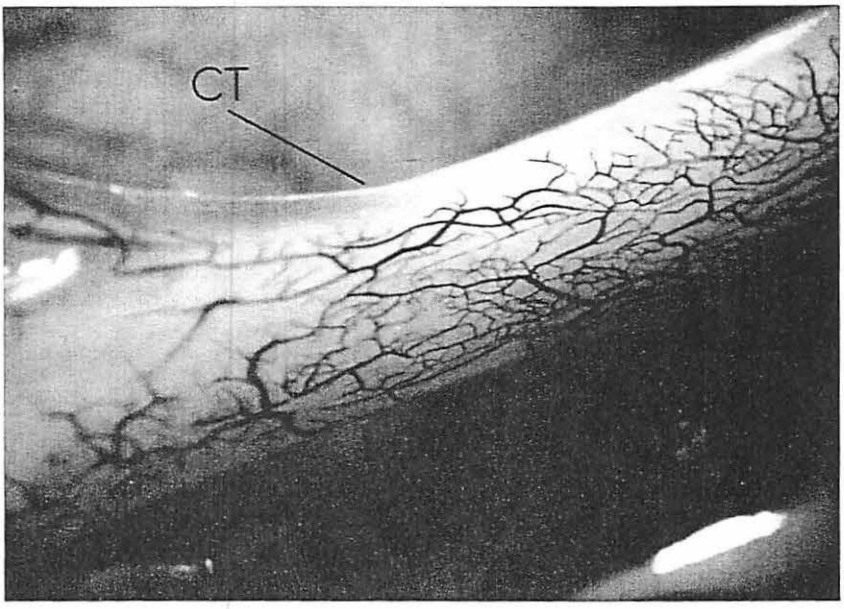

Fig. 5 Blood vessel network along a chorda tendinea (CT) in the left ventricle of a pig heart demonstrated by India ink injection. Magnification 10×.

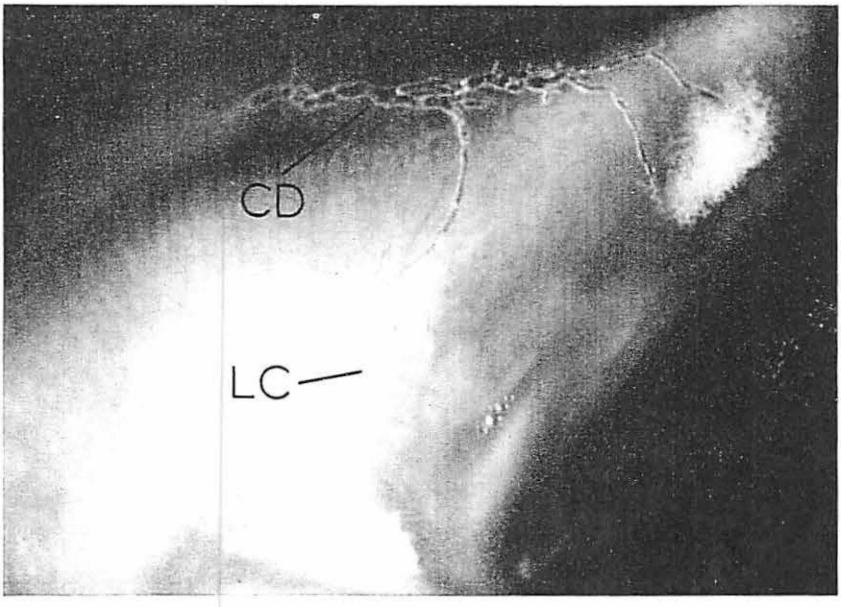

Fig. 6 Lymphatic channels (LC) in the mitral valve of a pig heart joining a collecting duct (CD) 170 microns in width in the valve annulus. White patches are artifacts due to frothing produced by the peroxide reaction. Magnification 7×.

The channels range in width between 20 and 30 microns and their irregular contour suggests that they contain valves. In some specimens blood vessel networks in the atrio-ventricular valves were injected with India ink and, after applying peroxide, lymphatics were demonstrated superficial to the blood vessels (Fig. 7).

Subendocardial lymphatics in the atria are not demonstrated as clearly as those in the ventricles but their pattern is similar, and channels 60–100 microns in width join the larger channels in the annuli of the atrio-ventricular valves.

In pig hearts India ink injected into the duct of the annulus of the mitral valve appears in the subepicardial duct of the atrioventricular sulcus. The connection between

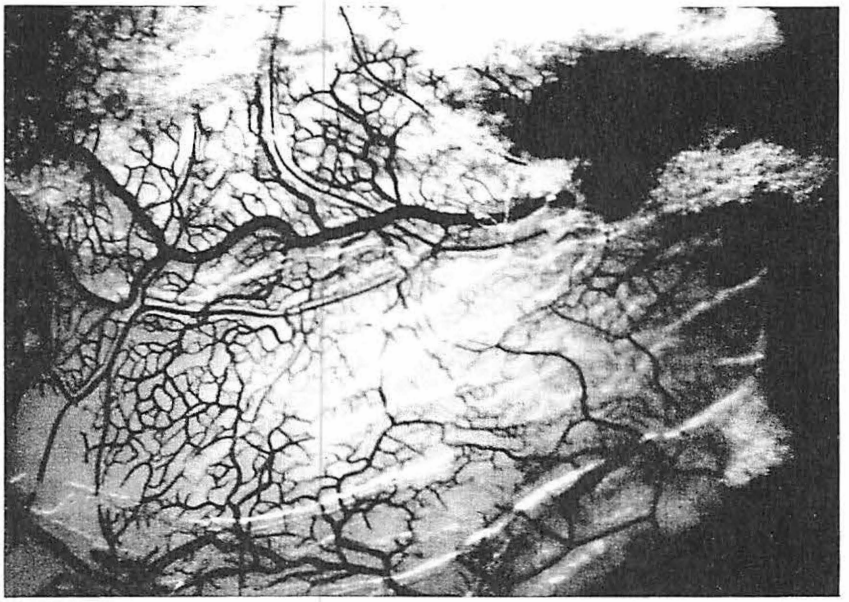

Fig. 7 Anterior leaflet of mitral valve in a pig heart in which blood vessels have been injected with India ink; lymphatics 20–30 microns in width, demonstrated by application of peroxide, are superficial to the blood vessels. This is a fresh specimen and has been neither formalin fixed nor cleared. Magnification 10×.

these channels is in the posterior atrioventricular junctional tissue near the interatrial septum and measures between 120 and 300 microns in width. Also in pigs, India ink injected into the apices of the anterior papillary muscles of the left ventricle appears in regional subepicardial lymphatics. A deep channel (60–100 microns in width) traverses the long axis of the papillary muscle, passes through the myocardium, and joins subepicardial lymphatic channels of similar magnitude (Fig. 8). Transmyocardial channels may be seen communicating with subepicardial lymphatics in specimens cleared and dissected after random injection of India ink beneath the endocardium.

Lymphatics could not be demonstrated in aortic and pulmonary valves or on the ventricular surfaces of mitral and tricuspid valves.

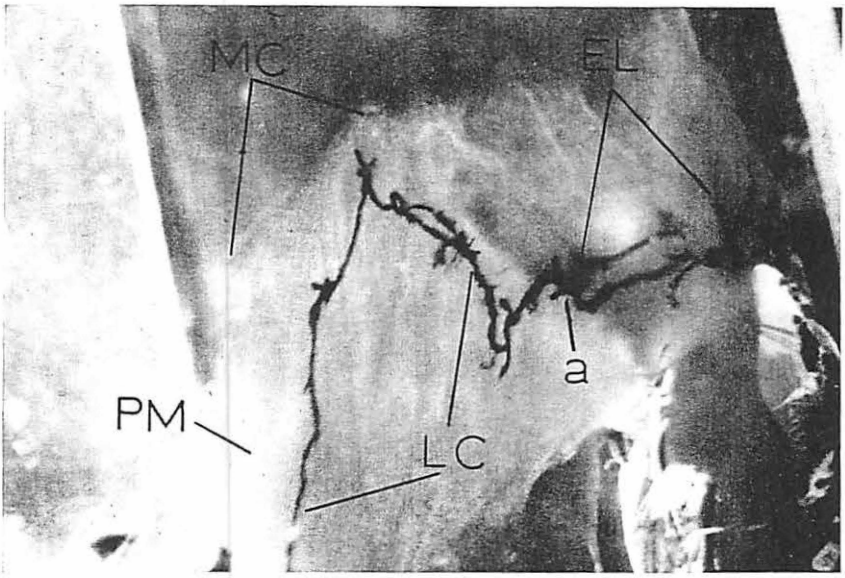

Fig. 8 Anterior papillary muscle of left ventricle in a pig heart which has been partially dissected. The photograph is made at an angle to demonstrate the junction (a) of subepicardial lymphatics (EL) with the lymphatic channel (LC) passing through the papillary muscle (PM) and myocardium (MC). Magnification 7×.

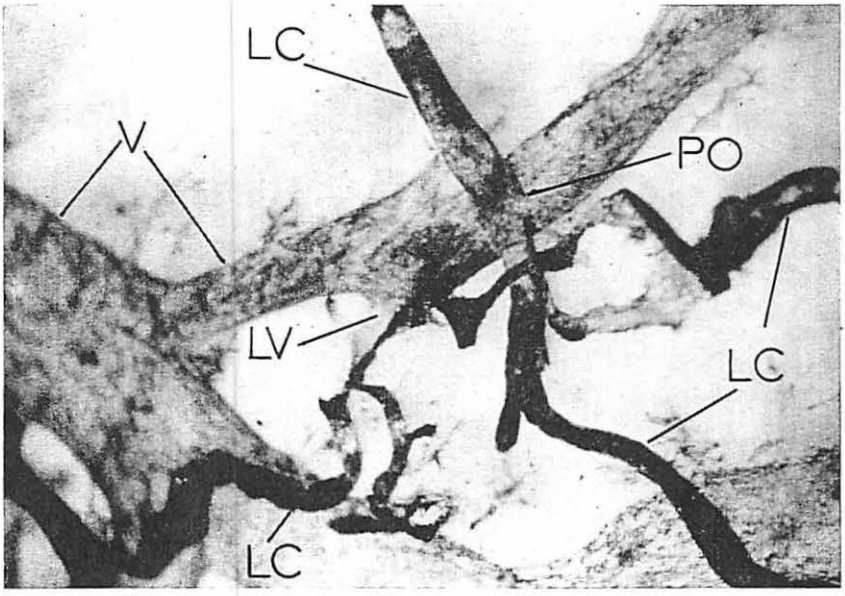

Fig. 9 Lymphatic-venous anastomosis in a dog heart. V-coronary vein and small branch; LC-lymphatic channels; PO-lymphatic channel passing over the venous branch; LV-site of lymphatic-venous anastomosis. At the anastomosis the venous branch is 400 microns in width and the lymphatic is approximately 120 microns in width. Magnification 20×.

In dogs and pigs injection masses introduced into the coronary artery or vein appeared promptly in a lymphatic capillary plexus at the apex of the heart and in the paracoronary and other large channels coursing toward the base of the heart. After clearing and dissection anastomoses were seen in the myocardium between small veins (1–2 mm in width) and lymphatics (100–300 microns in width) (Fig. 9). It is common to see arterial and venous injection masses intermixed in either vein or artery. Since communications between the arterial system and lymphatics have not been observed, arterial injection mass apparently occurs in lymphatics via arterial-venous anastomoses, then passes through venous-lymphatic connections. Due to the promptness with which these masses appear in the lymphatics and the absence of extravasation, it is not likely that the substances are absorbed by lymphatics from an extravascular source. Blood cells were not observed in lymphatic vessels.

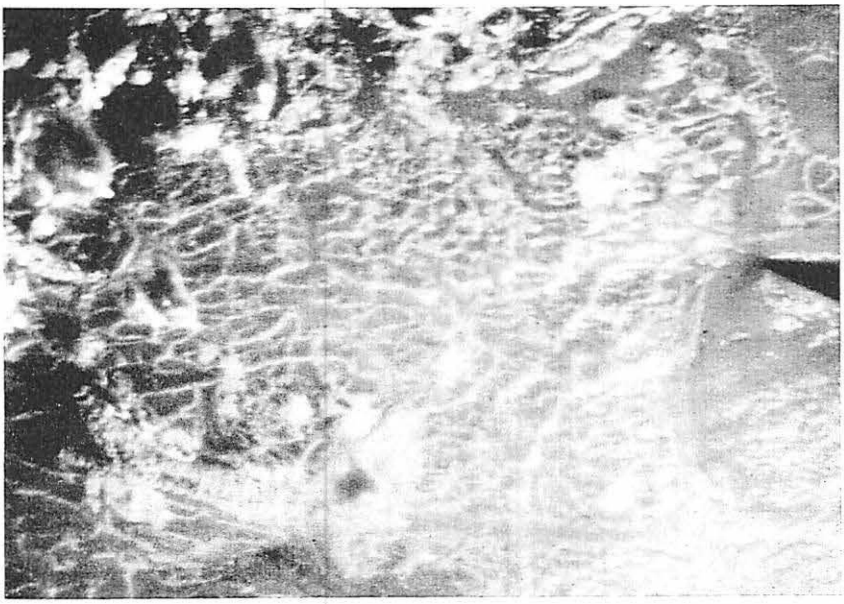

Fig. 10 Subepicardial lymphatic capillary network and small collecting channels over the left ventricle of a human heart demonstrated by application of hydrogen peroxide. Magnification 7x.

Observations in Human Hearts

The 20 human hearts studied were selected at random from autopsies and the causes of death varied. Clinical information concerning seven of them was not available, but of the remaining 13, eight were from males and five from females ranging in age from five to 72 years. Four adults had died of causes directly related to heart disease and nine of noncardiac causes, though most of them had some degree of asymptomatic atherosclerosis.

Subepicardial lymphatics demonstrated by application of hydrogen peroxide over the atria and ventricles in human hearts have the same general pattern as those in the other two species (Fig. 10). However, by injecting India ink beneath the epicardium

the network of lymphatic capillaries is denser in humans than in the other species (Fig. 11), but this was not observed in the subendocardial system. The phenomenon of lymphatics filling with coronary arterial or venous injection masses did not occur in the human hearts studied, although after clearing and dissecting communications were seen between small veins and lymphatics in the myocardium similar to those in the other two species.

In human hearts subendocardial lymphatics are observed in nearly all areas of the endocardium, though the system is demonstrated less clearly than it is in dogs and pigs. These networks are not as intricate as those in the other species, but lymphatic channels over apices of papillary muscle are similar in appearance to those in pig hearts. Beneath the endocardium of the ventricular walls and bodies of papillary muscles, lymph vessels

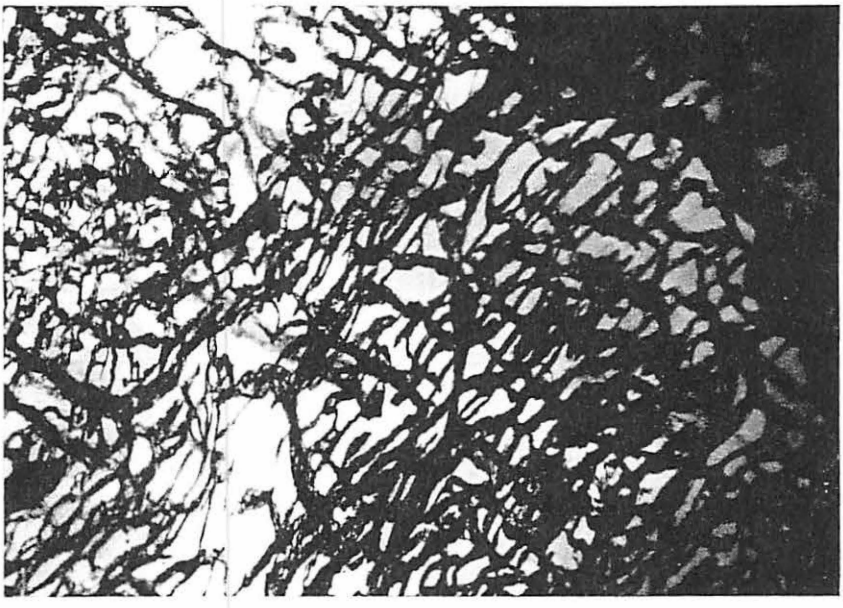

Fig. 11 Subepicardial lymphatics over the left ventricle of a human heart demonstrated by subepicardial injection of India ink. Magnification 30×.

measure between 20 and 45 microns in width with occasional channels up to 150 microns in width. Unlike those in the other species, the lymph channels are more nearly parallel to subjacent muscle bundles and have a bulbous appearance suggesting the presence of valves (Fig. 12). Just below the aortic and tricuspid valves, lymphatics measuring up to 250 microns in width course longitudinally deep to the valve rings toward the atrio-ventricular junction, but their termination has not been identified.

Lymphatics were not demonstrated in tricuspid or semilunar valves in any of the human specimens and were observed in mitral valves in only two instances. In one, a 65-year-old man who died with chronic congestive heart failure, the valves did not appear grossly to be involved by disease. Beneath the endocardium on the atrial surface of the posterior cusp of the mitral valve, lymphatics were traced from near the free

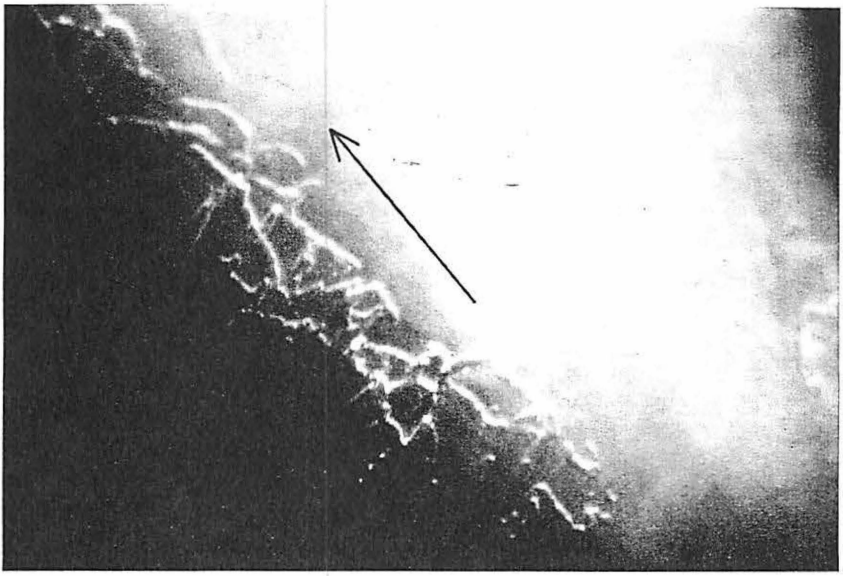

Fig. 12 Subendocardial lymphatic capillaries (20–40 microns in width) on a papillary muscle in the left ventricle of a human heart demonstrated by the application of peroxide. The arrow is oriented along the longitudinal axis of the papillary muscle and pointing in the direction of its apex. Magnification 25×.

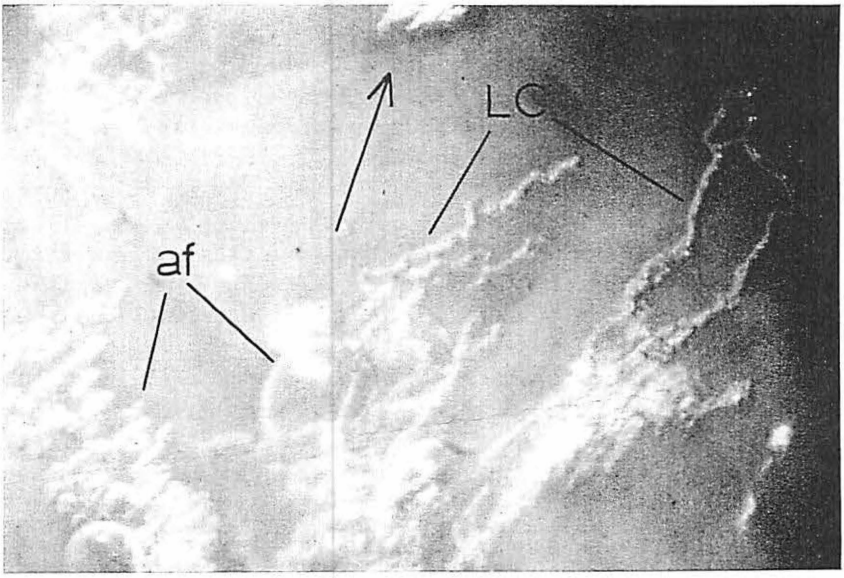

Fig. 13 Lymphatic capillaries (20–30 microns in width) on the atrial surface of the posterior cusp of the mitral valve in a human heart. The arrow is pointing in the direction of the valve annulus. The free edge of the valve cusp is beyond the lower margin of the illustration. LC-lymphatic capillaries; af-air bubble artifacts produced by the peroxide reaction. Magnification 30×.

margin to approximately half the distance to the valve ring. These vessels appeared slightly larger than those in pigs and dogs and measured between 20 and 30 microns in width (Fig. 13). The other case in which lymphatics were seen on the mitral valve was a 44-year-old woman with mitral stenosis who had died following commissurotomy. A small plexus of lymphatics on the atrial surface of the posterior cusp of the mitral valve was seen about halfway between its free edge and the valve ring.

Transmyocardial lymphatic channels and large ducts in the atrioventricular annuli like those seen in dogs and pigs were not identified in humans, but there were apparent collecting channels approaching the annuli from the atria.

Two hearts in the series were obtained from children. In one, a five-year-old boy who died with disseminated blastomycosis, subendocardial lymphatics were seen only on the apices of papillary muscles. In the other child, a six-year old girl who died with acute leukemia, no subendocardial lymphatics were demonstrated.

Discussion

The anatomical distribution of the lymphatics in the heart basically is similar in the three species studied. In general, there are subepicardial and subendocardial lymphatic capillary networks with transmyocardial communicating channels. In the subepicardial system collecting channels are directed toward the atrio-ventricular sulcus where they form a confluence from which the main cardiac lymph duct arises. Other investigators (7), however, have observed during in vivo studies in dogs that there are two main trunks that leave the heart separately. The left trunk, accompanying the left anterior descending coronary artery, exits between the left atrium and pulmonary artery. The right trunk, accompanying the right coronary artery, exits from the base of the heart over the aorta and through the preaortic fat pad and enters the mediastinum. Both these trunks course in the posterior portion of the superior mediastinum and enter the cardiac lymph node located between the inominate artery and superior vena cava. The in vivo observations may be more accurate since removal of specimens for study may destroy certain anatomical relationships.

Of particular interest are the differences in the lymphatic capillary networks between humans and the other two species. Although the general distribution of lymphatic capillaries is similar, the subepicardial lymphatic capillaries in humans appear to be denser than in dogs and pigs whereas subendocardial lymphatics are denser in dogs and pigs than in humans. In addition, the caliber of the subendocardial capillaries in humans is slightly greater than observed in the other species. These differences presently are unexplained. The relatively greater age of the humans studied in contrast to the youth and health of the dogs and pigs may indicate that these differences may be related to changes associated with aging. Several investigators have observed that the number of lymphatics in the heart and other organs tends to decrease with aging (22). Aging processes have been reported to cause thickening of the endocardium and auricularis layers of the atrio-ventricular valves (23) in addition to fibrosis and thickening within the cardiac skeleton, especially the annuli fibrosi (24, 25, 26).

Age changes of subendocardial lymphatics have not been reported specifically. Our series of young humans and old animals is too small in number to be conclusive. Extra-

polating from our observations in young animals and elderly humans, it appears that the subepicardial lymphatics become more numerous with age and subendocardial lymphatics become fewer. Possibly an aged, thickened cardiac skeleton results in impaired lymph drainage from the deeper areas of the heart and imposes an increased drainage load on the subepicardial system which is not involved directly with the fibrous framework of the heart. Lymph from the deeper areas of the heart not handled by lymphatics in this situation could be "squeezed" by the contractions of the myocardium toward the epicardium and absorbed by lymphatics there. To compensate, the capillaries in the subepicardial system possibly would increase in number. In elderly humans, the presence of these conditions may result in relative redundancy of the subendocardial lymphatic system which, in conjunction with thickening of the endocardium, may contribute to the anatomical features of subendocardial lymphatics observed in our studies as well as help to account for the difficulty in demonstrating lymphatics in valves, valve annuli, and the transmyocardial communicating system. Conceivably these circumstances may predispose the heart and valves to various diseases and resultant complications.

The possibility that alteration of cardiac lymph drainage is of clinical significance has been discussed elsewhere by a number of investigators (12, 13, 15, 27, 28, 29).

The occurrence of lymphatic venous communications is interesting but currently of undetermined significance in the heart and other anatomical regions (30, 31).

Acknowledgement

The author wishes to acknowledge the technical assistance of *J. R. Dumas* and Dr. *J. T. Russell.*

Summary

Some features of the anatomy of the lymphatic system of pigs, dogs, and humans are presented. In general, all three species have subepicardial and subendocardial networks with collecting channels. In dogs and pigs, the subepicardial and subendocardial systems communicate via transmyocardial channels and channels in the supporting structures of the atrio-ventricular valves; in humans, these communications have not been identified with certainty. Lymphatics are demonstrated readily in atrio-ventricular valves of dogs and pigs but have been seen only in the mitral valve in humans. Small differences in the subepicardial and subendocardial systems between humans and the other species are observed and their possible significance has been discussed.

In conclusion, it is quite obvious that acquisition of more factual knowledge from further anatomical, physiological, and pathological investigations is necessary to understand the functions of lymphatics of the heart and their implication in health and disease.

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The Ultrastructure of Pulmonary Lymphatic Capillaries of Newborn Rabbits and of Human Infants

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For a long time morphological studies of the pulmonary lymphatics have erroneously been considered as the simple search for a more precise knowledge of a problem which seemed only a delicate and intriguing anatomical puzzle. This usually involved the disputed presence (1–10) or absence (11–21) of true alveolar lymphatics, the localization and orientation of lymphatic valves and consequently the problem of the direction of lymphatic flow, and the differentiation of tissue clefts or small blood vessels from true lymphatics. Recent physiological (22–24), embryological (25), pathological (26–28, 82) and clinical (23, 24) investigations have revealed however that these problems have a basic and challenging importance in the understanding not only of the structure