Lymphatics of the Heart

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LYMPHATIC DRAINAGE of the heart has received little attention in the past but its potential importance has been recognized recently by several investigators.1-5 Review of studies of cardiac lymphatics4, 6-9 shows that many details of the anatomy of the system are still unknown, largely because of limitations of the technics of investigation. The small size and delicate structure of peripheral lymphatics and the presence of valves preclude their demonstration adequately by direct injection, and visualization in most instances has been by injection into living tissue of vital dyes which are removed by lymphatics. Using these technics, the most recent investigations have been those of Patek,9 Miller and associates,3 and Golab.10 An effective method for demonstrating lymphatics by direct application of hydrogen peroxide was described in 1922 by Magnus and Stubel11 and in 1963 by Parke and Michels,12 and the purpose of this report is to present observations of the cardiac lymphatic system obtained by this means. Injection and clearing technics have been utilized in some instances also.

Methods

Hydrogen peroxide initiates an oxidoreduction reaction with catalase and peroxidase in the tissue or lymph, or both, producing oxygen and water.13-15 The released oxygen causes distention of lymphatics and sometimes blood vessels; the two may be differentiated on the basis of their morphology. Lymphatics are superficial, transparent, and generally have a bulbous, irregular contour due to the presence of valves;7, 9, 16, 17 blood capillaries are of smaller diameter and constant size with their general arrangement more orderly than lymphatics. Artifacts produced by extravascular 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. Observation of subepicardial and subendocardial lymphatics was facilitated by cutting open the hearts according to a method described by Schlesinger,18 modified by leaving the ventricular septum intact. A 1 per cent solution of hydrogen peroxide applied topically with a cotton-tipped applicator resulted in distention of lymphatics in the region of application. The reaction was more effective in some hearts than in others but seemed to be improved in specimens refrigerated 24 hours or longer before study and still occurred in those refrigerated for more than a week. Frothing produced by the peroxide reaction was a complication that obscured some vessels but could be diminished by placing the specimens in 10 per cent formalin for 30 to 60 minutes prior to the use of peroxide. Whole specimens were immersed effectively in peroxide in some instances but, in general, results were best in unfixed tissue with local application.

Specimens were observed through a stereomicroscope or with the naked eye. As they became distended, lymphatics extended beyond the frothy area where peroxide was applied and were seen clearly. They remained distended for several minutes allowing time for photography and measurement with an ocular micrometer. The channels could be distended repeatedly by re-application of peroxide.

Photographs were made with Kodachrome II Professional Type A film and a 35-mm. Exacta camera back attached through one ocular of the stereomicroscope with magnification ranging from 7 to 30 times. Exposure times were determined by means of a photometer inserted into the other ocular of the stereomicroscope. Highlights of the vessels were 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 and clearing by the Spalteholz method were used in some instances.

Results

Observations in Dog and Pig Hearts

A dense network of subepicardial lymphatic capillaries measuring around 15 to 20 microns in diameter covers the outer surface of the heart. These join channels of intermediate

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size, some of which anastomose with larger channels accompanying the anterior and posterior coronary blood vessels. Others course longitudinally and join a large channel in the atrioventricular (AV) sulcus. The duct of the AV sulcus, from which the main cardiac lymph duct arises, is formed by continuation of the anterior and posterior paracoronary lymphatic ducts.\textsuperscript{8,19} The subepicardial capillary network over the atria is similar to that over the ventricles, and ducts of similar magnitude join the duct in the AV sulcus.

Beneath the endocardium of both ventricles, including the septum, small lymphatic vessels of relatively uniform caliber (15 to 20 microns in diameter) 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. 1). 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, and in dog hearts these channels form a denser network than in the pig heart. In both species, blood vessels course from the apices of the papillary muscles along the chordae tendineae and continue into the AV valve cusps, while lymphatics extend from the apices of the papillary muscles for only short distances along the chordae and communications with lymphatics in the AV valves are not demonstrated.

In the superior portions of the interventricular septum, channels (60 to 100 microns in diameter) 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 diameter between 110 and 225 microns (fig. 2). The networks on the valves are not so dense as those beneath the ventricular endocardium. The channels range in diameter between 20 and 30 microns and their irregular contour suggests that they contain valves. In some specimens blood vessel networks in the AV valves were injected with india ink and, after applying peroxide, lymphatics were demonstrated superficial to the blood vessels (fig. 3).

Subendocardial lymphatics in the atria were not demonstrated as clearly as those in the ventricles but their pattern was similar, and

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Subendocardial lymphatic capillaries (15 to 20 microns in diameter) overlying a portion of the anterior papillary muscle and surrounding endocardium in the left ventricle of a pig heart. The longitudinal axis of the papillary muscle extends from the left lower toward the right upper portion of the photograph. In the right upper field the apex has been cut and there is a fragment of severed chorda tendinea. The fluffy areas are artifact produced by the peroxide reaction. Magnification 8 x.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Lymphatic collecting duct (diameter 170 microns) in the mitral valve annulus of a pig heart. It is joined by smaller branches from the atrial surface of the valve leaflet. White patches are artifacts due to frothing produced by the peroxide reaction. Magnification 7x.}
\end{figure}
LYMPHATICS OF THE HEART

longitudinal channels (60 to 100 microns in diameter) joined the larger channels in the annuli of the AV valves.

In four pig hearts india ink injected into the duct of the annulus of the mitral valve appeared promptly in the subepicardial duct of the AV sulcus. After clearing, the connection between these channels was seen in the posterior AV junctional tissue near the interatrial septum and measured between 120 and 300 microns in diameter. In the same series of hearts india ink injected into apices of the anterior papillary muscles of the left ventricle appeared in regional subepicardial lymphatics. After clearing and dissecting, a deep channel (60 to 100 microns in diameter) was exposed traversing the long axis of each papillary muscle, passing through the myocardium, and joining subepicardial lymphatic channels of similar magnitude. In a few specimens cleared after random injection of india ink just beneath the endocardium, channels occasionally traversed the myocardium and joined subepicardial lymphatic ducts but their junction with the subendocardial system apparently was obscured by the ink.

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

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 5 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 were demonstrated over the atria and ventricles in all hearts and were of the same general pattern as those in the other two species studied (fig. 4).

In 16 of the 20 hearts subendocardial lymphatics were observed in nearly all areas of the endocardium, though the system was demonstrated less clearly than it was in dogs and pigs. These networks are not as intricate as those in the other species but lymphatic channels over apices of papillary muscles are similar in appearance to those in pig hearts. Beneath the endocardium of the ventricular walls and the bodies of papillary muscles, lymph vessels measured between 20 and 45 microns in diameter with occasional channels up to 150 microns. Unlike those in the other species, the lymph channels were more nearly parallel to subjacent muscle bundles and had a bulbous appearance suggesting the presence of valves (fig. 5). Just below the aortic and tricuspid valves lymphatics meas-

Figure 3
Anterior mitral valve in a pig heart in which the blood vessels have been injected with india ink; lymphatics, demonstrated by applying peroxide, are superficial to the blood vessels. Magnification 10×.

Figure 4
Subepicardial lymphatic capillary network over the left ventricle of a human heart. Magnification 7×.
uring up to 250 microns in diameter coursed longitudinally deep to the valve rings toward the AV junction but their termination has not been identified.

In the 13 patients for whom clinical information was available, subendocardial lymphatics were seen in hearts of three of the four dying from causes directly related to heart disease and in six of nine dying of noncardiac causes. Lymphatics were not demonstrated in tricuspid or semilunar valves 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 margin to approximately half the distance to the valve ring. They appeared slightly larger than those in pigs and dogs with diameters in the range of 30 to 40 microns (fig. 6). In the other instance, a 44-year-old woman with mitral stenosis who had died following commissurotomy, there was a small plexus of lymphatics on the atrial surface of the posterior cusp of the mitral valve about halfway between its free edge and the valve ring.

Transmyocardial ducts and large channels in the AV annuli like those seen in dogs and pigs were not identified in humans but there were channels approaching the annuli from the atria.

In a 5-year-old boy with disseminated blastomycosis, subendocardial lymphatics were seen only on the apices of the papillary muscles. In another child, a 6-year-old girl who had died with acute leukemia, no subendocardial lymphatics were demonstrated.

In this series subendocardial lymphatics were demonstrated in six of eight males and three of five females.

**Discussion**

Small differences in cardiac lymphatics of the three species studied are described. In dogs and pigs lymphatic capillary nets beneath the epicardium and endocardium join collecting ducts that course longitudinally and join a large subepicardial duct in the AV sulcus from which the main cardiac lymph duct arises. The subendocardial and subepicardial systems communicate through transmyocardial channels in the AV valve annuli, papillary muscles, and at random throughout the myocardium.

In humans the arrangement of the subendocardial and subepicardial systems is similar to that in the other species but communicat-
ing channels through the myocardium have not been demonstrated. Lymphatics in the AV valve annuli have not been identified but there is some evidence that they exist and they are seen easily in dogs and pigs.

There has been some question about the presence of lymphatics in valves, especially the mitral, since it is so often the seat of disease.\(^3\) Wearn et al.\(^{19}\) were of the opinion that human valves, both AV and semilunar, contain blood vessels normally and, because lymphatics are found usually in association with blood vessels,\(^7,20\) it seemed likely to us that normal valves should contain lymphatics and that modification of lymph drainage might be of importance in disease. The observations reported here have demonstrated the presence of blood vessels and lymphatics in mitral and tricuspid valves of normal dogs and pigs but not in aortic or pulmonary valves; in humans blood vessels have been seen infrequently in AV and semilunar valves but lymphatics have been demonstrated in only the mitral. The significance of this is not clear. In view of the relatively greater age of the humans studied and the presence of disease in most of them in contrast to the youth and health of the pigs and dogs, it may be that difficulty demonstrating valvular lymphatics in most of the human material, and subendocardial lymphatics in some, is a result of thickening of the endocardium and the auricularis layers of the AV valves as a consequence of aging or lymphatic obstruction, or both.\(^2,21\) Diminution of lymphatics with advancing age has been described in other organs.\(^{22}\) Chemical and enzymatic differences in the lymph among the three species may account for different responses to the technic used for demonstration of lymphatics, and the observation that the endocardium of the atria is normally thicker than that of the ventricles\(^{23}\) may help explain some of the differences.

The possibility that alteration of cardiac lymph drainage is of clinical significance has been suggested,\(^2,5,24\) and the observations reported here serve chiefly to support such speculation. The coarseness of lymphatic plexuses and the larger diameter of the individual lymphatic capillaries in the human subendocardium compared to those in dogs and pigs may be a reflection of impairment of lymph flow. Such impairment could result from fibrosis and thickening of some areas of the heart framework, especially the annuli fibrosi, which has been reported to occur.\(^{19,22,25}\) The results of partial or complete lymphatic obstruction at this level would be evident in nearly all areas of the heart including the valves. According to Gross et al.,\(^{25}\) the mitral valve ring may be affected early in rheumatic fever with subsequent involvement of aortic and tricuspid valves by contiguous spread along the fibrous framework which has elements in common with all three valves. Demonstration of lymphatics in valve rings of dogs and pigs suggests a means for spread of the rheumatic process. Inflammatory reaction with subsequent granulation and fibrosis in the annuli could produce impairment of lymphatic drainage of the AV valves and make them susceptible to infection, inflammation, and fibrosis. A mechanism of this sort may help explain thickening and the frequency of bacterial endocarditis in patients with rheumatic valvular disease, especially mitral.

Similarly, mechanical obstruction of lymphatics in valve rings and the AV sulcus following implantation of valve prostheses may be a factor having influence on the success or failure of these devices and other cardiac function during the postoperative period. Disturbance of subepicardial lymph flow may be significant in the development of postpericardiotomy syndromes as well as pericardial effusions.

Summary

The anatomy of the cardiac lymphatic system of pigs, dogs, and humans has been studied by topical application of hydrogen peroxide and also by injection technics. In general, all three species have extensive subepicardial and subendocardial networks with collecting channels directed toward large ducts in the AV sulcus continuous with the
main cardiac lymph duct. The subepicardial and subendocardial systems in dogs and pigs communicate via transmyocardial channels and channels in the supporting structures of the AV valves; in humans these communications have not been identified with certainty. Lymphatics are seen in both AV valves of dogs and pigs but have been seen only in the mitral valves of humans. The possible significance of these observations has been discussed.

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