Ventricular Endomyocardial Pathology Produced by Chronic Cardiac Lymphatic Obstruction in the Dog

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This work was undertaken on the hypothesis that endomyocardial fibrosis or endocardial fibroelastosis, or both, might be produced by chronically interrupting cardiac lymph flow. This concept developed from the fact that significant fibrosis occurs in areas of the body where substantial obstruction to lymph drainage occurs.

Some knowledge of the anatomy of cardiac lymphatics has been available since the 17th century, but even up to recent times little has been known about subendocardial lymphatics. Patke, in an excellent study and review of the literature, clarified the histology of the lymphatics of the heart. He was able to demonstrate significant subepicardial and subendocardial lymphatic plexuses, and noted that, in general, the lymphatics of the dog heart unite to form a single channel which he described as passing along the anterior surface of the pulmonary artery. Among the occasional exceptions seen was failure of the right and left cardiac lymphatics to unite to form such a single channel. Subsequently, Drinker and co-workers located the lymph node which usually receives the common cardiac lymphatic. We have used their terminology in referring to this node as the "cardiac lymph node." They used an anterior surgical approach, with removal of the sternum and upper 3 ribs.

Methods

Our preliminary anatomical studies demonstrated the feasibility of a left lateral surgical approach in the dog. With the dog under sodium pentobarbital anesthesia, the heart was exposed using aseptic precautions, generally through an incision in the fourth interspace. A small amount of T 1824 dye (about 0.2 ml.) was then injected through a 27-gage needle into the myocardium to visualize the lymphatic system. The injections were made through the intact pericardium into the anterior left ventricular wall, about halfway between the apex and the A-V sulcus. With appropriate dissection the cardiac lymph node could then be identified between the innominate artery and the superior vena cava. We found that this node usually received all of the lymphatics draining the heart, often by way of 2 entering channels. Not infrequently, a single cardiac lymphatic divided into 2 or 3 branches just before entering the node. On 1 occasion, we found a superficial lymph node anterior and to the left of the innominate artery which received part of the cardiac lymph drainage. The anatomical relationships most often seen via our surgical approach are shown in figure 1. We have not seen the blue dye entering the thoracic duct, nor have we seen any lymphatics ascend towards the thoracic duct. In 1 dog a small lymph channel was seen to bypass the cardiac lymph node and ascend towards the right lymphatic duct.

In the first 7 dogs operated on successfully, the lymphatics entering the cardiac lymph node were double-ligated and cut. In all subsequent experiments reported here the cardiac lymph node was resected, and in addition, the main lymphatic trunk lying posterior to the innominate artery was ligated just above the arch of the aorta. After completion of surgery, the chest was closed and the animals allowed to recover. All animals were given intramuscular penicillin postoperatively (600,000 units daily for 2 to 4 days and then 900,000 units every other day up to 3 weeks). Electrocardiograms were taken immediately before and after surgery, at weekly intervals thereafter, and just before the animals were killed.

The present report deals primarily with the
gross findings at autopsy, and histologic studies of the ventricular endocardium, subendocardium and myocardium. Blocks were routinely cut from the anterolateral area of the left ventricle and from the anterior area of the right ventricle. Additional blocks were taken from areas of gross pathology. The same sites were sampled in 15 control dogs. Previous study of sections from the dog's ventricles in animals subjected to other chronic experiments involving thoracotomy revealed no significant differences from the 15 control dogs reported here. All sections were treated with hematoxylin-eosin stain, and by orcein and Van Gieson stains. The latter is a combined stain to demonstrate elastic fibers and fibrous connective tissue.

Twenty-two dogs were operated on successfully. Six died within 5 weeks after surgery; in 3 of these dogs tissue decomposition prevented autopsy examination. Thus, 19 dogs were studied, 16 of them being sacrificed with intravenous sodium pentothal at varying times between 2 and 16 weeks after surgery. In these 16 animals, the degree of patency of the cardiac lymphatic system was determined by repeating the T 1824 dye injection just prior to sacrifice.

Results

Control Dogs
Fifteen unoperated stock dogs were studied as controls. Grossly, the right ventricle always showed a glistening, translucent endocardial surface, which was shiny, moist and delicate. The left ventricular endocardial appearance in 14 of the 15 dogs varied from glistening and translucent to one of slight whitish and opaque cast. The fifteenth dog showed a grayish-white opaque endocardial surface. Small verrucae, or small nodules, were commonly seen at the free edges of the mitral valve leaflets. In all, however, the valve leaflets proper were delicate, glistening and varied from transparent to translucent.

Microscopic study in the control animals revealed the right ventricular endocardium to be delicate, like a thin ribbon, with fine fibrous tissue beneath the endothelium and a thin elastic tissue layer. The left ventricular endocardium in the control dogs formed a narrow band, generally thicker than that from the right heart chamber, but significantly thinner than that of the left atrium. Figure 2 shows a representative section of the endocardium.
Left ventricle from a dog with obstruction to cardiac lymph flow (1 week postoperatively). Orcein-Van Gieson stain, X 150. A large subendocardial hemorrhage is present. The endocardium is thickened, the elastic layer is split, and there are fibrous connective tissue fibers in the hemorrhagic area. The hemorrhage penetrates into the subendocardial myocardium, which shows a few intact muscle fibers.

from the left ventricle in a control animal. The control dog with grossly visible endocardial whitish opacity of the left ventricular endocardium had a significantly thicker endocardium than the other dogs. Histologic study in this animal revealed an increase in both elastic and fibrous tissue. This was the only dog in which pulmonary artery worms (Dirofilaria immitis) were found at autopsy.

Thus, in 14 of the 15 stock dogs the left ventricular endocardium was similar to that described in the normal human heart.

Dogs with Cardiac Lymphatic Obstruction

Of the 19 experimental dogs, 2 had essentially normal lymph flow when tested by the T 1824 dye method prior to death. They were both completely normal on gross and microscopic examination. In 14 dogs, the T 1824 dye method of evaluating patency of the lymphatic system prior to death revealed decreased or essentially absent lymph flow. The other 3 dogs were not so tested since they died spontaneously.

On gross examination, left ventricular subendocardial hemorrhages were seen in 7 of the 17 dogs (fig. 3). This abnormality was found primarily in those studied within 5 weeks after surgery. Two of these animals also had right ventricular subendocardial hemorrhages. Eight dogs were considered to have thickening and increased opacity of the left ventricular endocardium as judged by gross inspection. Seven of the 8 were in the group studied 6 to 16 weeks after surgery. Since our experience in the early phases of this investigation was limited, we do not attach as much significance to this judgment as we do to the histologic findings (see below). Thickening and opacity of the mitral valve leaflets was seen in 7 dogs. Two animals studied 8 and 9 weeks postoperatively, respectively, also had thickening and shortening of the chordae tendineae of the mitral valve. The pericardium was normal in all the dogs studied.

Microscopic examination of the right ventricular endocardium failed to reveal significant histologic changes in the animals with lymphatic obstruction. A detailed analysis of the atrial obstruction is deferred, but no consistent changes were noted in the animals with obstruction of the lymphatics. Significant histologic changes were present in the
left ventricular endocardium (table 1). There was no significant correlation between the type of pathology noted and the length of life after surgery. Three of the 17 dogs showed no left ventricular pathology. Ten dogs were judged to have increases in both endocardial fibrous tissue and elastic tissue (figs. 4 and 5). Increases in elastic tissue were often seen as compaction of the elastic tissue layer with increased density (figs. 5 and 6). Increases in fibrous tissue were seen in both the subendothelial area, and in the layer beneath the elastic tissue (fig. 7). In 3 hearts, the fibrous tissue showed dipping into the subendocardial myocardium (fig. 5).

Three of the experimental animals developed clinical signs of impaired heart function 7 to 8 weeks after surgery. Two had pitting edema of the extremities; one also had ascites. The third dog manifested severe weakness and shortness of breath. One dog had subendocardial hemorrhages accompanying over-all thickening of the endocardium with increased elastic and fibrous tissue; another had small subendocardial hemorrhages; the third showed increased fibrous tissue in the left ventricular endocardium, thickening and opacity of the mitral valve leaflets, and shortening and thickening of the chordae tendinae. Electrocardiograms failed to reveal any consistent significant changes.

**Discussion**

This is a progress report of a study which is being continued. It is already clear, however, that significant endomyocardial pathology, primarily involving the left ventricle, is produced by chronic obstruction to cardiac lymph flow. The results, therefore, are compatible with our working hypothesis that chronic obstruction to cardiac lymph drainage leads to fibrotic and fibroelastic thickening in the ventricular endocardium. It is not surprising that the microscopic pathology showed no significant relation to the time after obstructive surgery within the period studied. The degree of lymph flow obstruction is variable from animal to animal, and probably does not remain complete because of collateral formation, and possibly recanalization. It will be necessary to study dogs surviving substantially longer than 16 weeks, and to do more detailed studies during the first 2 weeks after surgery. The desirability of a similar study in puppies is obvious.

Our observations are similar in some respects to those of Rusnjak, who reported...
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Figure 6
Left ventricle from a dog with obstruction to cardiac lymph flow (12 weeks postoperatively). Orcein-Van Gieson stain, X 150. The endocardium is thickened, mainly due to a diffuse increase in the amount of elastic tissue.

Figure 7
Left ventricle from a dog with obstruction to cardiac lymph flow (8 weeks postoperatively). Orcein-Van Gieson stain, X 150. There is thickening of the endocardium, mainly due to increased fibrous connective tissue beneath the elastic layer. There is also a slight increase in thickness above the elastic tissue layer, but without increased density.

many variations in the anatomy of the cardiac lymphatic system. However, he reported that certain of the cardiac lymphatics enter the thoracic duct, an observation at variance with our own experience. He produced obstruction to cardiac lymph flow in dogs by ligating the cardiac lymphatics, the right lymphatic duct, and the thoracic duct. The animals were kept alive up to 2 weeks after surgery. Of the 22 dogs so operated on, only 1 failed to show significant electrocardiographic changes.

Histologically, interstitial edema was found, most often with dilatation of lymph capillaries. In 3 dogs, there was disseminated focal necrosis in the myocardium. In 13 animals, the lymphatics and the coronary sinus were ligated simultaneously, following which 12 of the dogs died within 3 hours to 6 days. These animals showed severe and progressive changes in the electrocardiogram and at autopsy there was diffuse hemorrhagic muscle necrosis, interstitial edema, and markedly dilated lymphatics. Rusnjak's does not make clear how he was able definitely to identify certain vascular channels as "lymph capillaries."

Though the time sequence of pathologic change in our own experiments needs further study, the early finding of subendocardial hemorrhages may be an important clue to one of the possible mechanisms operative in endomyocardial fibrosis. Davies and Ball have previously suggested that the early pathologic change in endomyocardial fibrosis is thrombosis of subendocardial arterio-luminal vessels. Our findings suggest that subendocardial hemorrhage, as an early consequence of lymphatic obstruction, may predispose to fibrous tissue proliferation. Thus, the sequence leading to endomyocardial fibrosis may be lymphatic obstruction, subendocardial hemorrhage, and then fibrosis. Healing of necroses in the endocardial zone may play a role in its pathogenesis, as the results of Selye suggest. The assumption is, of course, that this disease entity may be related to impairment of cardiac lymph flow. There has been speculation that endomyocardial fibrosis may be due to parasitic involvement, a consideration which would be compatible with interference to lymph flow.

Our experimental findings also include endocardial hyperelastosis and increased fibrous tissue after chronic lymphatic obstruction in the absence of subendocardial hemorrhage. Some of these changes are suggestive
of the clinical counterpart of endocardial fibroelastosis. Numerous theories have been advanced concerning the origin of this disease, and considerable evidence suggests a genetic factor. The occasional occurrence of similar pathology in adults has been noted.

One of our 15 unoperated control dogs showed thickening of the left ventricular endocardium with increased elastic and fibrous tissue. Endocardial fibroelastosis has been reported by Eliot et al. in the dog and in the cat. In view of our experimental findings that chronic interference with cardiac lymph flow sometimes leads to left ventricular endocardial hyperelastosis, the possibility of a similar mechanism as causative for human endocardial fibroelastosis merits consideration. Inadequacy of the lymphatic system could be genetically determined, could be related to infection of the mother during pregnancy or could be due to infection of the fetus.

We do not claim the clear-cut experimental production of endocardial fibroelastosis or endomyocardial fibrosis, although some of our findings are highly suggestive. Rather, we emphasize the possible relation of our experimental findings to pathogenetic mechanisms for these disease entities. The cardiac lymphatics must be given proper consideration in cardiovascular physiology and pathology. Certainly they merit intensive investigation, since such as they surely have an important role in cardiac physiology and pathology. Their importance may encompass a far broader area than suggested above. They may be related to the so-called nutritional cardiopathies. The fibroplastic effects of serotonin might occur through an action on the cardiac lymphatics. Their involvement in endocarditis and myocarditis of certain types may be envisioned, for it is known that chronic lymphatic obstruction predisposes to recurrent inflammation and infection in the affected parts.

Summary

Chronic impairment of cardiac lymph flow was successfully accomplished in 22 dogs after preliminary anatomic studies revealed a feasible surgical technique. The anatomic and surgical details are presented. Gross and microscopic studies were possible in 19 of the operated animals, 3 of which died spontaneously, and 16 of which were sacrificed at varying time intervals between 2 and 16 weeks after surgery. Two of the 19 dogs were completely normal on gross and histologic examination. Abnormalities found in the remaining 17 animals included left and right ventricular subendocardial hemorrhages, increased elastic and fibrous tissue in the left ventricular endocardium, and opacification of the mitral valve leaflets.

It is concluded that chronic impairment of cardiac lymph drainage is productive of significant endomyocardial pathology. These observations are considered to be important as possible etiologic mechanisms in endomyocardial fibrosis and endocardial fibroelastosis.

The cardiac lymphatics merit continued intensive investigation, inasmuch as they surely have an important role in cardiac physiology and pathology. Their importance may encompass a far broader area than suggested above. They may be related to the so-called nutritional cardiopathies. Perhaps the fibroplastic effects of serotonin occur via an action on the cardiac lymphatics. Their involvement in endocarditis and myocarditis of certain types may be envisioned, for it is well known that chronic lymphatic obstruction predisposes to recurrent inflammation and infection in the affected parts.

Acknowledgment

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Summario in Interlingua

Obstruccion chronic del fluxo do lympha cardiac esseva complite a bon successo in 22 canes post que preliminnari studios anatomic habeva revelate un practic technica chirurgica. Lo detnlios anatomic e chirurgic es presentate. Studios macro- e micro-scopic essova possibile in 19 del operate animales. Tres de illos moriva spontaneamente. Le remanente 16 esseva sacrificate a varic intervales de tempore inter 2 e 16 septimanas post le chirurgia. Duo del 19 animales essova completemente normal in lo examino macroscopic e otiam histologic. Le anormalitates trovate in le altero 17 animales includeva hemorragias subendocardial sinistro- e dextero-ventricular, augmento de tissu elastic e fibrose in le endocardio sinistro-ventricular, e spissificazione e opacification del cuspides del valvula mitral.
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References

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