VENTRICULAR ENDOMYOCARDIAL CHANGES AFTER IMPAIRMENT OF CARDIAC LYMPH FLOW IN DOGS

BY

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The mammalian heart has an extensive lymphatic system (Patek, 1939) which has been given little attention in the published reports on cardiovascular systems. Our interest in the cardiac lymphatics was stimulated by the possibility that obstruction to their flow might cause endocardial changes. Reasoning by analogy with elephantiasis and similar conditions due to interference with lymph flow, we postulated that endomyocardial fibrosis or endocardial fibro-elastosis or both might follow chronic impairment of cardiac lymph flow. This was tested in the dog. Our preliminary studies (Miller, Pick, and Katz, 1960) established that endomyocardial changes are produced by chronic interference with the cardiac lymph flow. The present communication is a final report of a more extended series of observations in which we tested our hypothesis more precisely. This expanded survey also permitted a clearer definition of the sequence of pathological events that follow chronic impairment of cardiac lymph flow in the dog.

METHOD

In general, our surgical technique of interrupting cardiac lymph drainage was the same as that previously developed (Miller et al., 1960). In the first 22 operated dogs the lymphatics were ligated or the cardiac lymph node was resected. In subsequent experiments the entire lymphatic system was resected from caudad to the pretracheal node to cephalad to the cardiac lymph node. We continued to use a left lateral surgical approach. With the dog under sodium pentobarbital anaesthesia, the heart was exposed with aseptic precautions through an incision in the fourth intercostal space. A small amount of T1824 dye (about 0-2 ml.) was then injected with a 27-gauge needle into the ventricular myocardium through the intact pericardium. Shortly thereafter the cardiac lymphatic system could be identified by the blue dye, and the wholly extrapericardial resection of the cardiac lymphatic drainage system was accomplished. Fig. 1 shows a sketch of the cardiac lymphatic system as it is usually seen via our surgical approach.

In 13 dogs irritative polythenet™ strips were sutured into the surgical site after the lymphatic resection. Irritative polythene produces a rather intense fibrosis (Fishman et al., 1950) which it was hoped would retard lymphatic regeneration.

After completing the operation, the chest was closed and the animals were allowed to recover. All were given intramuscular penicillin post-operatively (600,000 units daily for 2 to 4 days and then 600,000 units every other day up to 3 weeks).

A total of 73 dogs were operated on to produce chronic impairment of cardiac lymph flow. Fifteen dogs died spontaneously at varying time intervals after operation to obstruct the lymphatics. In most of

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the other dogs coming to necropsy (after administration of a fatal dose of intravenous sodium pentobarbital), the degree of patency of the cardiac lymphatic system was assessed by repeating the myocardial T1824 dye injection at reoperation just before death.

In addition to the dogs operated on to produce chronically impaired lymph flow, the cardiac lymphatics were ligated in 9 dogs and the animals sacrificed within two hours. Five dogs were mock-operated, the operation consisting of a thoracotomy in the usual manner, injecting T1824 dye into the myocardium, and visualizing the lymphatic system, and then closing the chest. Fifteen unoperated stock dogs were used as controls.

The present report is primarily concerned with the gross findings at necropsy and with histological studies of the ventricular endocardium, subendocardium, and myocardium. Blocks were cut routinely from the anterolateral area of the left ventricle, from the anterior area of the right ventricle, and from both atria. Additional blocks were obtained from areas of gross abnormality. As a routine sections were taken from the anterior mitral leaflet, from the free edge to the valve ring. Sections were also usually taken from the tricuspid valve, and sometimes from the aortic valve.

The same sites were sampled in the control and mock-operated dogs, and in animals sacrificed within two hours of ligation of the cardiac lymphatics. In addition, the ventricular endocardium was examined in all other dogs coming to necropsy in this laboratory. When the gross examination revealed disease, blocks were taken from these hearts for microscopical study. All sections were treated with hematoxylin and eosin stain, and with orcein and Van Gieson stains, the latter being used to demonstrate elastic fibres and fibrous connective tissue.

The pathologist who performed the necropsies was not told about the nature of the previous surgical procedures. Gross and microscopical data were recorded separately, and the pathologist did not have access to the surgical or gross necropsy data until after the histological examinations were made and recorded. Assessment of lymphatic patency at necropsy was not used, since the T1824 dye injected by the surgeon at reoperation immediately before death of the animals diffuses out of the lymphatics within minutes after death, and the lymphatic pattern becomes distorted and blurred.

RESULTS

The data presented here include the animals previously reported (Miller et al., 1960).

Control Dogs. Grossly, the right ventricles in the 15 unoperated stock dogs used as controls all showed a glistening transparent to slightly translucent endocardial surface which was shiny, moist, and delicate. In 14 of the dogs the left ventricular endocardium varied from glistening and transparent-to-translucent to one with a slight whitish and opaque cast. The fifteenth dog showed a spontaneous abnormality described below.

Microscopical study in the 15 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 14 of the control dogs formed a narrow band, somewhat
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Fig. 2.—Normal left ventricular endocardium (Orcein-Van Gieson: ×170). The endocardium consists of loose fibrous connective tissue with a thin layer of parallel elastic fibres.

Fig. 3.—Spontaneous abnormality of the left ventricular endocardium. (Orcein-Van Gieson: ×170.) There is overall thickening of the endocardium with both the fibrous connective tissue and elastic tissue increased.

thicker than that from the right ventricle. Fig. 2 shows a section of the endocardium from the left ventricle of such a control dog.

One of five mock-operated dogs died 22 days after operation. Microscopically, minimal thickening of the left ventricular endocardium by increased fibrous and elastic tissue was noted. Necropsies were done on the remaining 4 dogs between 28 and 206 days after operation: microscopy revealed no endocardial abnormalities. None of the 5 dogs showed any gross endocardial changes.

None of the 9 dogs sacrificed within two hours of lymphatic ligation showed any gross endocardial changes. Microscopical study revealed slight left ventricular endocardial thickening, with increased fibrous and elastic tissue, in one dog. One other animal was thought to have a minimal increase in left ventricular endocardial fibrous tissue.

Dogs with Spontaneous Abnormalities of Ventricular Endocardium. It is about three years since this investigation was begun, and some 300 dog hearts have come routinely to necropsy. Our stock dogs are obtained from several sources and are usually about 3 years old. Among them, five were found with gross left ventricular endocardial greyish-white opacity. One of them had a patent ductus arteriosus. A second, given an injection of T1824 dye into the ventricular myocardium before an attempt to cannulate a cardiac lymphatic, demonstrated a very distorted and decreased lymphatic pattern. Death in this animal occurred from ventricular fibrillation during the experiment. Examination of the heart after death revealed endocardial whiteness and opacity involving both the right and left ventricle. A third dog, one of the 15 originally studied as controls, showed gross greyish-white left ventricular endocardial opacity. This dog had right heart Dirofilaria immitis. Otherwise, nothing unusual was noted in the other two dogs with "spontaneous" gross left ventricular endocardial whitening and opacity. Microscopically, these spontaneously abnormal dogs showed endocardial thickening due to increased fibrous and elastic tissue. Fig. 3 shows a section of the left ventricular endocardium from one of them.

Dogs Operated to Produce Chronic Impairment of Cardiac Lymph Flow. The Table shows the
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<table>
<thead>
<tr>
<th>Group</th>
<th>Days from surgical treatment to death</th>
<th>Number of dogs</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Total Subendocardial hemorrhages</td>
<td>Greyish-white endocardial opacity</td>
</tr>
<tr>
<td></td>
<td>LV RV</td>
<td>LV RV</td>
</tr>
<tr>
<td>A: decreased cardiac lymph flow before death*</td>
<td>&lt;14 7 1</td>
<td>4 1 4 5</td>
</tr>
<tr>
<td></td>
<td>14 to 56 14 2</td>
<td>4 3 10 2</td>
</tr>
<tr>
<td></td>
<td>56 to 112 12 2</td>
<td>8 2 9 1</td>
</tr>
<tr>
<td></td>
<td>&gt;112 12 2</td>
<td>8 2 9 1</td>
</tr>
<tr>
<td>B: cardiac lymph flow normal before death*</td>
<td>&lt;14 4 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>14 to 56 4 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>56 to 112 4 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>&gt;112 4 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>C: cardiac lymph flow not checked before death</td>
<td>&lt;14 7 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>14 to 56 7 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>56 to 112 7 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>&gt;112 7 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>D: spontaneous death</td>
<td>&lt;14 11 4</td>
<td>2 1 1 1</td>
</tr>
<tr>
<td></td>
<td>&gt;14 4 2</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>Totals</td>
<td>73 14 6</td>
<td>27 8 32 8</td>
</tr>
</tbody>
</table>

*Cardiac lymph flow was judged by the injection of T1824 dye as described in the text.*

LV = left ventricle; RV = right ventricle.

### TABLE

Endomyocardial Pathology Found in 73 Dogs Operated on to Produce Chronic Impairment of Cardiac Lymph Flow

Results in the 73 dogs operated on to produce chronic impairment to cardiac lymph flow. The results in the dogs that had irritative polythene sutured into the operative site were not significantly different from those in the entire group. They are therefore included in the Table without differentiation. In general, the results confirm the findings previously reported (Miller et al., 1960). Two major categories of abnormalities were found: (a) ventricular subendocardial hemorrhages, and (b) ventricular endocardial thickening due to increased fibrous and elastic connective tissue.

**a: Ventricular Subendocardial Hemorrhages.** Left ventricular subendocardial hemorrhages (Fig. 4) were present in 14 of the 73 operated animals. Right ventricular subendocardial hemorrhages were present in 6 dogs. Seven of the dogs with subendocardial hemorrhages had died spontaneously, 5 of them within less than 14 days of the operation. Eleven of the dogs showing subendocardial hemorrhages died spontaneously or came to necropsy within 5 weeks of operation. In 5 of the 7 dogs reoperated on just before death, the lymph flow had been judged to be decreased when checked by the T1824 dye method.

**b: Ventricular Endocardial Thickening due to Increased Fibrous and Elastic Connective Tissue.** Gross left ventricular endocardial greyish-white opacity was found in 27 of the 73 operated dogs (Fig. 6): it was accompanied by right ventricular endocardial opacity in 8 dogs. Of the 27 dogs, 23 were thought to have decreased cardiac lymph drainage at re-operation before death. Thus, of the total of 34 dogs judged to have had decreased cardiac lymph flow before death, 23 of them (68%) showed significant endocardial changes grossly. No gross endocardial opacity was found in any of the 12 dogs thought to have had normal cardiac lymph flow before death. No animal dying less than 14 days after operation had gross ventricular endocardial opacity. Gross endocardial whitening and opacity in the left ventricle was most pronounced on the apical, posterior, and septal surfaces. When the right ventricle was involved, it was most frequently on the septal and anterior surfaces. The gross changes seen in these animals were indistinguishable from the spontaneously abnormal dogs.
Fig. 4.—Left ventricular endocardium from a dog with chronic impairment of cardiac lymph flow (35 days after operation). (Haematoxylin and eosin: × 170.) A large subendocardial hemorrhage is seen under an oedematous endocardium. The hemorrhage penetrates into the myocardium.

Fig. 5.—Left ventricular endocardium from a dog with chronic impairment of cardiac lymph flow (85 days after operation). (Orcein-Van Gieson: × 170.) The endocardial thickening is due to a marked increase in fibrous and elastic connective tissue. Note the similarity to the spontaneously abnormal endocardium in Fig. 3.

Fig. 6.—Left ventricular endocardium from a dog with surgically produced chronic impairment of cardiac lymph flow (122 days after operation). Note gross whiteness and opacity of endocardium (A). A post-mortem tear of the endocardium (B) demonstrates its thickness.
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Microscopical examination revealed left ventricular endocardial thickening in 32 of the 73 dogs operated on (Fig. 5). Eight of these dogs also had thickening of the right ventricular endocardium. Of the 34 dogs judged to have had decreased cardiac lymph flow before death, 24 had left ventricular thickening. No dogs with normal cardiac lymph flow before death showed significant endocardial thickening. Of the 32 dogs that had left ventricular endocardial thickening 24 died spontaneously or were killed 56 days or longer after operation for lymphatic obstruction. Of the 8 dogs with right ventricular endocardial thickening, all had been killed 56 days or longer after the initial operation.

Of the 73 operated dogs, 35 had increased left ventricular endocardial fibrous tissue (Fig. 5, 7): in 8 of them increased fibrous tissue was present in the right ventricular endocardium. Of the 34 dogs judged to have had decreased lymph flow before death, 24 had increased fibrous tissue in the left ventricular endocardium. Two of the 12 dogs judged to have had normal cardiac lymph flow before death had increased left ventricular endocardial fibrous tissue, and in one of them there was also increased right ventricular fibrous tissue. Increased endocardial elastic tissue was present in the left ventricle in 36 of the 73 operated dogs (Fig. 5, 8); and in 7 it was accompanied by increased right ventricular endocardial elastic tissue. Occasionally, there was marked compaction of the elastic tissue layer of the endocardium, so that the increase in elastic tissue did not result in significant overall thickening of the endocardium. Twenty-five of the animals with increased left ventricular endocardial elastic tissue were in the group judged to have had decreased cardiac lymph flow before death.

Dipping of connective tissue from the endocardium into the subendocardial myocardium was occasionally observed (Fig. 7, 8). In 3 dogs a few areas of patchy myocardial necrosis were present in the left ventricular myocardium. In all the other animals studied the subendocardial

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**Fig. 7.**—Left ventricular endocardium from a dog with chronic impairment of lymph flow (78 days after operation). (Orcein-Van Gieson: × 170.) The endocardial thickening is mainly due to an increase in fibrous connective tissue which penetrates (dips) into the subendocardial myocardium.

**Fig. 8.**—Left ventricular endocardium from a dog with chronic impairment of lymph flow (83 days after operation). (Orcein-Van Gieson: × 170.) The endocardial thickening is mainly due to an increase in elastic tissue.
myocardium was essentially normal. In no instance was there evidence of left ventricular dilatation at necropsy. However, hypertrophy of the left ventricular muscle was present in a few dogs that had gross endocardial whitening and opacity.

In our initial study, microscopical examination of the right ventricular endocardium revealed no notable changes; but abnormalities were often noted during this more extensive investigation. These changes were primarily seen in those dogs allowed to live for 56 days or longer after the lymphatic-obstructive operation. Moreover, gross right ventricular endocardial greyish-white opacy almost invariably occurred in dogs with the more severe abnormalities of the left ventricular endocardium. It was our impression that predominant increases in endocardial fibrous tissue were more likely to be associated with considerable gross endocardial greyish-white opacity. Predominant increases in endocardial elastic tissue were associated with less gross changes. In a few dogs judged to have had a normal endocardium on gross inspection, microscopy revealed an increased amount of endocardial elastic tissue that was relatively much greater than the increase in fibrous tissue.

None of the 51 animals operated on in the second phase of this study evidenced any clinical changes suggesting congestive heart failure.

A more extensive experience with the histology of the mitral and tricuspid valves indicated considerable variability from one dog to another: we are, therefore, hesitant at this time to make any judgement about thickening or increased fibrous tissue in the atrioventricular valves (Miller, Pick, and Katz, 1961).

**DISCUSSION**

Our results have shown that chronic interference with cardiac lymph flow in the dog produces (a) ventricular subendocardial hemorrhages, and (b) thickening of the ventricular endocardium due to increased amounts of fibrous and elastic connective tissue. Early after lymphatic-obstructive surgery, ventricular subendocardial hemorrhages were frequent. A high percentage of dogs surviving the surgical procedure developed increases in ventricular endocardial fibrous and elastic connective tissue, which resulted in abnormal increases in ventricular endocardial thickness. Many of these animals showed gross greyish-white opacity of the ventricular endocardial surface. It is noteworthy that we observed the spontaneous occurrence of a similar gross ventricular endocardial whitening and opacity in 5 of our stock dogs. Furthermore, the histological changes in these 5 dogs were indistinguishable from those in the dogs with lymphatic obstruction produced by operation. A fortuitous circumstance allowed us to prove that the cardiac flow was impaired in one of these spontaneously abnormal dogs.

It appears that our analogy of elephantiasis to chronic impairment of cardiac lymph drainage and endocardial fibrosis has merit. It seems reasonable to conclude, in the light of our experimental findings, that chronic impairment of cardiac lymph flow in man may play an important role in the production of endocardial fibrosis. Clinically, such lymph flow obstruction could occur in the mediastinum outside the heart, on the epicardial surface, or within the myocardium proper.

Davies and Ball (1955) have suggested that thrombosis of the arterio-luminal vessels may be the early pathological change in endomyocardial fibrosis (as they observed it in Uganda). Our studies show that impairment of lymph flow from the heart frequently results in early ventricular subendocardial hemorrhages. It is possible that such hemorrhages may predispose to endocardial fibrosis, particularly in the presence of continued inadequacy of lymph drainage. Rusznyak (1957) has reported interstitial edema and at times disseminated focal necroses in dogs after obstruction to cardiac lymph flow. Healing of areas of necrosis might play a role in the pathogenesis of endomyocardial fibrosis (Selye, 1958), although our findings do not support this possibility. It has been suggested that endomyocardial fibrosis may be due to parasitic involvement of the heart (Lancet, 1952). Such a concept would be compatible with our experimental findings, since it is known that certain parasites infest and occlude lymphatics.
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Significant increases in ventricular endocardial elastic tissue were also seen in the dog after interference with cardiac lymph drainage. At times this was the most dramatic change observed. This increase in endocardial elastic tissue in the dog may shed some light on the pathogenesis of human endocardial fibro-elastosis, which is thought to be congenital in origin (Rosahn, 1955). In congenital fibro-elastosis, inadequacy of the lymphatic system could be genetically determined, or could be initiated while the foetus was in utero by an infectious or inflammatory process. The resultant pathological sequence could be gradual, starting either in utero or shortly after birth. It is known that insufficiency of the lymphatic system draining certain bodily parts may be inherited (Donald, Deas, and Wilson, 1952) as well as acquired.

We have speculated on the mechanisms of occurrence of human endomyocardial fibrosis and human congenital fibro-elastosis based upon our studies in the dog, although the change seen in the dog after chronic obstruction of cardiac lymph flow is not identical with that seen in either of these two diseases. Moreover, the pathology in endomyocardial fibrosis is substantially different from that of congenital endocardial fibro-elastosis. The location of the most severe endocardial involvement is different in each. Nevertheless, it is possible that the adult dog, the human infant, and the human adult react to a similar pathogenic insult (impairment of cardiac lymph flow) with different pathological end-results. The occurrence of subendocardial haemorrhages may be an important factor in the pathological sequence leading to fibrosis when there is continued impairment of lymph flow. We have found no evidence in support of the theory that ventricular dilatation leads to endocardial fibro-elastosis (Black-Schaffer, 1957), though the possibility exists that dilatation might impair endocardial lymph drainage.

Our studies present substantial evidence that chronic impairment of cardiac lymph drainage in the dog results in endomyocardial disease. No such evidence has previously been forthcoming. What this means in relation to diseases in man is not yet established, and will undoubtedly have to await improved techniques for evaluating human cardiac lymphatics. Nevertheless, it is pertinent to consider that certain diseases of unknown aetiology in man may be related to impairment of cardiac lymph flow, based upon these experiments in dogs. Certainly the cardiac lymphatics merit proper attention in human cardiovascular pathology; and they call for further extensive investigation, for their significance may well extend beyond our present and past speculations (Miller et al., 1960; 1961).

Summary

Chronic impairment of cardiac lymph flow was successfully produced in dogs by a wholly extrapericardial surgical technique. Gross and histological studies were made in 73 operated animals, as well as in appropriate controls. The endomyocardial pathological changes that resulted consisted primarily of early ventricular subendocardial haemorrhages, and of thickening of the ventricular endocardium due to increased fibrous and elastic connective tissue. Gross greyish-white ventricular endocardial opacity occurred in a large percentage of the dogs judged to have decreased cardiac lymph flow before sacrifice. It was a rare finding in other dogs.

It is concluded that chronic impairment of cardiac lymph flow in the dog produces significant endomyocardial disease. These observations in the dog are considered to be important in evaluating the pathogenesis of certain human endocardial diseases of unknown aetiology. In particular, attention is focused on endomyocardial fibrosis and endocardial fibro-elastosis.

The cardiac lymphatics are undoubtedly important in myocardial physiology and pathology and deserve continued investigation. It is well known that impaired lymph drainage from a part is associated with fibrosis and predisposition to infection and inflammation in that part. Interference with cardiac lymph flow may play an important role in the pathogenesis of endocardial disease, and may also be important in the pathogenesis of various forms of myocarditis.
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REFERENCES


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