The Susceptibility of Dogs with Chronic Impairment of Cardiac Lymph Flow to Staphylocoecal Valvular Endocarditis

By Albert J. Miller, M.D., Ruth Pick, M.D.,
Irwin K. Kline, M.D., and
Louis N. Katz, M.D.

Previous reports from this laboratory have described an effective method of producing impairment of cardiac lymph flow in the dog.\(^1\).\(^2\).\(^3\) One of our dogs, operated upon to produce chronic impairment of cardiac lymph flow, died spontaneously 82 days after surgery. A mediastinal abscess and a vegetative endocarditis of the mitral valve were found at autopsy. The valve changes being a unique occurrence in our laboratory, it was decided to investigate the susceptibility of dogs with chronic impairment of cardiac lymph drainage to the development of staphylocoecal endocarditis.

Spontaneously occurring bacterial endocarditis is rare in dogs.\(^4\) Though experimental bacterial endocarditis has been produced in apparently normal dogs, the technic has required multiple large injections of bacteria given over long periods of time.\(^5\) In one study, the exposure of the heart valve to bacteria within a special capsule suspended in the heart caused endocarditis in some apparently normal animals.\(^6\) However, most methods of creating experimental bacterial endocarditis in dogs have involved heart valve trauma or the introduction of various types of stressful interference with the cardiovascular system. In particular, the production of large arteriovenous fistulas by Lillehei et al.\(^7\) caused a marked susceptibility to bacterial endocarditis.

Angrist and Oka\(^8\) recently summarized some of the problems concerning the pathogenesis of bacterial endocarditis. They indicate that bacterial endocarditis is preceded by interstitial, edematous, and cellular valvular "distortion." This alteration in the valve substance is accompanied by platelet vegetations, at times with fibrin and on occasion with collagen and fibrinoid alteration. They describe these changes after various types of "stress" in experimental animals, and quote Keefer\(^9\) to the effect that surface contamination of bland vegetations leads to bacterial endocarditis. Others\(^10\) have also described valve surface alterations that they considered to predispose to bacterial endocarditis.

Degenerative, noninflammatory alterations in the valve substance and on the valve surface are not rare in stock dogs.\(^4\) In our experience these changes are easily distinguishable from acute valvulitis and vegetative endocarditis and do not represent active disease or lead to disability.

Methods

Stock dogs were anesthetized with intravenous sodium pentobarbital (25 mg./K.). By a left lateral surgical approach, the heart was exposed through an incision in the fourth interspace. Artificial respiration was maintained via tracheal intubation with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. A small amount of T-1824 dye (about 0.2 ml.) was injected with a 27-gage needle into the left ventricular myocardium through the intact pericardium. Shortly thereafter the mediastinal lymphatic drainage

From the Cardiovascular Institute, Michael Reese Hospital and Medical Center, Chicago, Illinois.

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### Table 1

*Endocarditis and Myocarditis in Dogs Operated on to Produce Impairment of Cardiac Lymph Flow and Paired Controls*

<table>
<thead>
<tr>
<th>Cardiac lymph-obstructed dog number</th>
<th>Control dog number</th>
<th>Dog weight in Kg.</th>
<th>Days between surgery and first injection of staphylococci</th>
<th>Dosage of staphylococci in ml. suspension</th>
<th>Number of intravenous injections of staphylococci</th>
<th>Days between first injection of staphylococci and death</th>
<th>Death: spontaneous (S) induced (I)</th>
<th>Endocarditis of mitral valve</th>
<th>Ventricular myocarditis</th>
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<td>1C</td>
<td>17.7</td>
<td>139</td>
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<td>3</td>
<td>243</td>
<td>I</td>
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<td>—</td>
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<td>34</td>
<td>S</td>
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<td>135</td>
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<td>29</td>
<td>I</td>
<td>—</td>
<td>—</td>
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<tr>
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<td>S</td>
<td>++++†</td>
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<td>121</td>
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<td>S</td>
<td>++++†</td>
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<td>5</td>
<td>S</td>
<td>++++†</td>
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<td>2.0</td>
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<td>7</td>
<td>I</td>
<td>—</td>
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</tr>
<tr>
<td>16</td>
<td>—</td>
<td>15.4</td>
<td>133</td>
<td>2.0</td>
<td>1</td>
<td>7</td>
<td>I</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*++ — Without gross vegetation.  †+++ — Including vegetations.  † This dog also had tricuspid valvulitis.
IMPAIRMENT OF CARDIAC LYMPH FLOW

Table 2
Summary of Results in Dogs Operated on to Produce Chronic Impairment of Cardiac Lymph Flow

<table>
<thead>
<tr>
<th>Number of dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total studied</td>
</tr>
<tr>
<td>Endocarditis of mitral valve</td>
</tr>
<tr>
<td>Also with tricuspid valvulitis</td>
</tr>
<tr>
<td>Also with ventricular myocarditis</td>
</tr>
<tr>
<td>Ventricular myocarditis without valvulitis</td>
</tr>
<tr>
<td>Total with valvulitis or myocarditis, or both</td>
</tr>
</tbody>
</table>

tween 0.5 and 2.0 ml. of a saline suspension containing approximately 1 million bacteria per ml.) were started in the operated dogs between 6 and 170 days postoperatively. The control nonoperated dogs were given injections at the same times as their paired mates. Pertinent details are given in Table 1.

Those dogs that did not die spontaneously were killed with intravenous sodium pentobarbital. After the autopsy examination, blocks were taken from the left ventricle, from the mitral, tricuspid and aortic valves, and from other areas grossly suspect of pathology. Material for culture was obtained from the mitral valves showing gross changes in two dogs. Slides were prepared with hematoxylin-eosin and with orcein-Van Gieson stains. The pathologist examining the heart was not aware of whether the dog was a control or a cardiac lymph-obstructed animal at the time of necropsy. The histologic sections were studied without knowledge of the previous experimental procedures or gross autopsy findings.

Results

There was severe vegetative bacterial endocarditis of the mitral and the aortic valves in the dog that died spontaneously 82 days after cardiac lymphatic resection (fig. 1). Myocarditis involving the left ventricle was present, and subendocardial hemorrhages were seen in both ventricles.

Table 1A shows the experimental results in the 10 dogs operated on to produce obstruc-

![Figure 1](image)

Vegetative endocarditis of the anterior mitral leaflet in a dog dying spontaneously 82 days after surgical obstruction to cardiac lymph drainage. Note fibrin (pale in figure) and clumps of bacteria (dark in figure) on the valvular endocardium and severe infiltration with polymorphonuclear leukocytes throughout the valve. Hematoxylin and eosin stain, × 60.

* We gratefully acknowledge the generous supplies of procaine penicillin made available by Dr. K. Kohlstedt of Eli Lilly and Co.

† Fifty milliliters of nutrient broth were inoculated with 1 loop of bacteria (Craberg strain) and incubated with gentle shaking for approximately 16 hours at 37°C. The cultures reached final optical densities of between 0.650 and 0.700, and a pH of 6.5 to 6.8. Ten milliliters of the culture were centrifuged, the sediment was washed with sterile saline and then resuspended in 10 ml. of sterile saline.
tion of cardiac lymph flow and their 10 paired controls. Table 1B gives the results in the six operated dogs that did not have controls. Table 2 summarizes the results in all the dogs operated on to produce obstruction to cardiac lymph flow. Cultures from the valves of two of the dogs with gross and histologic bacterial endocarditis yielded staphylococci.

Vegetative endocarditis was diagnosed grossly when soft, tan, friable nodules were present on the valve leaflet, mostly near the free edge. Histologically the vegetations consisted of large basophilic masses containing clumps of bacteria and fibrin (fig. 2). The affected valves were invariably severely infiltrated by polymorphonuclear cells. Occasionally hemorrhages and fibrinoid material separated the vegetations from the valvular stroma (fig. 3). In two instances large distended lymph channels filled with polymorphonuclear leukocytes were identifiable (fig. 4). The changes in the valve substance were usually diffuse, and were seen in parts of the leaflet with a normal endocardium as well as adjacent to the vegetations. In two dogs no vegetations were found, and the diagnosis of acute valvulitis was made because of severe polymorphonuclear cell infiltration throughout the leaflet (fig. 5). Myocarditis, always of the acute variety, was diagnosed in six dogs with diffuse interstitial collections of polymorphonuclear leukocytes that separated muscle fibers and occasionally formed small abscesses (fig. 6). Patchy ventricular necrosis was also noted in four of the operated dogs; in two of them these areas showed calcification.

Ventricular endocardial fibroelastosis developed in seven of the 16 operated animals. Four of these seven dogs were among those that developed either valvulitis or myocarditis. One control dog showed some left ventricular endocardial fibroelastosis histologically. None of the 10 control dogs developed bacterial valvular endocarditis or myocarditis.

Ventricular subendocardial hemorrhages were seen in six of the cardiac lymph-structured dogs and in one of the controls. Hemorrhages occurred in the mitral valves in four of the operated animals, and in one

![Figure 2](http://circ.ahajournals.org/)

Figure 2
Mitral valve of dog no. 5 (table 1A). Histologic picture similar to figure 1. Hematoxylin and eosin stain, × 150.

![Figure 3](http://circ.ahajournals.org/)

Figure 3
Mitral valve of dog no. 9 (table 1A). Note the clear, eosinophilic staining area between the vegetations and the stroma of the valve. A second area of heavy bacterial invasion (dark in figure) and polymorphonuclear leukocyte-filled stroma is clearly visible. Hematoxylin and eosin stain, × 150.
of the controls. Evidence of pyelonephritis was present in nine of the dogs with impaired cardiac lymph drainage and in five controls. Small abscesses in the kidneys were frequent.

Three of the control dogs died spontaneously, and because of the plan of the experiment the paired cardiac lymph-obstructed dogs were then killed. As is shown in table 1A, none of these three dogs had either endocarditis or myocarditis. Seven of the dogs operated on to produce chronic impairment of cardiac lymph flow died spontaneously; six of them had bacterial endocarditis of the mitral valve (table 1).

**Discussion**

Persistent interference with the lymph drainage from a part of the body results in edema and fibrosis, and predisposes to inflammation and infection. The mammalian heart has an extensive lymphatic system, and we have definitively demonstrated an extensive lymphatic plexus in the anterior leaflet of the mitral valve in dogs with chronic impairment of cardiac lymph flow. The results of the present study indicate that impairment of cardiac lymph flow predisposes to valvular bacterial endocarditis after limited exposure to staphylococci. The nature of the experimental method and the criteria demanded for the diagnosis of endocarditis were such as to make the results meaningful.

It is not known exactly how impairment of cardiac lymph flow predisposes to bacterial endocarditis. It is possible that edema of the heart valve is associated with surface transudation that allows the bacteria to adhere more readily to the valve surface. Also, lymph
stasis may permit the growth of bacteria that are carried into the valve substance. The histologic findings in two of the dogs with bacterial endocarditis suggested that the infection began in the valve interstices, rather than on the surface.

It is possible that some of the “cardiovascular stresses” that predispose to bacterial endocarditis operate via impairment of local lymphatic drainage. It has been suggested that large arteriovenous fistulas predispose to bacterial endocarditis by altering the endothelium of the heart in some way. Small arteriovenous fistulas do not predispose to bacterial endocarditis. Dogs with large arteriovenous fistulas develop cardiomegaly and a rise in central venous pressure. Recently Wegria et al. have proved that the flow of thoracic duct lymph decreases markedly as the central venous pressure is raised. The lymph drainage of the heart may be expected to be similarly impaired when the central venous pressure is raised by the production of large arteriovenous fistulas.

Previous studies from this laboratory have shown that chronic impairment of cardiac lymph flow produces ventricular endocardial fibroelastosis in the dog. In preliminary studies (unpublished), we have found that marked constriction of the superior vena cava has also caused ventricular endocardial fibroelastosis in a few dogs surviving over 6 weeks after the surgery. These preliminary results provide indirect evidence that cardiac lymph flow is impaired by elevation of central venous pressure.

Our experimental findings may shed some light on why acute rheumatic fever predisposes to recurrent rheumatic carditis and to bacterial endocarditis. Rheumatic fever does involve lymphatic tissue. Also, it is well documented that the vascularity of valves affected by the rheumatic inflammation is increased. Grossly visible capillaries may at times be seen on the atrial surface of the mitral valve involved. In our study of the lymphatics of the mitral valve in dogs, we always found the grossly demonstrable lymphatics to be on the atrial surface, and have previously raised the question of whether some of the supposed blood capillaries seen in man may actually be lymphatics. Recent postmortem studies of human hearts have also been reported to show lymphatics on the atrial surfaces of the atrioventricular valves.

The inflammatory involvement of lymphatic vessels could be within the valve substance, within the myocardium, on the epicardium, or in the mediastinal lymphatics draining the heart. Impairment of cardiac lymph flow due to rheumatic inflammation would in turn predispose to further inflammation and to fibrosis. The fibrosis subsequent to the inflammation and to lymph stasis would tend to further impair lymph flow. A vicious cycle could be set up that would serve to perpetuate the impairment of lymph flow, and thus predispose to recurrent inflammation and to bacterial adherence and invasion. Figure 7 is a schematic presentation of the hypothesis concerning some of the factors that may be operative.

The hypothesis offered is based solely on experimental work in dogs. It is apparent that it may be applicable to infectious and inflammatory diseases of the heart other than rheumatic fever and bacterial endocarditis. A similar pathogenetic mechanism might operate in chronic myocarditis, for example. Degenerative changes in the cardiac lymphatic system might be a factor in the predisposition of older individuals to acute bacterial endocarditis after certain surgical procedures.
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We have previously demonstrated that impairment of cardiac lymph flow is associated with histologic evidences of increased and protracted inflammatory reactions around foreign bodies, and with increased scar formation after autologous blood injection into the ventricular myocardium. The size of the infarct after coronary ligation is also augmented by chronic lymph obstruction.

Further observations in human material are necessary to test the hypothesis presented. Though some of these studies will require the development of technics to better visualize lymphatics in postmortem material, more critical evaluation may in itself be revealing. It would be important to know, for example, whether the cardiac and pretracheal lymph nodes in the mediastinal cardiac lymphatic system are affected by rheumatic fever in patients dying with this disease. Finally, we would like to emphasize that while the lymphatics play a role in cardiac inflammatory and infectious diseases, other factors are obviously also of importance. Like other biological processes, inflammatory and infectious processes are multifaceted.

Summary

Nine of 16 dogs (56 per cent) with surgically produced chronic impairment of cardiac lymph flow developed acute endocarditis of the mitral valve or acute myocarditis, or both, after intravenous injections of staphylococci, while none of 10 unoperated control dogs did. The results show that chronic impairment of cardiac lymph flow predisposes to staphylococcal endocarditis and myocarditis.

Impairment of cardiac lymph drainage may be important in explaining certain poorly understood pathologic processes in man. Thus, impaired lymph flow may predispose to bacterial endocarditis, recurrent rheumatic carditis, and myocarditis. It may be one of the alterations which perpetuates chronic inflammatory processes in the heart valves and in the myocardium. Inflammation and fibrosis in turn may predispose to impairment of lymph flow, and the latter in its turn will predispose to further inflammation, infection, and fibrosis. Thus a vicious cycle is established. A hypothesis concerning the role of cardiac lymphatics in infection, inflammation, and fibrosis is outlined. There is great need for careful and detailed observations of disease involvement of the cardiac lymphatic system in man.

Acknowledgment

We wish to acknowledge the technical assistance of James Rodgers and Richard Gray. The authors are indebted to Drs. Sidney Cohen and Robert Keller of the Department of Microbiology, Michael Reese Hospital and Medical Center, for supplying the suspensions of staphylococci and for their helpful advice.

References


Neurogenic Control of the Heart

In 1845 the brothers E. F. and Ernst Heinrich Weber, repeating with the advantage of more exact physiological knowledge the experiments of R. Lower in 1669, showed that stimulation of the vagi slowed and inhibited the heart’s action, and the accelerating effect of the sympathetic was pointed out in 1863 by von Bezold; it was then naturally assumed that the cardiac contractions depended on the activity of intracardiac ganglia and nerves, and were nervous in origin rather than merely controlled by the nervous system. This neurogenic hypothesis, though modified by Engelmann, eventually was superseded by the myogenic conception of the cardiac contractions as the result of the work of W. H. Gaskell (1847-1915), following J. G. Romanes’ (1848-94) demonstration of the block that can be produced to the passage of contraction waves in medusae. Gaskell (1883) proved that the cardiac contractions arise in the muscular tissue independently of the nerves and ganglia, and that the auricles and ventricles have their own automatic rhythm, though normally obedient to the influence of the sinus. This was to some degree foreshadowed by Harvey’s observation that if the ventricle of an eel or that of various fish is cut into pieces contractions continue to occur in the several portions.—SIR HUMPHRY DAVY ROLLESTON. The Harveian Oration. Great Britain, Cambridge University Press, 1928, p. 122.
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