Production of Endocarditis with Staphylococcus Aureus and Streptococcus Mitis in Dogs with Aortic Insufficiency

By Benjamin Highman, M.D., Joseph Roshe, M.D., and Paul D. Altland, Ph.D.

Following the induction of aortic insufficiency, 15 dogs received intravenous injections of either Staphylococcus aureus or Streptococcus mitis. The clinical course and pathologic picture resembled those of acute and subacute bacterial endocarditis respectively. The valvular lesions were confined largely to contacting surfaces of aortic and mitral leaflets. The dogs given S. mitis often developed an acute diffuse proliferative glomerulonephritis. No such valvular or renal lesions were noted in 10 control dogs, including 2 with sham operations, given similar bacterial injections.

STUDIES on bacterial endocarditis have been handicapped by lack of a suitable large laboratory animal in which the disease could be produced consistently. Recently, one of us (J.R.) has devised a method for the controlled production of graded aortic insufficiency in dogs. The present study was undertaken to determine the susceptibility of such animals to experimental endocarditis.

METHODS AND MATERIALS

Twenty-five mongrel dogs weighing 7.5 to 15.0 Kg. were obtained from our animal hospital after being quarantined, vaccinated against distemper, and treated for worms and vermin. Aortic insufficiency was produced in 15 of these dogs. In this technique, an aortic leaflet punch is introduced through an incision in the ascending aorta into the sinus of Valsalva during a brief period of inflow occlusion. A disc of tissue of known diameter is removed from the base of the desired aortic leaflet, leaving the free edge of the cusp intact. Thus, varying degrees of incompetence can be produced safely; the important factors are the weight of the dog and the amount of cusp tissue removed. In this study, defects from 3.9 to 4.9 mm. in diameter were produced. The right coronary cusp was perforated in every dog, and in two (D-1 and C-3 in table 1) the left coronary cusp in addition. Eight dogs serving as controls had no surgery, while two controls had sham operations involving incision and suture of the ascending aorta leaving the aortic cusps intact.

Eight to 150 days after surgery, bacteria were injected intravenously. 4 to 5 times weekly for one, two, and, in one instance, 3 weeks (tables 1 and 2). The first injection consisted of 1 ml. and subsequent injections consisted of 2 ml. of a 5 hour culture of Streptococcus mitis JH-26 (Lancefield D) or Staphylococcus aureus in beef heart infusion broth containing 0.5 per cent dextrose. These organisms were derived from the same cultures used in previous endocarditis studies. Eight dogs with aortic insufficiency were given S. mitis JH-26, while seven dogs with aortic insufficiency and 5 controls, including one sham, received S. mitis JH-26, while seven dogs with aortic insufficiency and 5 controls, including one sham, received Staph. aureus. All survivors except two were killed within two weeks after beginning the injections.

Blood cultures were made before and after beginning the series of bacterial injections. Blood cell counts, sedimentation rates, and hematocrits, were made in many instances. Percutaneous femoral arterial pressures were registered before and at intervals after beginning the injections, using a Statham transducer and direct recorder. At autopsy, blood cultures were taken from the left ventricle and the heart was opened following the blood flow pathways. The aorta was opened by an incision bisecting the anterior mitral leaflet and extending upwards between the left and noncoronary cusps through the wall of the aorta. The heart and blocks of other organs were fixed in 10 per cent aqueous solution of buffered formalin (pH 7.0). Routine paraffin sections were prepared and stained with hematoxylin azure eosin and, in some instances, for bacteria, fibrin, hemosiderin, calcium, collagen and elastic tissues as described in previous studies.

CLINICAL COURSE

All dogs with aortic insufficiency developed endocarditis and all except dog R-11 (table 1)
developed positive blood cultures. The sedimentation rates and white blood counts increased markedly. All controls survived and none showed endocarditis. This finding is important since development of spontaneous endocarditis has been reported.1'7'8

The dogs with aortic insufficiency which received *Staph. aureus* usually became acutely ill within 48 hours, deteriorated rapidly, and, excepting dog R-11, died or were killed prematurely in about one week. Within a few days, the diastolic murmurs increased in intensity, and peripheral pulses became more bounding; femoral arterial pulse pressures widened markedly (table 1). One could predict regularly an acute necrotizing valvulitis with increasing aortic incompetence on the basis of these typical changes. The pulse pressure of dog R-11 was only slightly elevated (table 1); at autopsy, the opening in the right aortic leaflet was found to be blocked partially by a tag of tissue.

In contrast, the dogs which received *S. mitis* JH-26 exhibited a milder course resembling clinical subacute bacterial endocarditis. Excepting 2 dogs that died at 6 and 11 days after the first injection, these animals appeared to be in fair condition when sacrificed 10 to 14 days after the first injection. One animal (J-1) was not sacrificed and died with pulmonary edema 11 days after the last of 9 injections. In nearly all dogs, new and changing murmurs were heard within a few days after the first injection. A short harsh blowing apical systolic murmur suggesting mitral insufficiency usually appeared early and either diminished, became louder as in dog J-1, or remained unchanged. Less commonly, a high pitched systolic sea-gull murmur was heard at the base suggesting partial obstruction of the aortic valve by vegetations. Peripheral pulses became more bounding in some and diminished in others. These changes correlated well with femoral arterial pressure pulse recordings (table 1).
EXPERIMENTAL ENDOCARDITIS

Fig. 1A. Heart of dog C-3 which received Staphylococcus aureus and died in 6 days. The base of the right coronary cusp is extensively ulcerated; a needle passes through original defect in this cusp and points to a small vegetation on the ventricular endocardium. The surgical defect in the left coronary cusp shows little gross involvement. There is a massive hemorrhage in the noncoronary cusp; above is a bacterial vegetation on the suture line in the ascending aorta. B. Kidney of dog C-3 showing numerous hemorrhagic lesions and infaracts.

Fig. 2A. Section through surgical defect of right coronary cusp of dog C-3 shown in figure 1A. Above is distal portion of leaflet; its lower portion marginating the surgical defect is markedly thickened, ulcerated, and invaded by bacteria. A few bacterial colonies are seen at the base of the surgical defect which borders the left ventricle below. (Hematoxylin azure eosin; X 9.) B. Posterior mitral leaflet of dog R-25 showing colonies of staphylococci along the surface near the line of closure and extending deeply into the substance of the leaflet; the latter shows extensive necrosis. (Hematoxylin azure eosin; X 25.) C. Higher magnification of auricular surface of 2B showing bacteria in surface cells. The subjacent valvular stroma is necrotic and infiltrated below mainly by leukocytes. In the right lower corner is the border of a large bacterial mass invading the deeper stroma. (Hematoxylin azure eosin; X 385.)

Pathologic Findings
All dogs with aortic insufficiency given bacteria developed lesions similar in many respects to those found in human endocarditis and to those produced by the same organisms in rats exposed to high altitudes. The gross and microscopic findings are depicted in the figures and are summarized in table 2. Nearly
Fig. 3. Gross specimens of hearts of dogs given *Streptococcus mitis* JH-26 (Table 2). A Anterior mitral leaflet of dog R-21 showing marked deformity and a huge vegetation on the auricular surface. B Heart of dog R-29 showing vegetations on the three aortic cusps and on the bisected anterior mitral leaflet; the chordae tendineae are thickened. The large vegetation protruding above the free margin of the right coronary cusp bridges the right border of the surgical defect (see Fig. 4.4). C Deformed anterior mitral leaflet of dog J-1 showing rupture of 2 chordae tendineae (arrows). D Heart of dog R-40 showing marked deformity and large vegetations marginating opening in right coronary cusp and extending across the commissure to involve the noncoronary cusp. Outflow obstruction is evident and femoral arterial pulse pressure was narrowed (see Table 1). In right lower portion of figure is an infarct margined by an irregular gray line (arrow). There are a few small vegetations on the bisected anterior mitral leaflet below and to either side of the aorta.

All the animals also showed superficial inflammatory foci in the tricuspid valve, but these were less severe than the nonbacterial lesions in the mitral and aortic valves. These nonbacterial lesions (+ in Table 2) represent early developing, mild, or regressing lesions.

**Dogs with Aortic Insufficiency Given Staphylococcus Aureus**

The surgical defect in the cusps frequently became ulcerated, ragged, enlarged, and marginated by a narrow zone studded with minute friable vegetations. Similar vegetations often developed on the outflow surfaces of the intact aortic cusps, particularly on areas in contact with the surgical defect or regurgitant stream during diastole. Vegetations were noted also on the auricular surface of the mitral leaflets, on the suture line in the aorta and on the ventricular endocardium opposite the regurgitant stream. Endocardial hemorrhages were frequent, particularly near the apices of the papillary muscles of the left ventricle, in the endocardium adjoining both atrioventricular rings, and in the leaflets.

Hemorrhagic and suppurative lesions were found in nearly all organs, and multiple infarcts were noted frequently in the spleen and kidney (Fig. 1B).

**Microscopic Findings.** The bacterial vegetations were flat and composed of masses of bacteria, largely intracellular, admixed with a
Fig. 4A Section through large vegetation bridging border of surgical defect in right coronary cusp of dog R-29 illustrated in 3B. (Hematoxylin azure eosin; X 8.) B Higher magnification of vegetation in 4A at its junction with the contact surface of the upper portion of the leaflet. The valvular stroma is seen in the left lower corner. Below are numerous leukocytes and some fibroblasts invading the fibrinous matrix. Above are large bacterial colonies and satellite colonies grading into bacteria laden macrophages. Some large mononuclear cells are seen within some of the larger colonies. (Hematoxylin azure eosin; X 385.)

small amount of eosinophilic material (fig. 2B and C). There was an extensive valvulitis with marked cellular proliferation and infiltration by inflammatory cells. Ulceration occurred mainly in lesions involving the suture line and the rim of the surgical defect (fig. 2A). The lesions frequently extended from the contact surfaces to other surfaces of the leaflets and to the chordae tendineae. Significant regressive changes were noted only in dog R-11; the fibrous tag blocking the surgical defect contained lysed colonies of bacteria.

Valvular Blood Vessels and Hemorrhages.
Small blood vessels were noted frequently in the leaflets, particularly near the base, but occasionally as far as the middle third. In infected leaflets, the vessels were frequently engorged or laden with leucocytes. Hemorrhagic extravasations were frequent even in leaflets showing little or no inflammation. They occurred chiefly in the proximal portion. Occasionally, as in dog C-3 (fig. 1A), there was a massive hemorrhage extending from the base nearly to the free margin of the cusp. The extravasations did not appear to communicate with the surface of the leaflet.

Dogs with Aortic Insufficiency Given Streptococcus Mitis
The valvular vegetations varied markedly in size, shape, color, and configuration (fig. 3). Their distribution was similar to that described above. Dog J-1 showed severe aortic and mitral insufficiency with ruptured chordae tendineae (fig. 3C). Aortic and mitral insufficiency are mutually aggravating; this may account for the early death of this dog.

The changes in other organs were less striking than in the dogs given Staph. aureus. There were infarcts in the kidneys, spleen, and heart (fig. 4D) and a few punctate hemorrhages, chiefly in the kidneys and lungs.

Microscopic Findings. The vegetations were composed chiefly of an eosinophilic fibrillar
matrix staining like fibrin; this matrix was admixed with blood and contained numerous colonies of bacteria at various levels and along the surface (fig. 4). Ulceration and necrosis of the substance of the leaflet and invasion of endothelial and stroma cells by bacteria were less evident than in staphylococcal valvulitis; the inflammatory changes and the number and size of the hemorrhagic extravasations were usually also less marked. In several instances, marked regenerative changes were noted with lysis and phagocytosis of bacteria and beginning organization of the vegetation. The changes were similar to those reported in rats with endocarditis due to viridans streptococci.

Other Lesions. In addition to the changes described grossly, bacterial emboli in intertubular and glomerular capillaries and focal glomerular changes were found occasionally. Four of the 8 dogs showed a diffuse proliferative glomerulonephritis with a marked increase in cellularity of most of the glomerular tufts. The changes were essentially similar to those reported by Lillehei and his associates in dogs with arteriovenous fistulas and experimental endocarditis. These authors considered the renal lesions to be similar to those occurring in the kidney in association with human bacterial endocarditis. Our studies indicate that dogs with aortic insufficiency may be useful in the production and study of experimental glomerulonephritis.

**DISCUSSION**

A method for the consistent production of experimental endocarditis, using dogs with aortic insufficiency, has been presented. The exact nature of the increased susceptibility of such animals to endocarditis is unknown. Among contributing factors one must consider the added stress of an increased left ventricular stroke volume, deformity of the aortic valve and possibly an impaired coronary blood flow. Previous investigators have postulated that an increased cardiac work load may be an important factor in rendering resistant laboratory animals susceptible to endocarditis. In dogs with aortic insufficiency, the vessels in infected leaflets were frequently engorged and laden with leucocytes suggesting that the leaflet is dependent in part on such vessels for its metabolic and defensive needs. Coronary blood flow normally depends mainly on an adequate diastolic pressure. Since the mean and diastolic pressures are markedly reduced in dogs with aortic insufficiency, it seems likely that the coronary and valvular blood flow may be impaired in aortic insufficiency; this may be an important factor in lowering the resistance of the leaflets to infection.

**SUMMARY**

Aortic insufficiency was induced in 15 dogs by removing a disc of tissue from the base of one or both coronary cusps of the aortic valve. Bacterial endocarditis was produced readily in all of these dogs by intravenous injections of broth cultures of either *Staphylococcus aureus* or *Streptococcus mitis* JH-26. Similar injections failed to produce endocarditis in 10 control dogs including 2 with sham operations. The lesions were confined largely to the contacting surfaces of the aortic and mitral leaflets and contiguous areas. Dogs with aortic insufficiency given *S. aureus* developed large ulcerating and necrotic lesions, and bacteria were found in the interior of the leaflet. They developed an acute fatal infection with splenic and renal infarcts and extensive hemorrhagic and suppurative lesions in nearly all organs. The dogs given *S. mitis* developed a more insidious subacute infection with large bacterial valvular vegetations and infarcts in the kidney, spleen and heart. They often developed a diffuse proliferative glomerulonephritis and focal glomerular lesions. It is postulated that the marked susceptibility to endocarditis is due mainly to an increase in cardiac workload. A deficient coronary, and possibly valvular, circulation present in aortic insufficiency may also be a predisposing factor.

**Acknowledgment**

Milton Parker, Robert L. White and Edwin C. Thompson rendered valuable technical assistance.

**SUMMARIO IN INTERLINGUA**

Insufficientia aortic esseva inducite in 15 canes per remover un disco de texitu ab le base de un o ambe eusides coronari del
valvula aortic. In omne iste canes endocarditis bacterial esseva facilemente producita per injectiones de cultura a bouillon de Staphylococcus aureus o Streptococcus mitis JH-26. Simile injectiones non produciva endocarditis in 10 canes de controlo, includente duo con pseudo-operationes. Le lesiones eseva restrin-gite in grande mesure al superficie de contacto del foliolos aortic e mitral e a areas adjacente. Canes con insufficientia aortic que recepiva S. aureus disveloppava grande lesiones ulcerante e necrotic, e bacterios eseva trovate al interior del foliolo. Illos disveloppava un acute infection mortal con infarcimentos splenic e renal e extense lesiones hemorrhagic e suppurative in quasi omne lor organos. Le canes que recepiva S. mitis disveloppava un plus insidioso infection subacute con grande vegeta-tiones valvular bacterial e infarcimentos de ren, splen, e corde. Illos frequentemente disveloppava un diffuse glomerulonephritis proliferative e focal lesiones glomerular. Nos postula que le marcate susceptibilitate a endocarditis es debite principalmente a un augmento del carga de labor cardiac. Un defecte circulation coronari e possibilemente valvular que es presente in insufficientia aortic pote etiam ager como factor predisponente.

REFERENCES


Production of Endocarditis with Staphylococcus Aureus and Streptococcus Mitis in Dogs with Aortic Insufficiency

BENJAMIN HIGHMAN, JOSEPH ROSHE and PAUL D. ALTLAND

Circ Res. 1956;4:250-256
doi: 10.1161/01.RES.4.3.250

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1956 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/4/3/250

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/