Experimental Bacterial Endocarditis Due to Streptococcus Mitis

I. Method of Induction

By Robert A. Rabens, M.D., Joseph E. Geraci, M.D., John H. Grindlay, M.D., and Alfred G. Karlson, Ph.D.

It is important in order to advance the increasingly successful attack on subacute bacterial endocarditis to be able to produce the disease experimentally and to treat it with new and improved therapeutic regimens. Since endocarditis caused by Streptococcus mitis has not been consistently produced previously in the dog, a study was undertaken in which cardiovascular stress was created by the establishment of aortico-inferior vena caval and bilateral iliofemoral arteriovenous shunts. After a period of antibacterial treatment and of stabilization, daily intravenous injections of broth cultures of S. mitis were begun. In 50 per cent of 24 animals bacterial endocarditis developed, simulating that found in clinical cases at necropsy. These animals had widely patent fistulas and 11 showed evidences of congestive heart failure. Of the remaining 12 animals, five with patent fistulas died of the effects of multiple infected pulmonary emboli. Seven others whose shunts had closed were killed and found to have no significant lesions other than healed caval infections.

The evaluation of new treatment regimens for bacterial endocarditis would be greatly facilitated by the development of a simple method of producing the disease in animals. In the past most of the methods employed in the production of experimental endocarditis involved one major approach; that is, the intravenous injection of organisms into the animal, either with or without preceding injury to one or several of the heart valves. More recently, attempts have been made to increase the cardiac work load by various procedures followed by the injection of organisms. Although positive results were sometimes obtained by each of these procedures, Streptococcus mitis endocarditis in the dog has not been consistently produced.

The purpose of this paper is to present our experience with the production of bacterial endocarditis with a culture of a penicillin-sensitive Streptococcus mitis isolated from a human case of subacute bacterial endocarditis. Utilizing the method of Lillehei and his colleagues, aorto-inferior vena caval anastomoses were created in dogs followed by intravenous injections of the organism.

Materials and Methods

1. Preparation of Animals.—Twenty-four mature, healthy, vaccinated (antidistemper) dogs were used.* In 22 animals an aorto-inferior vena caval shunt was prepared by making a side-to-side anastomosis of 10 to 12 mm. between the lower part of the aorta and the inferior vena cava just above their bifurcations.† A strip of ilaval plastic sponge about 2 mm. in diameter was wrapped around the anastomotic site. A distinct thrill was palpated, and a marked whorling of arterialized blood was visualized in the vena cava immediately after the proximal arterial clamp was removed. In two animals a two-stage operation was done with the construction of bilateral iliac and femoral shunts each 12 mm. in length.

On the day of the operation and continuing for four consecutive days, the animals were given an intramuscular injection of penicillin (200,000 units) and dihydrostreptomycin (0.3 Gm.) to prevent bacteremia from postoperative wound infection and to eradicate any organisms which may have already been present. After a lapse of two days to permit

* Nine other animals were not included owing to premature deaths of seven and closure of the fistula shortly after operation in two others.
† We are indebted to Drs. J. M. Janes and R. Thors for the creation of the majority of the fistulas.
excretion of the antibiotics, blood cultures were made on three successive days followed by two days of observation. The blood cultures, which were negative, were done to insure that no bacteremia existed before the experimental infection was started. This 12-day preinfection period served as a stabilization phase in which many of the more important physiologic changes occurring as a result of the stress imposed by the fistula took place.

2. Administration of Organisms.—On the twelfth postoperative day, 6 ml of blood were obtained for culture from the jugular vein of each animal. With the needle in place, an immediate injection was then given of a whole 24-hour dextrose-brain broth culture of *Streptococcus mitis*. This procedure was repeated for at least 14 consecutive days in the 15 animals whose daily blood cultures remained positive for the streptococcus. Blood cultures were made every other day thereafter in these animals. In the case of the nine animals whose blood cultures remained negative or were only occasionally positive, the daily 10 ml injections of culture were continued for periods of up to 30 days. Five animals were given additional injections consisting of two daily injections of 10 ml of whole culture for 11 to 20 days followed by three daily doses of 20 ml for 14 to 17 days. In only two of these five animals was bacteremia established.

3. Clinical Examinations.—A daily examination was made just prior to the drawing of blood for cultures and the injection of bacteria. This was done in order to determine the effects of the surgical procedure and also to determine the onset and course of the endocarditis. The examination included the following: daily weights, rectal temperatures, auscultation of the heart and lungs, auscultation and palpation of the abdominal and inguinal regions for bruits and thrills and inspection of the body for edema, evidences of collateral circulation and petechiae.

4. Termination of Experiment.—In 11 animals signs of endocarditis developed and all 11 died from congestive heart failure and the complications incident to their endocarditis. One additional animal with healed endocarditis was killed.* Of the remaining 12, 5 had a consistent bacteremia and died from the effects of their septicemia and of widespread pulmonary embolism. The other seven were killed* after having negative blood cultures for at least six weeks. A necropsy was carried out on every animal. Small portions of valvular and caval vegetations (opposite the site of anastomosis) were cultured on blood agar and in dextrose brain broth. Smears of these areas were stained with Gram's stain.†

For purposes of comparison, the animals were divided into two groups (table) depending on the presence (group I) or the absence (group II) of endocarditis as determined at necropsy. Each group was further divided into two subgroups: those that died of their induced disease (A) and those that were killed (B).

### Results

1. Clinical Findings.—It was found that the diagnosis of bacterial endocarditis was not difficult to make once the process was well established. However, the exact time of the onset of the disease was difficult to ascertain. This was true for several reasons: (1) cardiac murmurs, systolic in time, were present shortly after the surgical procedure, (2) bacteremia due to *S. mitis* usually occurred after several days of injections, and (3) transient hyperthermia occurred as a result of the bacteremia alone.

In all animals, regardless of the eventual development of endocarditis, there was a soft,

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals</th>
<th>Blood Cultures</th>
<th>Major Cause of Death</th>
<th>Contributory Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. With bacterial endocarditis</td>
<td>12</td>
<td>Consistently positive</td>
<td>Congestive heart failure</td>
<td>Embolic complications, pneumonia</td>
</tr>
<tr>
<td>A. With active endocarditis</td>
<td>11</td>
<td>Consistently positive, then negative for final 6 weeks</td>
<td>Killed</td>
<td></td>
</tr>
<tr>
<td>B. With healed endocarditis</td>
<td>1</td>
<td>Consistently positive</td>
<td>Septicemia</td>
<td></td>
</tr>
<tr>
<td>II. Without bacterial endocarditis</td>
<td>12</td>
<td>Positive, then negative for final 6 weeks</td>
<td>Pulmonary infarction, pneumonia</td>
<td></td>
</tr>
<tr>
<td>A. With fatal septicemia</td>
<td>5</td>
<td>Septicemia</td>
<td>Killed</td>
<td></td>
</tr>
<tr>
<td>B. With stenotic fistulas</td>
<td>7</td>
<td>Septicemia</td>
<td>Killed</td>
<td></td>
</tr>
</tbody>
</table>

* The animals were killed with a barbiturate given intravenously.

† Gross and histopathologic studies have been made on the various organs from each animal. The results will be reported in a separate communication.
apical systolic murmur appearing shortly after the operation. For several weeks after operation significant tachycardia (120 to 200 beats per minute) and an increase in the intensity of both heart tones were noted in all animals. The Nicoldoni or Branham sign was positive for the life of the animals whose fistulas remained widely patent.

In animals of group IA (11 animals with active endocarditis) there was a close correlation between the clinical signs and the necropsy findings in six of eight animals with aortic valvulitis. These animals had loud grade 2 to 4 (basis of 1 to 4) basal systolic murmurs followed by softer early diastolic murmurs at the base. In the remaining five animals with active endocarditis a softer systolic apical murmur of grade 2 intensity was heard. The single animal of group IB (healed endocarditis) had no significant murmurs. In the group without endocarditis (group IIA and B) there were, nevertheless, signs of cardiac stress such as soft, apical systolic murmurs graded 1 in all 12 animals in the postoperative period. These murmurs persisted in seven animals. At necropsy, cardiac dilatation and predominantly left ventricular hypertrophy were noted in animals of this group.

Thus, the onset of definite, loud precordial murmurs, more persistently elevated temperature and consistently positive blood cultures signaled the presence of bacterial endocarditis. The time between the initial injection and the probable onset of infection of the heart was variable, the onset of infection occurring 7 to 70 days after the injections commenced (average 18 days). Blood cultures became positive in 1 to 11 days (average 4.4 days) and remained so for the duration of the life of these animals. These animals lived from 7 to 128 days (average 41.7 days), once the diagnosis was established.

The single animal with healed endocarditis was not suspected of having bacterial endocarditis despite the presence of promptly and persistently positive blood cultures for five months. This animal was killed six weeks after the cultures became negative, some 202 days after the onset of injections.

In 12 animals endocarditis did not develop. Of the 5 animals that died of septicemia and of pulmonary infarction (group IIA), four died 46 to 58 days after the first injections (average 48.5 days). The clinical diagnosis of endocarditis was not considered in these animals, although in one, a persistent to-and-fro, grade 1 apical murmur was heard. The fifth animal died from the effects of a ruptured cerebral mycotic aneurysm 184 days after the injections were started. In all of these five, the blood cultures became positive in 7 to 24 days and remained positive. The remaining seven animals (group IIB) had healed, scarred caval lesions, closed or markedly stenotic arteriovenous fistulas and no endocarditis. These animals presented a variable intermittent bacteremia which finally cleared completely. They were killed 86 to 213 days (average 135 days) after initial injections. Two of these seven were remarkable because of the persistence of positive blood cultures for five and six months respectively, finally presenting a sterile blood stream for the final six weeks.

Auscultation of the abdomen and groin proved to be a decided diagnostic aid. The loudness of the bruit depended on the size of the fistula, the distance of the latter from the stethoscope and the presence or absence of local intravascular lesions. A loud systolic bruit graded 3 to 4 (basis 1 to 4) and a distinct thrill which persisted until death were found in animals with a patent shunt (17 animals, comprising all of groups I and IIA). On the other hand, a gradually decreasing bruit and thrill, especially when coupled with subsidence of peripheral edema and collateral venous circulation, pointed, in the seven animals of group IIB, to functional and anatomic closure of the fistula.

Daily weight records showed a gradual loss of weight through the terminal period of congestive heart failure in infected animals. The animals with active endocarditis (group IA) lost an average of 16.4 per cent of their body weight. The five animals dying of pulmonary infarction and sepsis (group IIA) had a comparable loss of 15.7 per cent of body weight. Of the seven animals of group IIB, three gained weight, two others lost an average of only 6.9 per cent of their body weight and in two the
weight remained constant. The general physical appearance of the animals with valvulitis was not significantly different from that of other animals until late in the course of the infection. Then, marked anorexia, listlessness, dyspnea, orthopnea and unsteadiness of gait accompanied the development of effusions, moist pulmonary rales, progressive dependent edema, distention of the veins and weakened and irregular heart tones. Daily rectal temperatures were found to be most consistently elevated in the animals with endocarditis, but this tendency was not pronounced or maintained until the final few weeks of life. The induced bacteremia caused a transient fever. Terminally, temperatures ranging from 102.5 to 105 F. were the rule in animals with bacterial endocarditis. Definite evidence of splenomegaly and of embolic phenomena was searched for but not found.

2. Bacteriologic Observations.—Positive blood cultures for S. mitis were obtained during and following the period of injections, but persistently positive cultures were seen only in those animals with a localized infected lesion. Such lesions included the vegetations in the inferior vena cava and on the heart valves.

In the 12 animals with bacterial endocarditis the blood cultures became positive in 1 to 11 days after injections were started. Heavily positive cultures (11 to innumerable colonies per plate) were obtained in animals with endocarditis, especially during the final two weeks of life. In one animal with healed endocarditis positive blood cultures developed after the first injection and the cultures remained positive for five months, finally reverting to negative for the six weeks before the animal was killed. One other animal required supplemental injections of 20 cc. thrice per day to initiate bacteremia occurring after five days of these injections.

Group IIA consisted of five animals in which endocarditis did not develop. In all these animals blood cultures became persistently positive after 7 to 24 injections and remained positive until death. These animals had friable vegetations at the site of the fistula and widespread areas of infarction of the lungs. One of these five animals required additional injections of 10 ml. twice per day for 20 days and 20 ml. thrice per day for 17 days before blood cultures became consistently positive. This animal died from a ruptured cerebral mycotic aneurysm six months after the first injection. Group IIB consisted of seven animals that presented healed, scarred caval lesions with closed or markedly stenotic arteriovenous fistulas and no evidence of bacterial endocarditis. These animals had extremely variable intermittent bacteremia of a mild nature (1 to 10 colonies per plate) and were killed on an average of three months after the beginning of injections. Three of these seven received supplemental injections in a vain effort to infect the blood stream.

The organism was demonstrated in post-mortem specimens by stain and by culture. Smears were made of lesions in 10 of the 11 hearts with active endocarditis and all had gram-positive, short-chained cocci as the only organism present. Cultures from these vegetations yielded S. mitis in all. The cultures of the vena caval vegetations revealed S. mitis in 16 of the 17 animals with patent fistulas.

Comment

Observations on endocarditis in man by Abbott showed that usually a damaged valve or an arteriovenous shunt and bacteremia are necessary for the development of bacterial endocarditis. Circulating bacteria seem to have a selective affinity for the slightly damaged, insufficient valve and for the endothelial surface that is traumatized by a vigorous blood impact. Illustrative examples are the endocarditis involving mild aortic or mitral insufficiency, the endocarditis associated with a ventricular septal defect and the endarteritis resulting from a patent ductus arteriosus or a peripheral arteriovenous fistula. In this study, the lesions of the inferior vena cava are examples of infection due to trauma caused by increased blood flow. Traumatic arteriovenous fistulas and aneurysm are a rare cause of bacterial endocarditis and endarteritis in man. Rienhoff and Hamman, Williams and Cutler and Wolf have reported such cases. The latter authors emphasized the sclerotic thickening of the free edges of the aortic cusps in aortic in-
sufficiency as a substrate for endocarditis. Allen\(^1\) studied human hearts affected by subacute bacterial endocarditis. He was impressed by the constancy of the location of the vegetations on the outflow surface of the valve and especially at the lines of closure. The idea was presented that hemic impact and contact factors are operative in this disease. These factors may explain the localization of valvar and vascular lesions observed in the present study.

In a review of experimental bacterial endocarditis those studies which utilize the principle of cardiovascular stress are pertinent to this discussion. Nedzel\(^6\) injected Pitressin and streptococci intravenously, producing a proliferative valvulitis. The drug was thought to increase the cardiac work and the force of valve closure, causing a bland endocarditic lesion on which bacteria were deposited. However, Vischer and Henschel,\(^1\) using epinephrine and Pitressin and the intravenous injection of bacteria, were unable to confirm this work. Highman and Aitland\(^1\) found that intermittent exposure of rats to low pressure simulating a 25,000-foot altitude yielded thickened valves in nearly all of the animals, and fibrinous vegetations in 35 per cent of the group. These workers\(^2\) subjected a group of rats to similar environmental conditions for four hours a day for 30 or more days, following which the rats received massive single injections of streptococci from human cases of subacute bacterial endocarditis. In 66 per cent of these “high altitude” rats bacterial endocarditis developed as compared with a control group of 15 per cent. Lillehei and his co-workers\(^1\) noted that bacterial endocarditis developed “spontaneously” in 70 per cent of animals with iliofemoral arteriovenous fistulas. They then initiated a study in which the creation of bilateral fistulas and injection of beta hemolytic streptococci produced bacteremia in 100 per cent of dogs.\(^2\) They concluded that the original stress was more important than infection in producing the disease and postulated an increased endothelial susceptibility as one result of that stress. Gowdy and his associates\(^3\) were able to produce endocardial lesions within 6 to 12 hours after huge (2 to 4.5 cm.) aortocaval shunts had been created in dogs. These large fistulas were noted to prolong definitely the time required to remove the bacteria from the blood stream.

Holman\(^1\) has fully considered the important cardiovascular changes that occur when a large arteriovenous fistula is opened. The pathogenic relationships involved in the production of the endocarditis in dogs can be summarized as follows: the fistula causes an overloading of the venous system by allowing the shunting of large volumes of arterial blood. An increase in the circulating blood volume, the cardiac rate and the cardiac work then occurs. The shunt also causes a local vena caval lesion to form which becomes infected by the repeated intravenous administration of organisms. The blood stream is thus continuously supplied with bacteria. The exact mechanism whereby the increased cardiovascular stress sets up the valvular endothelial localization is not known, but it would appear that increased hemic trauma to the valve is, at least partly, responsible. The observations of the spontaneous closure (functional or anatomic or both) and of the healed caval lesions of the fistulas in the seven dogs without endocarditis, in spite of the administration of great numbers of bacteria, makes the following conclusions possible: One, that a maintained stress in the form of a widely patent fistula is one of the chief factors necessary to infect the valves. Two, that the first lesion to form is probably a sterile “jet” lesion whose subsequent fate depends on the continued stress and on bacteremia. Our experience confirms that of Holman\(^1\) and of Lillehei and co-workers\(^1\),\(^2\) that the size of the anastomosis is an important consideration in experiments of this type. There is a tendency for shunts smaller than the “critical” size (probably 10 mm. in aortocaval fistulas in dogs) to undergo fibrotic closure, while shunts larger than this tend to increase in size from the increased blood volume and enlargement of the proximal segment of the artery with more blood being shunted. Thus, a vicious cycle is instituted.

The most important factor in the failure to produce bacterial endocarditis in some of these dogs is the spontaneous closure of the fistula. Another factor may have been the type of organism, since it is known that *Streptococcus*...
mitis is a relatively avirulent organism in comparison with the hemolytic streptococci used in the majority of other studies. Increased dosage of bacteria beyond a certain point did not seem to be a factor in the production of endocarditis. This conclusion is in agreement with the experience of the Lillehei group. Five dogs died from causes other than endocarditis. All had early and persistent blood stream infections. They lived as long as did the dogs with endocarditis and all had widely patent fistulas at necropsy. It is difficult to explain why endocarditis did not develop in these animals.

Evaluation of therapeutic regimens of this type of experimental endocarditis, in which a weakly virulent organism is used, would require a method in which endocarditis developed in a larger number of animals. Further work in this direction is justified before attempting studies on the effects of antibacterial therapy and the surgical closure of the anastomoses.

**Summary**

Streptococcus mitis endocarditis was produced in 50 per cent of 24 dogs by the intravenous administration of this organism following the creation of aorto-vena caval shunts. Infection was accomplished by daily injections of 10 ml. of a 24-hour broth culture of Streptococcus mitis for at least 14 consecutive days. Persistent bacteremia became manifest within 11 days. In two animals (one with endocarditis) additional injections of 20 cc. of the culture three times per day were required to establish bacteremia. Failure to produce bacterial endocarditis in 50 per cent of the animals was associated with contracted or closed fistulas in seven of these twelve animals. In the remaining five animals, death occurred from other causes. It thus appeared that the presence of an adequately patent fistula is necessary for the development of endocarditis. Eleven animals died from their endocarditis. Five others died from pulmonary and embolic septicemia. Streptococcus mitis was consistently recovered from the valvular and vena caval vegetations. The pathogenesis of this experimentally induced bacterial endocarditis is discussed briefly.

**Summario in Interlingua**

In 12 casos ex un(gruppo de 24 canes, endocarditis a Streptococcus mitis resultava del administration intravenose de ille organismus post le creation de derivationes inter aorta et vena cave. Le infection esseva complite per le injection diurne, durante un continuo periodo de al minus 14 dies, de 10 ml de un cultura a bouillon de S. mitis de un maturitate de 24 horas. Bacteremia persistente se manifestava intra 11 dies. In duo animales—un con endocarditis—bacteremia resultava solo de injectiones additional de 20 cc del bouillon tres visas per die. Inter le 12 animales que non disveloppava endocarditis bacterial il habeva septe casos in que iste facto esseva associate con le presentia de fistulas contrahite o claudite. In le remanente cinque casos de iste gruppo altere causas resultava in le morte del animal. Per consequente il pare que le disveloppamento de endocarditis require le presentia de un fistula a apertura adequate. Dece-un del animales moriva de lor endocarditis. Cinque alteres moriva de septicemia pulmonar e embolic. In omne casos S. mitis esseva constantate in le vegetation del valvulas e del vena cave. Es presentate un breve discussion del pathogenese de iste typo experimental de endocarditis bacterial.

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Experimental Bacterial Endocarditis Due to Streptococcus Mitis: I. Method of Induction
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