Experimental Bacterial Endocarditis in the Opossum (Didelphis virginiana)

III. Comparison of Spontaneously Occurring Endocarditis with That Induced Experimentally by Pyogenic Bacteria and Fungi

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It was possible to induce bacterial endocarditis in opossums with single intravenous injections of *Streptococcus viridans* or *Staphylococcus aureus*. Fifty-eight percent of those animals given *Streptococcus viridans* developed bacterial endocarditis in which most of the lesions were on the left side of the heart. The experimentally induced streptococcal disease was similar to that which may occur spontaneously in opossums, both with respect to the distribution and structure of the vegetations. Single injections of *Staphylococcus aureus* resulted in endocarditis in 100% of the test animals. These lesions differed from those due to streptococci by having a relatively high frequency of right- as well as left-sided valvular disease and by being somewhat smaller than those due to streptococci. Endocarditis could not be successfully induced with injection of three different fungi. (Amer J Path 64:513–520, 1971)

THE OPOSSUM (*Didelphis virginiana*) is subject to spontaneously occurring bacterial endocarditis resembling that seen in man with respect to both the distribution of valvular lesions and the causative organisms.[1,2] Since endocarditis was not found in newly captured opossums, it was postulated that the disease in this species is a result of captivity and intimate contact with other laboratory animals and man.[1] This concept was supported by showing that spontaneously occurring bacterial endocarditis could be all but eliminated from a colony of opossums by isolation of these animals from other species, proper control of temperature and humidity and daily sterilization of cages.[3–5]

Our laboratory has recently utilized these "clean" opossums as a model for the study of experimentally induced bacterial endocarditis. Early investigations demonstrated that bacterial endocarditis in opos-

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sums could be caused by a single injection of *Streptococcus viridans*.[2,3,5] More recently, we have been concerned with endocarditis induced with other organisms. This report compares spontaneously occurring endocarditis with that induced by *Streptococcus viridans, Staphylococcus aureus, Candida albicans, Candida guilliermondii* and *Torula glabrata.*

Materials and Methods

A total of 133 adult male opossums, weighing 2.5 to 3.6 kg each, were used in these studies. The incidence of naturally occurring disease has already been reported in 53 of these animals.[1] The remaining 80 opossums were used to study experimentally induced bacterial endocarditis. Each of these experimental opossums was kept in an individual stainless-steel cage (22 × 20 × 15 inches) in a room where temperature and humidity were controlled (24 C and 50–60% humidity).

Phencyclidine-HCl (25 mg/kg) was given intramuscularly to induce anesthesia.[4,5] Injections of microorganisms were made into the jugular vein, which had been exposed surgically. Similarly, blood for bacteriologic and immunologic studies was obtained from the jugular vein. One-milliliter suspensions of organisms were injected immediately after removal of the first blood sample. The organisms and their concentrations used were

1. *Streptococcus viridans:* 52×10^6 viable cells/ml; given to 21 animals
2. *Staphylococcus aureus* (coagulase-positive): 56×10^6 viable cells/ml; given to 23 animals
3. *Candida albicans:* 55×10^6 viable cells/ml; given to 2 animals
4. *Candida guilliermondii:* 51×10^6 viable cells/ml; given to 2 animals
5. *Torula glabrata:* 51×10^6 viable cells/ml; given to 2 animals

Bacterial studies were done using serial blood samples and portions of valvular vegetations from each opossum. Both aerobic and anaerobic cultures were performed using trypticase soy broth, thioglycolate and citrate media.

Complete anatomic studies were done on each opossum, with representative tissues from each animal being fixed in 10% buffered formalin. These tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Routine sectioning was done at 5 μ but the kidney specimens were also sectioned at 3 μ Thin sections of kidneys were stained with PAS and periodic acid–methenamine silver as well as with hematoxylin and eosin. The Brown and Brenn stain was used to identify bacteria in valvular vegetations.

Out of the 80 opossums used, 30 animals were assigned as controls. Each subject was given 1 ml of sterile broth intravenously at the same time that test groups were inoculated with either bacterial or fungal strains.

Results

Valvular Morphology

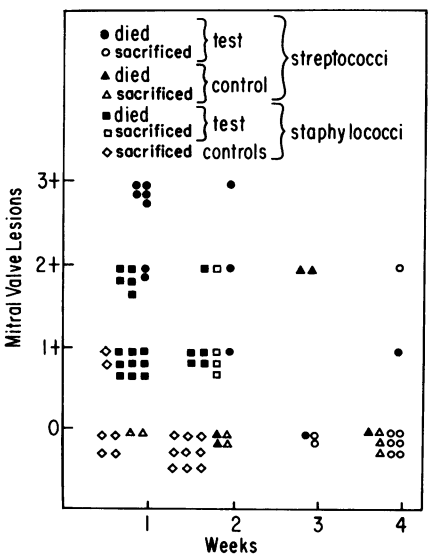
The same grading system was used to define the degree of valvular involvement throughout these experiments.[4] Valvular lesions varied in severity from intact but thickened and edematous valve leaflets in-

filtrated with moderate numbers of lymphocytes and neutrophils (1+) to destructive valvulitis with large, poorly organized masses of inflammatory cellular exudate, bacteria and fibrin (3+). Right-sided cardiac lesions were uncommon, especially in the streptococcal-infected animals. However, the severity of endocarditis was generally greater in the streptococcal group than in the staphylococcal group. Also, in general, the vegetations were larged in opossums given staphylococci, but in other respects the valvular lesions were indistinguishable.

Frequency of Valvular Lesions and Endocarditis

Twelve of 21 test animals and 2 of 12 control animals had endocarditis when they were examined after a single injection of *Streptococcus viridans* or sterile broth, respectively. Endocarditis was found in all 23 opossums infected with *Staphylococcus aureus,* and in 2 of the 12 control animals that had received sterile broth. The mitral valve was most often involved with the aortic valve being the next most frequently affected.

Text-fig 1 compares the time of appearance and severity of mitral endocarditis in the streptococcal and staphylococcal experiments. The time in weeks after injection of the bacteria is plotted against the severity of the mitral lesions, which have been classified as indicated above and as described previously in more detail.[4] All of the animals given straphylococci and most of those given streptococci died within 2 weeks after the injection. However, the control groups in these two



TEXT-FIG 1—Appearance and degree of severity of mitral endocarditis in opossums after a single inoculation of 52 × 10^6 *Streptococcus viridans* or 56 × 10^6 *Staphylococcus aureus.*

experiments were comparable, with 2 animals in each group having spontaneous bacterial endocarditis.

Table 1 compares the findings in spontaneously occurring bacterial endocarditis with those in endocarditis induced by the injection of pyogenic organisms. Table 1 also shows that, although the mitral valve was affected in each of the animals having endocarditis, it was not involved alone in any of the staphylococcal infections. The spontaneous group and the streptococcal group were very similar in that with rare exceptions, the right side of the heart was not involved with endocarditis. In contrast, opossums given staphylococci showed a greater frequency of right-sided valvular vegetations. Text-fig 2 illustrates the rates of occurrence of pathologic changes in groups of animals injected with bacteria compared to those in controls. The control groups showed significantly lesser incidence of endocarditis, brain abscesses and kidney abscesses compared to the animals injected with bacteria.

Other Organ Lesions

Brain abscesses were frequently found in animals given either streptococci or staphylococci but they were much more common in the latter. Kidney abscesses were found exclusively in opossums intravenously injected with staphylococci (Text-fig 2).

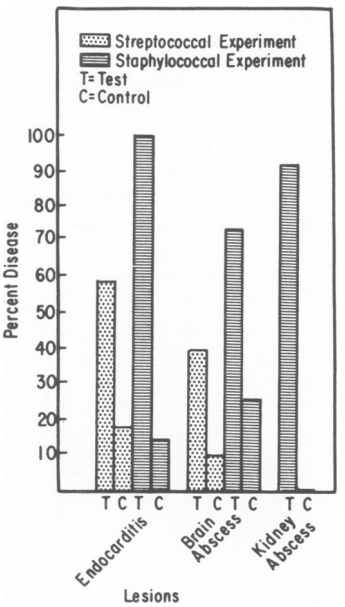
Bacteriologic Findings

In our past experience with *alpha hemolytic streptococci*, the homologous organism was cultured from the valvular vegetations in 68% of those opossums that had been given bacterial inoculum.[2,4,5] As for staphylococcal experiments, the homologous organism was cultured from 91% of these animals. In the control group, however, *Staphylococcus epidermidis* was cultured from the heart valves of only 1 opossum.

Table 1—Distribution of Valvular Vegetations in Spontaneous and Experimentally Induced Endocarditis

	Presence of vegetation (% of total No. seen in each group)		
Valves*	Spontaneous	Streptococcal	Saphylococcal
M	39	42	0
M, A	56	42	4.5
M, T	5	0	13
M, A, P	0	8	4.5
M, A, T	0	8	39
M, A, T, P	0	0	39

* M = mitral; A = aortic; T = tricuspid; P = pulmonic.



TEXT-FIG 2—Comparative rates of pathologic changes in opossums with endocarditis induced by single injection of 52 $\times$ 10^6 streptococci or 56 $\times$ 10^6 staphylococci.

In Table 2, the recovery of homologous organisms from heart valves of individual animals is correlated with the occurrence of bacterial endocarditis in opossums given either streptococci or staphylococci and in their controls. The overall incidence of valvulitis was higher in opossums given *Staphylococcus aureus* since endocarditis was identified in 100% of these animals, compared to 58% recorded from streptococcal experiments.

Fungal Inoculum

Three groups of 3 opossums each were given intravenous injections of either *Candida albicans, Candida guilliermondii or Torula glabrata.* One member of each group was sacrificed at 10, 20 and 30 days after inoculation. Endocarditis was absent from all of these 9 opossums. Attempts were made also to isolate the organisms from the animals' heart valves but no growth was observed.

Discussion

The striking resemblance between human bacterial endocarditis and that occurring spontaneously in opossums held in captivity has already been noted.[1,2] In each, the mitral valve is most commonly involved and the right side of the heart is only rarely involved. In each, *Streptococcus viridans* is a common etiologic agent.

It has been found possible to induce bacterial endocarditis in adult

Table 2—**Streptococcus viridans** and **Staphylococcus aureus** Cultured from Heart Valves in Two Groups of Opossums After A Single Inoculation*

Streptococci				Staphylococci			
Animal No.	Heart valve culture	Endocarditis	Survival (days)	Animal No.	Heart valve culture	Endocarditis	Survival (days)
Experimental							
1	+	+	3	1	+	+	5
2	+	+	3	2	+	+	5
3	+	+	4	3	+	+	5
4	—	+	6	4	+	+	5
5	+	+	7	5	+	+	5
6	+	+	7	6	+	+	6
7	+	+	7	7	+	+	6
8	+	+	8	8	+	+	6
9	+	+	10	9	+	+	6
10	NC	—	14	10	+	+	6
11	—	+	18†	11	+	+	7
12	NC	—	18	12	+	+	7
13	—	—	21	13	+	+	7
14	+	—	25	14	+	+	7
15	—	+	28†	15	+	+	8
16	+	—	28†	16	—	+	8
17	+	+	28†	17	+	+	8†
18	—	—	28†	18	+	+	9
19	+	—	28†	19	+	+	9
20	+	—	28†	20	+	+	9
21	—	—	28†	21	+	+	10†
				22	—	+	10†
				23	+	+	10†
Controls							
1	NC	—	3†	1	+	+	6†
2	NC	—	3†	2	—	+	6†
3	NC	—	8†	3	+	—	6†
4	NC	—	9†	4	NC	—	7†
5	—	+	14	5	NC	—	7†
6	NC	—	18	6	NC	—	7†
7	NC	+	21	7	—	—	9†
8	+	—	29†	8	—	—	9†
9	—	—	29†	9	—	—	9†
10	—	—	29†	10	—	—	9†
11	—	—	29†	11	—	—	9†
12	—	—	29†	12	—	—	10†
				13	—	—	10†
				14	—	—	10†
				15	—	—	10†

* NC = not cultured. † Animal was sacrificed

opossums by using a single intravenous inoculum of pyogenic microorganisms. This is in sharp contrast to the situation in most other animal species where multiple injections of bacteria, valvular injury or altered hemodynamics are required for the production of bacterial endocarditis.[6-9]

Although both streptococci and staphylococci may easily produce bacterial endocarditis in the opossum, the hosts differ materially in the distribution and incidence of valvulitis and in the complications that develop. The disease induced with streptococci is nearly identical to that which occurs naturally in both man and in captive opossums. Staphylococcal-induced disease is markedly different from these in that a much higher proportion of the infected animals develop endocarditis and the disease is not as sharply localized to the left side of the heart. It is of particular interest that the fungal organisms tested failed to cause any recognizable endocarditis.

The reason for the ready susceptibility of the opossum to pyogenic organisms is not known. Two major immunoglobulins (IgM and IgG) have been identified in opossums as well as a subclass of IgG.[10,11] It is of interest that opossums infected with streptococci produced only low levels of serum antibodies since it is known that the opossum responds relatively slowly to certain antigens.[5,12] Conversion from IgM to IgG antibody activity is slow and it is even incomplete after a second injection of antigen. Although little is known about the state of cellular immunity in opossums, personal observations suggest that they respond to tuberculin sensitization as do other mammals. This may account for what appears to be an effective defense against fungal infection.

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