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Experimental Bacterial Endocarditis in the Opossum (*Didelphis virginiana*). II. Induction of Endocarditis with a Single Injection of *Streptococcus viridans*

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The high rate of occurrence of spontaneous bacterial endocarditis in adult opossums (*Didelphis virginiana*) and its elimination under appropriate laboratory conditions suggested that this animal might be an excellent model for the study of experimental endocarditis [1-3]. This paper reports the induction of bacterial endocarditis in opossums by a single injection of *Streptococcus viridans*.

Materials and Methods

The adult male opossums (2.5-3.6 kg) used in this study were kept in individual stainless steel cages (22 inches × 20 inches × 15 inches) in a room with a constant temperature of 24 C and the humidity at a level of 50%-60%. Alpha-hemolytic streptococci isolated from a spontaneous case of bacterial endocarditis in an opossum served as the infectious agent. The 1-ml bacterial inoculum used in each animal was derived from a freshly grown culture and contained 52×10^6 viable organisms. The cellular concentration was measured as total wet volume with a Hopkins tube, and viability of the bacteria was estimated by colony-counts.

Twenty-one animals made up the test group, and 12 others were controls. The jugular vein was approached aseptically for the intravenous injection of bacteria and for collection of blood

samples. Phencyclidine HCl (25 mg/kg) was used to facilitate handling of the opossums. Antibiotics were not used.

Blood samples for bacteriologic, immunologic, and hematologic studies were taken immediately before infection and twice weekly throughout the 28-day experimentation. Aliquots of the blood were placed in trypticase soy broth, thioglycolate, and citrate tubes. Aerobic and anaerobic cultures were done in a routine diagnostic laboratory. Serum was prepared from clotted blood, and total proteins were measured by the biuret method, with BSA as a standard [4]. Zonal electrophoresis was carried out on the millipore apparatus with 0.1 M barbital buffer (pH 8.6). Ponceau S was used to stain the proteins. Their relative concentrations were then estimated by an analytrol. Levels of antibody were estimated by agglutination, with serial 2-fold dilutions of serum as described by others [5]. Total counts of leukocytes were done with the Fisher autocyto-meter, and the packed cell volume was determined by the micro-method with capillary tubes and the Clay-Adams microcentrifuge. Smears of peripheral blood were made in each case, and differential cell counts were performed after Wright's stain. Representative tissues from each animal were fixed in 10% buffered formalin, processed in the usual way, embedded in paraffin, and sectioned. Hematoxylin and eosin was used as the routine stain, and the Brown-and-Brenn stain was used to identify bacteria in valvular vegetations.

Results

Pathology. Changes were observed in the hearts, kidneys, and central nervous systems of both test and control animals (figure 1). Endocarditis was found in 12 of the 21 test animals and in 2 of the 12 controls. The mitral valve was

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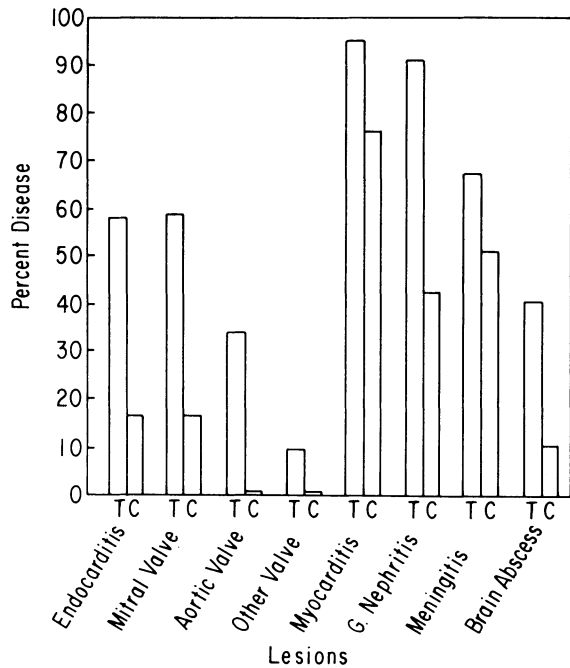


Figure 1. Distribution of organ injuries in opossums following single inoculation of alpha-hemolytic streptococci.

affected in each animal with endocarditis. The aortic valve was the next one most frequently involved. One opossum had mitral, aortic, and pulmonic valvulitis, and another had mitral, aortic, and tricuspid valvulitis. The morphology of these lesions has been described in detail [6]; they were found to be identical to those observed in spontaneous bacterial endocarditis in this species [2]. Myocarditis either was characterized by lymphocytes in aggregates or was more diffusely distributed. There was no correlation between the occurrence or severity of myocarditis and endocarditis.

Meningeal inflammation was found in 14 test opossums and 6 controls. This inflammation was mild and consisted of scattered lymphocytes. Brain abscesses were found in 8 test animals but in only 1 of the controls. These lesions were not easily recognized on gross examination but appeared as small, poorly defined areas of necrosis and polymorphonuclear leukocytes.

Proliferative glomerulonephritis also appeared frequently in 19 test animals, but was present in only 5 of the 12 control animals. No differences between the morphologic features of glomerulonephritis in test opossums and those in the controls were observed.

Bacteriology. Table 1 summarizes the bacteriologic and pathologic findings in all animals. There were 3 positive blood cultures in test animals and 1 in the controls before the injection of bacteria; of the former group, only 1 opossum developed endocarditis. The incidence of bacteremia was greatest during the first 3 weeks of the experiment, a finding corresponding to the highest rate of development of bacterial endocarditis. Alpha-hemolytic streptococci were isolated from the vegetations in 9 of the 12 cases of endocarditis (75%). That alpha-hemolytic streptococci were not eliminated from control animals is indicated by positive blood cultures, which were found in 8 of 41 samples (19%). However, only 2 of these animals developed endocarditis, the vegetations were small and confined to the mitral valve, and no bacteria were recovered from either vegetation. In only 1 control animal were streptococci found in cultures of heart valves.

Serology and hematology. Low levels of anti-streptococcal antibodies were common in both test and control animals, and infection was not fol-

Table 1. Alpha-hemolytic streptococci cultured from blood samples and heart valves and pathologic evidence of endocarditis in opossums during 28-day experimentation following single inoculation of alpha-hemolytic streptococci

Time (days)	Blood cultures (positive/total)		Heart valve cultures (positive/total)		Endocarditis (positive/total)	
	Tests	Controls	Tests	Controls	Tests	Controls
0-7	3/21*	1/12*	5/6	0/2	6/6	0/2
8-13	6/15	2/10	3/3	0/2	3/3	0/2
14-20	4/12	2/8	0/3	0/2	1/3	1/2
21-27	3/9	3/6	1/3	0/1	1/3	1/1
28	0/6	0/5	4/6	1/5	1/6	0/5

* Blood culture taken before injection of bacteria.

lowed by a rise in antibody activity. Similarly, there was no difference between test and control animals with regard to total serum protein or its fractions as studied by zonal and immunoelectrophoresis. The peripheral white counts were high compared with human values, and no change with infection was observed in total count, differential count, or packed cell volume.

Discussion

Bacterial endocarditis can be induced in most species of animals only after they have been subjected to hemodynamic alterations, other stress, or the injection of multiple doses of bacteria [7–11]. In contrast, bacterial endocarditis can be induced in opossums with ease. This was done in the present study without hemodynamic change, with stress minimized, and with the injection of only a single bacterial inoculum.

It should be pointed out that adult opossums develop bacteremia readily, but the spontaneous occurrence of bacterial endocarditis can be all but eliminated in these animals under appropriate laboratory conditions. Although 19% of the blood cultures in our control animals were positive, only 2 opossums developed endocarditis, and neither infection was thought to be due to streptococci. No bacteria were obtained from the endocardial lesions of these latter 2 animals. However, the direct participation of the injected organisms in the pathogenesis of valvulitis seems certain, since 31% of blood cultures were positive in the test group, 12 of 21 animals developed endocarditis, and streptococci were easily recovered from 75% of these vegetations. In the 25% of vegetations without alpha-hemolytic streptococci, beta-hemolytic streptococci were isolated from only 1 animal, and no growth was seen in the remaining 2 test opossums.

The ready susceptibility of opossums to bacterial infection has not been explained fully. Recent studies of their immune system indicate that they have 2 major classes of humoral antibodies [12]. However, opossums respond to antigenic stimulation more slowly than do more conventional mammals [12–14]. There is no evidence that cellular immunity is impaired in these animals.

The relationships between bacterial endocarditis, brain abscesses, and glomerulonephritis in these animals are of particular interest. The adult opos-

sum often has a minor degree of chronic meningitis. However, brain abscesses were observed only in those animals having bacterial endocarditis and can be attributed most likely to emboli arising from the infected heart valves. The pathogenesis of the renal lesions is less easily explained. Although proliferative glomerulonephritis was seen more often in the kidneys of the test animals, it was also present in several controls.

Summary

Bacterial endocarditis was induced in unmodified adult opossums (*Didelphis virginiana*) by administration of a single intravenous injection of alpha-hemolytic streptococci. Animals were kept in individual cages and in a controlled environment preceding and during the 28-day experimentation. Bacteriologic, immunologic, hematologic, and pathologic findings have been presented. The results suggest that bacterial endocarditis can be induced successfully in this species by a single bacterial inoculum without subjecting animals to any cardiovascular stress or hemodynamic alterations.

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