

Bacterial Clearance and Endocarditis in American Opossums

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The American opossum is the only experimental animal that regularly develops bacterial endocarditis spontaneously. There was no relation between the ability of opossums to clear bacteria from the bloodstream and the subsequent development of endocarditis.

The American opossum (*Didelphis virginiana*) has been shown in captivity to acquire bacterial endocarditis spontaneously (4, 6). We studied the ability of opossums to clear the bloodstream of bacteria in an attempt to relate this host factor to the subsequent development of bacterial endocarditis.

Animals. Adult opossums (1.5 to 3 kg) were purchased within 3 days of capture by local trappers or were hand-caught along country roads south of Houston. They were housed individually in stainless-steel cages in an air-conditioned room and fed cat food and water ad libitum supplemented with oral ferrous sulfate and horsemeat once each week. Cages were cleaned each weekday but were not sterilized, a procedure which greatly reduces the incidence of bacterial endocarditis in captive opossums (5). Animals were examined and weighed two to three times each week.

Bacterial clearance studies: mutant strains. Temperature-sensitive mutants of *Staphylococcus epidermidis* and *Salmonella typhimurium* were prepared by Michael Gough and Jane Scott, Baylor College of Medicine. These mutants grew in brain heart infusion to 10^8 to 3×10^8 organisms per ml in 48 h at 25 C. *Staphylococcus* did not form visible colonies on a brain heart infusion agar plate at 37 C; *Salmonella* grew into visible colonies in 96 h. The rate of reversion to normal growth at 37 C was <1 per 10^6 .

Virulent strain. A beta-hemolytic strain of *Streptococcus faecalis* was isolated from the bloodstream of an opossum the day after capture. This animal died 3 days later and was found to have vegetations containing *S. faecalis* on aortic, mitral, and tricuspid valves. The organism was grown in brain heart infusion agar and kept frozen at -70 C until use. Overnight growth in brain heart infusion at 37 C yielded 6×10^8 to 8×10^8 bacteria per ml.

Preparation of animals. Sodium pentobarbital (50 mg/kg of body weight) was injected intravenously into a tail vein to produce light anesthesia. The inside aspect of one hind leg was shaved and scrubbed with povidone-iodine. Sterile dissection exposed an artery and vein each of which was cannulated with no. 50 polyethylene tubing. Blood was obtained for a baseline culture. Lines were kept open by flushing with saline which contained 10 U of heparin per ml; both were free of preservative.

Preparation of bacteria. The mutant strains were grown in brain heart infusion agar at 25 C, centrifuged at 2 C at $3,000 \times g$, washed once, and suspended in saline to give a final concentration of about 2×10^8 per ml. For each experiment, actual bacterial counts were determined by culturing appropriate 0.1-ml portions after serial 10-fold dilution.

Measuring clearance of bacteria. Bacteria (10^8 in 0.5 ml of saline) were injected rapidly into the cannulated vein, and the catheter was flushed with an additional 1 ml of saline. Blood was aspirated from the artery for 15 s and then 5, 10, 15, 20 min later. Tenfold dilutions were carried out, and appropriate portions were streaked onto brain heart infusion agar plates, which were then incubated at 25 C. Duplicate specimens of baseline blood and of undiluted blood, obtained at 30 min, were incubated at 25 and 37 C. Colonies were counted after 48 h, and clearance curves were constructed.

Statistical analysis. The analysis of variance was used with the alpha level having been set at 0.05 before initiation of the study.

Clearance of mutant strains. The results of clearance studies in 26 opossums with *S. epidermidis* are summarized in Fig. 1. Nineteen appeared to be clinically normal at the time of the study and had at least a 2-month survival thereafter. Seven died within 4 weeks of the procedure; two of these had had a positive

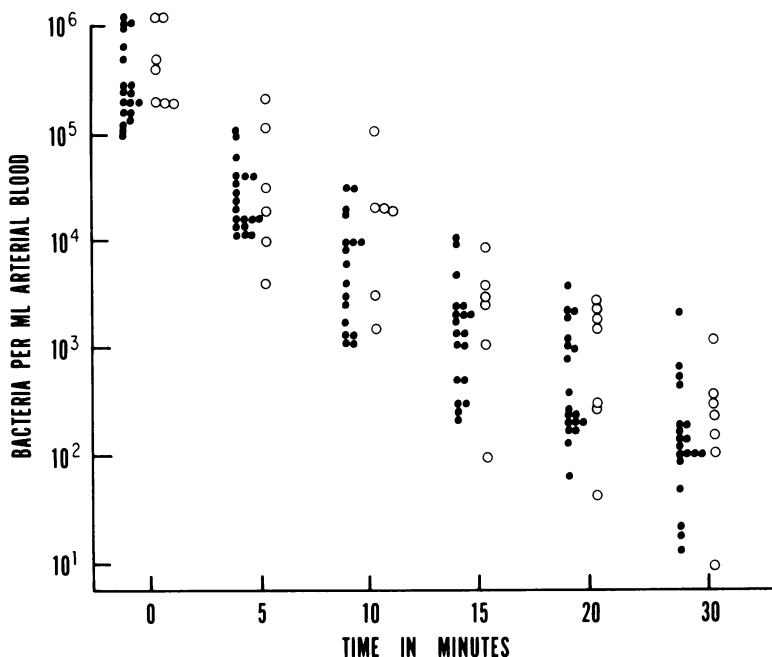


FIG. 1. Clearance of *Staphylococcus epidermidis* by 26 opossums during a 30-min period of observation. Nineteen opossums that appeared to be clinically normal and survived for at least 2 months (●). Seven opossums that appeared to be sick at the time of study or that died within 3 weeks (○).

baseline blood culture at the start of the clearance study. Six of the seven had bacterial endocarditis at autopsy; culture of their heart valves at 25 C did not yield the mutant strain of *S. epidermidis*. In all animals the number of bacteria in arterial blood declined from 10^5 - 10^6 to 10^2 - 10^3 in 30 min. Application of the analysis of variance revealed no difference in the ability of the two groups to clear bacteria.

S. typhimurium was cleared somewhat more rapidly than was the *Staphylococcus*. No difference was detected in clearance by three opossums that survived or one that died 9 days later with bacterial endocarditis caused by *E. coli* and *Staphylococcus aureus*. *S. typhimurium* was not recovered from the heart valves either at 25 or 37 C. Use of the mutant *Salmonella* was discontinued when we found that slow growth took place in vitro at 37 C.

Clearance of *S. faecalis*. Preliminary studies showed that groups of three opossums survived intravenous injections of 10^5 and 10^6 *S. faecalis* and three of four survived 10^7 organisms. Seven freshly captured opossums, all of which had sterile baseline blood cultures, were challenged intravenously with 10^8 *S. faecalis*. Clearance during 40 min of observation proceeded at a rate similar to that observed for *S. epidermidis* (Fig. 2). Venous blood cultures were intermittently

positive for enterococci (<10 organisms per ml) in all animals during the next 2 days. Thereafter these organisms were detected in increasing numbers in the blood of three animals which went on to develop generalized sepsis and died within 7 to 13 days. Bacterial endocarditis was present in each at autopsy. Enterococci were not detected again in the blood of the four remaining opossums; these then survived for at least 2 months. There was no significant difference in the rate of clearance by animals which developed bacterial endocarditis and those which survived.

The initiating event in bacterial endocarditis is thought to be bacteremia which is then followed by implantation of organisms on the valvular endothelium (1, 2). Factors that might predispose to bacterial endocarditis include: (i) the presence of preexisting valvular lesions, which have not been previously described in opossums (4, 6); (ii) an increased incidence of spontaneous bacteremia, which we have been unable to document in long-term studies of captive opossums (D. Musher and Y. Richie, unpublished observations); or (iii) decreased ability to clear bacteria from the bloodstream. The studies described above indicate that there was no correlation between bacterial clearance at 30 to 40 min or 24 to 48 h and the subsequent

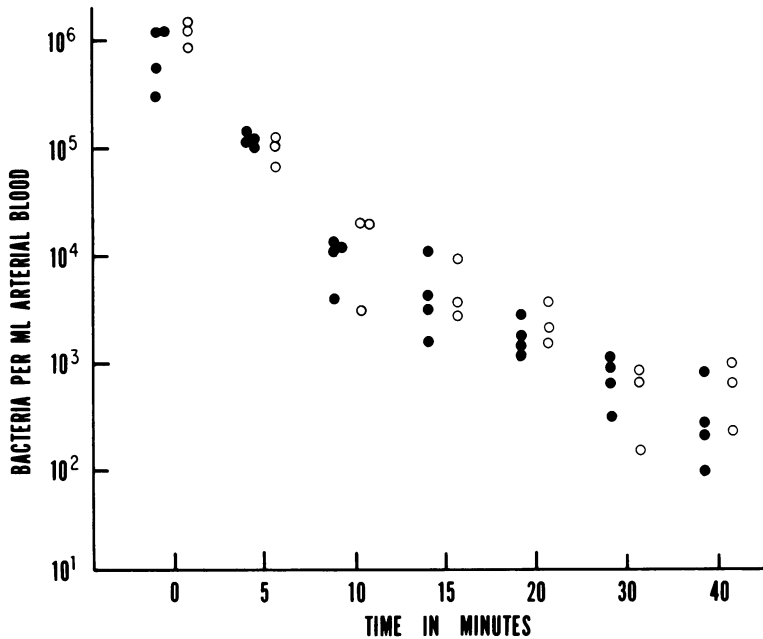


FIG. 2. Clearance of *Streptococcus faecalis* by seven opossums during a 40-min period of observation. Four opossums that remained healthy (●). Three opossums that died within 7 to 13 days of generalized enterococcal infection including BE (○).

development of bacterial endocarditis. This finding appeared to be true for both relatively nonvirulent gram-positive organisms and virulent gram-positive and gram-negative ones. These data are consistent with the hypothesis (3) that the outcome of bacteremia depends more on its cause or on other host factors such as the status of the cardiac valves and bactericidal capacity of phagocytizing cells than on the ability of the host to clear bacteria from the bloodstream.

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