Fate of Staphylococci in Fresh Rabbit Blood and Serum* (32940)

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The bactericidal activity against gram-positive bacteria present in normal serum is not found in plasma carefully preserved from clotting by anticoagulants or hydrophobic surfaces and appears to be released from platelets only during blood clotting (1–3). These experiments suggest a lack of appreciable activity *in vivo*. Because this conclusion is contrary to a common assumption that serum bactericidal activity is an important factor in host defenses against gram-positive bacteria, a reexamination for possible activity in circulating blood with a different assay technique was attempted. This communication measures the rate of death of staphylococci in fresh rabbit blood before and after clotting has occurred. Assuming that unclotted blood examined immediately after bleeding resembles blood in the animal, then a comparison of bactericidal kinetics for fresh blood and for serum should reveal whether circulating blood is as bactericidal as serum.

Methods. All experiments were performed with a serum-susceptible *Staphylococcus epidermidis* in logarithmic growth phase accomplished by incubating a 1/40 dilution of an overnight broth culture for 70 min in a 37°C rotary water bath and adjusting the absorbance to 0.015 with 1% peptone–0.5% NaCl diluent (5×10^7 bacteria/ml). All procedures were performed at room temperature (24°C). Approximately 7 ml of blood were withdrawn by cardiac puncture from a 3 kg rabbit into a plastic syringe containing 2.3 ml of adjusted bacterial culture at 37°C. The total volume was read and the contents were mixed by inversion. At intervals, 1.0-ml portions were delivered into 19.0 ml of water to stop by dilution the bactericidal action. Following a subsequent 1/10 dilution, 0.5 ml was added to 19.5 ml of broth in 50-ml Erlenmeyer flasks. After an incubation period of 7.5 hours in a rotary 37°C bath the cultures were in logarithmic growth phase and the absorbancies were measured at 600 mμ. The photometric assay had a precision of ± 5% and a linear relationship was found between inoculum size and absorbance. Optical assays are accurate and not significantly affected by bacterial clustering (4). Viable counts were also measured on a 1/10 dilution of the contents of the flasks before incubation and these results agreed with those from the photometric assay. The assay for survivors was similar in all studies. Controls were obtained by making similar dilutions of the same culture without blood. The bactericidal action of serum was studied by adding 2.0 ml of adjusted staphylococci to a mixture of 6.0 ml of pooled rabbit serum and 2.0 ml of diluent at 37°C. This mixture was placed at 24°C and 1.0-ml portions were removed at intervals and added to 19.0 ml of water and the survivors were assayed.

Rabbit β-lysin was prepared from serum by Seitz filtration (5) and dialyzed against 0.15 M NaCl. The preparation was equilibrated to 37°C before addition to blood. Immediately after blood was drawn by the described procedure, 2.0 ml of β-lysin was also drawn into the syringe and mixed with the fresh blood and staphylococci. Bactericidal activity of β-lysin in the absence of blood was assayed in 0.3% $NaHCO_3$ because bicarbonate potentiates its activity against staphylococci.

Results. The percentage of bacteria surviving in fresh rabbit blood and serum plotted as a function of the time after mixing with bacteria is shown in Fig. 1. With fresh blood there was no decrease in the number of staphylococci until the appearance of appreciable clotting. At this time the number of staphylococci decreased sharply, either through release of bactericidal factors or the trapping of the bacteria in the clot. The time of onset of blood clotting was dependent on the proficiency of cardiac puncture and usually was observed at about 7 min, with full

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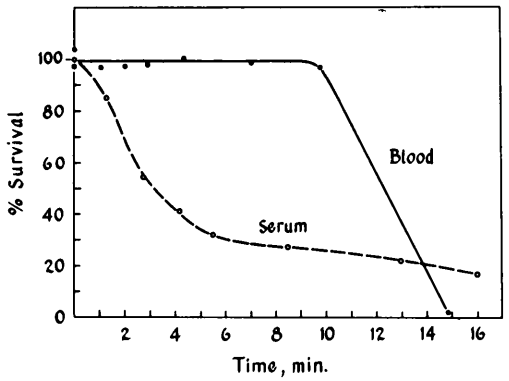

FIG. 1. A comparison of bactericidal kinetics for fresh blood and serum of rabbits against *Staphylococcus epidermidis*.

clotting at 12 min. Similar results were obtained in six rabbits. The bactericidal activity of serum began immediately and proceeded rapidly.

Discussion. These results have shown the system found in serum that is bactericidal for staphylococci is inactive in freshly shed rabbit blood. Thus, our data are consistent with reports that serum bactericidal factors are activated during clotting and until then are not appreciably active. This does not prove that the bactericidal system has no role in natural immunity, since the possibility exists for activation with or without blood clotting under conditions such as may be found after trauma or bacterial challenge. In this regard, it has been shown that platelet bactericidal factors are not identical with a number of blood clotting factors (3) and may be released from platelets by endotoxin (6). Experiments attempting activation independent of blood clotting may be performed with the kinetic assay technique described. In one such attempt the activity of rabbit serum was checked 4 hours after injection of 9 mg of brucella antigen (US Dept. Agriculture) into normal and brucella-immunized rabbits. The freshly shed blood of these rabbits was inactive although the serum was active. This indicates that either the endotoxin content of these organisms could not release appreciable amounts of β-lysin or that any released β-lysin was neutralized. Purified β-lysin was used to check for additional inhibitors in blood besides those which cause β-lysin to bind to platelets. Blood was not inhibitory to platelet-released β-lysin since a combination of β-lysin and freshly shed blood killed 40% of bacteria in 2.5 min compared with 15% killed by β-lysin alone.

The inactivity of blood relates to staphylococci and likely to other gram-positive bacteria but not to the complememt-dependent bactericidal system active against gram-negative bacteria. Similar experiments indicated that the factors which kill *Brucella abortus* are operative in unclotted blood and thus probably *in vivo*.

Summary. A method is presented to enable measurement of the initial bactericidal activity in freshly-drawn blood. Although *Staphylococcus epidermidis* was quickly killed by rabbit serum, whole blood did not become bactericidal until it clotted. The β-lysin which is presumably responsible for the death of gram-positive bacteria in serum could not be released from platelets into the circulating blood by injection of endotoxin into sensitized rabbits. No inhibitor for β-lysin was found in unclotted blood.

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