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Effect of High Oxygen Concentration on Virulence of *Staphylococcus aureus*

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Cultures of *Staphylococcus aureus* were agitated and treated with oxygen or air for a period of 29 days. At intervals of 1, 3, 9, 15, and 29 days, samples of washed cells were tested for coagulase activity and for abscess-producing ability. It was found that aeration with agitation initially increased abscess formation and that the increase was of the same magnitude for oxygen and air. Continued treatment beyond 1 day resulted in a progressive decrease in this activity which was more pronounced in the oxygenated cultures. Throughout the treatment, the bound coagulase activity remained constant. Thus, there appeared to be no quantitative relationship of coagulase titer with virulence as expressed by lesion formation.

The application of closed environmental systems to manned space missions has necessitated studies of the effects of these modified environments on the virulence of infectious microorganisms. Of particular interest is the effect of increased partial pressures of oxygen. Staphylococci were selected for study because they are present on body surfaces continuously exposed to the gaseous environment. These bacteria produce a number of toxic substances and enzymes which have often been correlated with pathogenicity. Coagulase is generally recognized as one of the more important virulence factors elaborated by these microorganisms. The purpose of this study was to determine the influence of a high oxygen concentration on the virulence of *Staphylococcus aureus*, as evidenced by abscess production, and to compare this with the coagulase activity of the cells.

MATERIALS AND METHODS

A 1-ml amount of a 24-hr broth culture of phage type 80 strain of *S. aureus* was transferred to each of four 500-ml Erlenmeyer flasks containing 250 ml of Trypticase Soy Broth (BBL). One of the cultures was treated with oxygen (95%, plus 5% nitrogen), and another, with room air by continuously bubbling the gas (300 ml/min) under the surface of the medium through a glass tube. Additionally, both cultures were constantly agitated on a mechanical shaker [128 0.5-inch (1.27-cm) strokes/min]. The two remaining cultures served as controls; one was agitated but not aerated, and the other received neither treatment. All of the cultures were incubated at 37 C. Each succeeding day throughout the study period, 1 ml of the 24-hr growth in the individual flasks was transferred to a similar vessel containing sterile broth, and the indicated treatment was applied to the fresh culture. At intervals of 1, 3, 9, 15, and 29 days, the 24-hr cultures were examined for coagulase activity and abscess-forming capacity. Cells in 20 ml of the broth were sedimented and washed twice with buffered saline. Appropriate dilutions of the cells were plated in triplicate on Nutrient Agar (Difco) to determine the number of colony-forming units. The remaining washed cells were stored at 4 C overnight. They were then diluted to yield 2×10^7 colony-forming units/ml and were tested for bound coagulase activity with Coagulase Plasma (Difco), according to the instructions provided with the test plasma. Quantitation of activity was accomplished by testing serial twofold dilutions of the bacteria. Titers were expressed as the reciprocal of the highest dilution causing the plasma to clot. The time required for clot formation by the undiluted washed cell suspension was also recorded.

The abscess-forming ability of the organisms was determined by subcutaneous inoculation of mice with 0.1 ml of appropriate dilutions. Injections were made on the shaved abdominal wall of the animals. At the intervals specified, male Swiss-Webster mice, 10 to 12 weeks old, were arranged in four groups of 28 animals each. Bacteria from each of the four cultures were tested in one of these groups of mice. Each animal received a dose of 0.1 ml containing 2×10^6 colony-forming units of bacteria and was examined daily for the presence of a demonstrable lesion. It was found in earlier studies with the test strain that the dose given would produce a local abscess in approximately 50% of mice inoculated. When an abscess was observed within 7 days, a positive result was recorded.

RESULTS AND DISCUSSION

As shown in Table 1, the coagulase activity of the cultures was not affected by any of the test conditions. No statistically significant difference was found in the coagulase titer or in the time

TABLE 1. *Abscess formation and coagulase activity of Staphylococcus aureus treated with oxygen or air*

Aeration (days)	Mice per group	Abscess formation (per cent positive)[a]		Coagulase activity[a]			
				Oxygen		Air	
		Oxygen	Air	Min to clot	Titer[b]	Min to clot	Titer[b]
0	28	—	50.0	—	—	60	32
1	28	85.7[c]	78.6[d]	65	32	45	32
3	28	64.3	64.3	50	64	45	32
9	28	46.4	60.7	55	32	40	64
15	28	21.4	50.0	60	64	45	64
29	28	7.1[c]	28.6	45	32	50	32

[a] No change was observed in cells from nonaerated stationary or agitated control cultures.
[b] Reciprocal of highest dilution producing plasma clot.
[c] Difference from control significant at 1% level.
[d] Difference from control significant at 5% level.

required for clotting. Treatment of the cells with oxygen or air did, however, produce an increase in their abscess-producing capacity. The increase was observed after only 1 day of aeration and was of the same magnitude for air or oxygen. Subsequently, a progressive loss in activity which was more pronounced in the oxygenated cultures was noted. In fact, after 29 days, the abscess-producing ability of the organisms treated with oxygen was significantly lower than that of the controls. Cells treated with air also showed a decrease in activity after the initial rise, but at 29 days they had not reached a value significantly below that of the controls. All values for the controls remained essentially constant throughout the period of observation.

The number of mice developing abscesses after injection with the nonaerated culture (0 days) was compared by use of a chi-square test with the number observed in the groups receiving aerated cultures. The increase related to oxygen treatment is statistically significant at the 1% level and for air at the 5% level. Similar comparisons were made of the results among animals injected with cultures aerated for 29 days. It was found that the oxygenated cells had an abscess-producing ability significantly less than had the nonaerated controls. Linear regression equations were applied to the data beginning with day 1, by use of the percentages shown as well as probit transformation values. These analyses provide estimates of the number of days during which the aerated cells in the dose used were capable of producing an abscess in 50% of the mice inoculated. These estimates were 8.8 and 15.3 days for oxygen and air, respectively, and a statistical test of the difference between them is significant ($P < 0.05$). Thus, oxygen is more effective than air in reducing the abscess-producing potential of the bacteria.

We have found no consistent quantitative relationship between bound coagulase titer and abscess formation. The incidence of abscesses in the test groups increased initially and then decreased, but the coagulase activity remained constant. Brown (1) suggests that coagulase production is necessary for pathogenicity but concludes that the usefulness of the coagulase test by itself has been greatly exaggerated. Kapral and Li (4) do not agree that either bound or soluble coagulase is essential for virulence of staphylococci.

Fahlberg and Marston (3) reported that aeration of cultures or treatment with 10% carbon dioxide did not significantly affect coagulase titers, but that shaking of the cultures did increase soluble coagulase production. Edwards and Turner (2) demonstrated increased coagulase activity in cultures which were both aerated and agitated. They found that peak titers were achieved within the first 8 hr and that rapid destruction of activity occurred when growth was not stopped at that time. Only low titers were detectable after 24 hr of incubation. These workers made no distinction between bound and soluble coagulase.

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