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SUPEROXIDE INVOLVEMENT IN THE BACTERICIDAL EFFECTS
OF NEGATIVE AIR IONS ON STAPHYLOCOCCUS ALBUS

The physical nature of small air ions is well established and it is recognized that they can produce a variety of biological effects.¹ However, in only a few instances have portions of the underlying biochemical changes been detected^{2,3,4}. In consequence, we lack a precise understanding of the events taking place in any given case of air ion action. Theoretically, one can consider the hydrated superoxide radical anion $(O_2^-)(H_2O)_n$ with $n \approx 4-8$ as a likely candidate for a biologically active species of negative air ion⁵, and a vast literature exists as to the chemical and biological reactivity of superoxide⁶. This includes a leading role in bacterial killing caused by radiation^{7,8}, in which superoxide dismutase (SOD), an enzyme that catalyzes the reaction:

protected markedly. Other studies have also demonstrated the bactericidal effect of O_2^- ^{9,10,11}. Inasmuch as the bactericidal action of small negative air ions has been repeatedly confirmed, we decided to test for the involvement of O_2^- in this phenomenon by evaluating the protective effect of SOD.

Figure 1 illustrates the apparatus used in our experiments. To insure that all negative ions went through the solution to the ground wire, we insulated the ground wire using heat shrink tubing and teflon tubing to a point well below the solution's surface. A Modulon corona discharge type negative ion generator provided the ionizing potential; to maintain a uniform current we adjusted the ionizing potential using separate variable transformers on the A.C. lines of each generator.

Inoculated flasks, containing approximately 10^6 cells/ml of Staphylococcus Albus in 40 mls of sterile 3.75 mM NaCl with 1.25 mM KPi, pH 7.8 buffer, were maintained at $22 \pm 1^\circ\text{C}$ in a waterbath for the duration of the experiment. A vacuum pump with airflow regulator exhausted air from the flasks at a rate of approximately 500 ml per minute. One ml aliquots were removed hourly, serially diluted, and then plated as previously described¹².

We isolated superoxide dismutase from bovine liver using the procedure of McCord and Fridovich¹³. This yielded pure enzyme of high specific activity (approx. 3.500 units/ml). Catalase was purchased from Boehringer Mannheim Biochemicals and extensively dialyzed before use. We sterilized these enzymes by passing them through a millipore filter immediately before addition to the bacterial inoculum.

Table I illustrates that the exposure of bacteria to negative air ions caused a substantial loss in viability in as short a period as two hours, with a total loss of viability seen after five hours of ion treatment. Control samples, incubated under identical conditions but with no negative ionization, retained full viability after 5 hours. The addition of $10 \mu\text{g/ml}$ SOD protected bacteria completely against negative ion killing while catalase or denatured SOD had no significant protective effect. The non-effectiveness of catalase in this system appears in marked contrast to that seen in most systems which involve O_2^- toxicity. For example, both O_2^- and H_2O_2 were required for lipid peroxidation or erythrocyte lysis¹⁴ where their toxicity manifests through their interaction to produce hydroxyl radical and singlet oxygen. In the case of negative air ion bacterial kill the toxicity of O_2^- appears H_2O_2 independent. A possible mechanism might involve O_2^- acting as a nucleophile on the phospholipid bilayer, causing a de-esterification

of fatty acids¹⁵. This could lead to an increase in surface charge and a weakening of the membrane, which under hypotonic conditions might lead to cell lysis and death.

The experiments reported here support the concept that the negative air ions responsible for bacterial death consist solely or in part of hydrated superoxide anions. Paradoxically, while negative air ions have a reputation as a rather beneficent species¹, superoxide radical anion apparently acts as one of the most toxic species produced in aerobic metabolism⁶. Perhaps the resolution of this paradox lies in the relative amount of O_2^- required for an effect. Negative air ions can cause physiological effects at extremely low levels, with clean country air having a negative ion concentration of only about $10^3/cm^3$, an amount which more closely parallels that of pheromones than of chemical reactants. In our system we have greatly amplified the flux of negative ions to a level which could produce, theoretically at least, 0.3 mMoles O_2^- /hour, which we channeled through a total of 40 ml of solution. Even so, in vitro systems testing for O_2^- reactivity have invoked a flux hundreds of times greater. Thus the effects of superoxide as a negative air ion although dependent on its chemical reactivity may be greatly amplified by a biological sensitivity in the hormonal range. Hopefully, our tentative identification of $(O_2^-)(H_2O)_n$ as the negative air ions responsible for killing microorganisms can provide a more solid base for investigating the effects and mechanisms of negative air ion effects in other biological systems.

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BACTERIAL KILL BY NEGATIVE AIR IONS: PERCENT VIABILITY

Conditions *	Incubation Time (hours)					
	0	1	2	3	4	5
no (-) ions (control)	100	123±3.4	115±22.5	112.5±1.0	111.6±22.3	105.6±11.0
(-) ions	100	116±3.5	72.3±9.9	29.2±24.9	2.5±2.3	0
(-) ions +10 µg/ml SOD	100	129.0±1.0	106.2±18.8	134.0±13.0	109.0±25.1	104.8±14.2
(-) ions +10 µg/ml (autoclaved) SOD	100	87.9±7.1	70.0±18.1	27.1±1.1	5.5±1.4	0
(-) ions +10 µg/ml Catalase	100	105.2±23.8	56.7±10.4	21.5±4.4	11.9±3.4	1.0±1.0

* Negative ion flux maintained at approximately 5 microamps in all experiments. † Figures indicate the range between duplicate experiments.

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FIGURE 1

1. Air exhaust tube. 2. Ion discharge needle. 3. Cotton plug.
4. Air inlet tube. 5. 18 gauge hypodermic needle. 6. Ground wire tube.
7. Ground wire. 8. Heat shrink tubing. 9. 1 mm teflon insulating tube.
10. Bare platinum tip. 11. 1 mm teflon sample tube. 12. Magnetic
stirring rod. The apparatus was constructed in a 500 ml erlenmeyer flask
fitted with a #10 rubber stopper.

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