

MATTERS ARISING

Superoxide involvement in negative air ion effects

INTEREST in research on the biological effects of small negative air ions has been due to the variety of claims made for them, ranging from lethal effects on microorganisms to therapeutic effects on humans¹⁻³. In spite of the promising results reported, there has been a lack of understanding of the underlying biochemical basis. This is not surprising because the exact nature of the air ions has not been defined by those concerned. Consequently, there has been no investigation of the interaction between well defined ionized air species and biological systems of different degrees of complexity, which could have provided the basis for understanding the possible biological impact. Recently, however, experiments have been reported by Kellogg *et al.*⁴ which "support the concept that the negative air ions responsible for bacterial death consist solely or in part of hydrated superoxide anions". This conclusion was based on the observation that the enzyme superoxide dismutase (SOD) completely protected bacteria against killing by air ionization. This enzyme is known to remove O₂⁻ efficiently by catalysing its disproportionation into hydrogen peroxide and molecular oxygen. The negative air ions in that study were produced by a corona discharge-type negative ion generator.

We suggest caution in adopting this conclusion. The negative ion species generated in air by corona discharge have been identified accurately by mass spectrometry⁵. At atmospheric pressure, in static or dynamic conditions, CO₃⁻ is the dominant species produced. Such ions were found to be due to small concentrations of CO₂ in air. The CO₂ apparently reacts with O⁻ or O₃⁻ to form CO₃⁻. It was estimated that only about 40 p.p.m. of CO₂ is needed at atmospheric pressure to convert all oxygen ions to CO₃⁻ species. Superoxide anion, O₂⁻, was detected in small amounts only at a gas pressure lower than 200 torr, a maximum of about 15% O₂⁻ being produced at 20 torr.

We know of no published report that CO₃⁻ can be converted to O₂⁻ in water. We have tested⁶ the possible generation of O₂⁻ from ionized air in aqueous medium by simple and straightforward chemical assay such as reduction of nitroblue tetrazolium (NBT) to the corresponding formazan⁷. A 5-day exposure of an aqueous solution of NBT (10⁻⁵ M) to the ion generator (Modulion Corona discharge-type operated at a maximum discharge current of 0.1 mA) provided no indication of conversion to formazan. Also, no evidence could be obtained of the production of hydrogen peroxide, the product of O₂⁻ self-disproportionation

($k = 4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, ref. 8) in water exposed to the ion generator (1 ml water, 10 cm distance from the needle, 24-h exposure).

Finally, the negative ion flux in the work of Kellogg *et al.*⁴ was 5 μA . This corresponds to about 3×10^{13} electron charges per s or 1.1×10^{17} electron charges per h. Even if all the negative charges produced O₂⁻, and neglecting its decay, this is equivalent to 1.8×10^{-7} mol O₂⁻ per h. This differs significantly from 3×10^{-4} mol O₂⁻ per h as the stated level of O₂⁻ in the same work.

We have no ready explanation for the protection by SOD against bacterial killing by negative air ions observed by Kellogg *et al.*⁴. Only by carefully duplicating their experiments and manipulating the variables in their system could one hope to explain the observed data. Whatever the explanation, however, it is very unlikely to be mediated by superoxide radical anion.

Regarding the general mechanism of action of negative ion generators (which are almost exclusively of corona discharge-type), we find it surprising that no attention has been paid to the striking resemblance between them and electrostatic precipitators. These latter devices, well known in the chemical industry, efficiently collect dust, fumes and mists by charging the particles in an electrostatic field created by negative corona discharge electrodes⁹.

We suggest that the same effects attributed to some undefined small negative air ions could be explained by the electrical field produced by the generation of ions. Such a field could cause, for example, the enhanced migration of microbial cells, reminiscent of electrophoresis, which ends in agglutination or adsorption to the walls, resulting in a lower plate count.

The claimed feeling of well-being produced by ion generators¹⁻³ could be due to an atmosphere free of aerosols and dirt, cleaned by electrostatic precipitation. Would not any other air-filtration device give the same results?

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1. Knoll, M., Fichmeier, J. & Schön, R. W. *Adv. Electron. Electron Phys.* **19**, 178-244 (1964).

- Gualtierotti, R., Kornbluh, I. H. & Sirtori, C. *Bioclimatology and Aeroionotherapy* (Carlo Elba Foundation, Milan, 1968).
- Kreuger, A. P. & Reed, E. J. *Science* **193**, 1209-1213 (1976).
- Kellogg, E. W., Yost, M. G., Barthakur, N. & Kreuger, A. P. *Nature* **281**, 400-401 (1979).
- Shahin, M. M. *Appl. Opt. Suppl.* **3**, 106-110 (1969).
- Rosenthal, I., Juven, B. J., Gordin, S. & Ben-Hur, E. J. *Appl. Bact.* **46**, 451-454 (1979).
- Beauchamp, C. O. & Fridovich, I. *Analyt. Biochem.* **44**, 276-287 (1971).
- Bielski, B. H. J. & Allen, A. O. *J. phys. Chem.* **81**, 1048-1050 (1977).
- Kirk, R. E. & Othmer, D. F. (eds) *Encyclopedia of Chemical Technology* Vol. 5, 646-665 (1954).

KELLOGG, YOST AND KRUEGER REPLY—We have several points to make in reply to the comments by Rosenthal and Ben-Hur. First, no one has yet definitely characterized the chemical nature of negative air ions. The work by Shahin¹ on the ionic species produced by corona discharge seems quite interesting, and he does clearly identify the predominant negative ion made in his system (at atmospheric pressure) as CO₃⁻. However, his system differs from ours² in several ways, including the maximal current used (1 mA compared with our 0.01 mA) and the corona discharge voltage (12 kV compared with our 5 kV). We cannot determine whether these differences would affect the negative ions produced. However, even if our system did make CO₃⁻ as the principal negative ion species, Shahin's work does not preclude the production of HO₂, which as an uncharged species would go undetected by mass spectroscopic methods, and which would give rise to O₂⁻ in solution.

We do know of a number of studies that support the O₂⁻(H₂O)_n negative air ion concept. For example, Mohnen³ summarized his work as follows: "An attempt has been made to determine theoretically a reaction scheme to explain the formation of negative small ions in the troposphere and lower stratosphere. The terminal ions are found to be hydrated O₂⁻ and CO₄⁻. The degree of hydration depends on temperature, and the mean number of water molecules attached to those ions is of the order of 3 or 4. As soon as the water vapor concentration is less than the concentration of carbon dioxide (about 8 km), the terminal ion is found to be hydrated CO₄⁻. The reaction scheme is based on measured and reasonably estimated reaction rate constants determined by using U.S. standard atmosphere for the neutral gas composition." Regardless of the identity of negative ions in air, this does not preclude their transformation to other ions, such as O₂⁻ on addition to solution. Our data, which showed strong protection against negative ion bacterial kill by superoxide dismutase (SOD), but not by denatured SOD, strongly indicated O₂⁻ involvement in this negative ion effect, which occurred whether one considers negative air ions as O₂⁻(H₂O)_n or not.