Journal of Applied Microbiology 1999, **87**, 889–897

Sensitivity of *Candida albicans* to negative air ion streams

J.M. Shargawi, E.D. Theaker, D.B. Drucker, T. MacFarlane and A.J. Duxbury
Department of Dental Medicine and Surgery, University of Manchester, Manchester, UK

7158/04/99: received 7 April 1999 and accepted 16 August 1999

J.M. SHARGAWI, E.D. THEAKER, D.B. DRUCKER, T. MACFARLANE AND A.J. DUXBURY. 1999.
Negative air ions (NAIs) are known to kill *C. albicans*; however, their precise mechanism of action is uncertain. Elucidation of this has been hampered by a lack of reproducibility between results obtained by different investigators. The aim of this study was to determine the influence of variation in experimental parameters on the sensitivity of *C. albicans* to negative air ions and the role of ozone in this process. Ten strains of *C. albicans* were exposed to NAIs generated at different emitter distances, exposure times, relative humidities and under aerobic and oxygen-free conditions. In further experiments, ozone levels were measured under the same conditions. The effect of NAIs on *C. albicans* growth was assessed by measuring the area of the zone of inhibition generated around the electrode of the ionizer. There was a significant reduction in area of zone of inhibition with increasing emitter distance ($P < 0{\cdot}05$), relative humidity($P < 0{\cdot}05$) or under oxygen-free conditions($P < 0{\cdot}05$). Increases in exposure time resulted in a significant increase in growth inhibition ($P < 0{\cdot}05$). Ozone levels increased with increasing exposure times ($P < 0{\cdot}01$) but were significantly reduced as emitter distance increased ($P < 0{\cdot}01$). When utilized in a nonventilated room, levels of ozone produced did not exceed recognized safety limits. These results (a) demonstrate the importance of careful control of experimental parameters if reproducibility of studies involving NAIs is to be achieved, and (b) highlight the possible role of ozone in the microbicidal effects of NAIs.

INTRODUCTION

Negative air ions (NAIs) have been shown to have a lethal effect on various micro-organisms (Krueger and Reed 1976). This field of research was initiated by Tchijevski (1933, cited by Krueger and Reed 1976), who demonstrated inhibition of growth of *Staphylococcus aureus*, *Vibrio cholerae* and *Salmonella* sp. following exposure to NAIs, and subsequently developed by Krueger and Smith (1957, 1958) and Krueger *et al.* (1959). The latter demonstrated that air ions have a significant and reproducible effect on the viability of microbial cells, findings which are supported by the work of Kingdon (1960) with respect to *Escherichia coli*. More recently, Marin *et al.* (1989) have demonstrated inhibition of growth of both *E. coli* and *Staph. aureus* on exposure to NAIs. However, little attention has been paid to the effect of NAIs on *C. albicans* (Cousins *et al.* 1991).

Correspondence to: D.B. Drucker, Oral Microbiology Laboratory, Department of Dental Medicine and Surgery, University of Manchester, Higher Cambridge Street, Manchester, M15 6FH, UK (e-mail: David.Drucker@man.ac.uk).

The action of NAIs on micro-organisms cultured on an agar surface has been variously attributed to the physical displacement of cells (Rosenthal *et al.* 1979), to cell agglutination (Rosenthal 1981), to accelerated desiccation of the agar gel (Kroling 1985), or to an effect associated with the ion discharge itself (Pethig 1984). The latter is initially composed of free electrons which react with oxygen to generate oxygen radicals. These radicals interact with oxygen and carbon dioxide to yield O_3^-, O_2^- and CO_3^- anions (Pethig 1984), the generation of which, by corona discharge, has been confirmed using mass spectrometry (Shahin 1969). The bactericidal effects of NAIs have been attributed to both CO_3^- (Rosenthal 1981) and O_2^- ions (Kellog *et al.* 1979). Moreover, the generation of ozone, nitric oxide and nitrous oxide is also feasible (Pethig 1984).

Conducting a properly controlled laboratory study of the effects of NAIs on micro-organisms has presented practical difficulties. The control and measurement of temperature and humidity are essential (Phillips *et al.* 1964). Krueger and Reed (1976) have attributed the 'uneven quality' of the published research on negative air ion streams to faulty

experiment design. In particular they highlighted the failure of investigators (a) to take into account the production of ozone and nitrous oxides, (b) to monitor and control ion densities, temperature, humidity, and (c) to hold the experiment at ground potential. Rosenthal *et al.* (1979) have suggested that inadequate results were a consequence of the poor technical design of ion generators, complicated by incomplete and inaccurate descriptions of experimental conditions. These factors have made reproducibility of experiments difficult to achieve.

The potential value of a commercially available, corona-discharge ion generator designed for use in the oral cavity has been confirmed, *in vitro*, with respect to the treatment of dental caries (Burke *et al.* 1995). Similarly, based on their observations of the lethal effect of NAIs *in vitro* on a variety of Gram-positive and Gram-negative bacteria, Cousins *et al.* (1991) concluded that the use of such an ion generator could result in the elimination of bacteria from carious lesions, periodontal pockets, contaminated surfaces and instruments. However, to date there are only limited published data on the effects of the factors highlighted by Krueger and Reed (1976) on the sensitivity of micro-organisms to NAI streams and on the effect of NAIs on *C. albicans* (Cousins *et al.* 1991). Thus the aim of this study was to measure the sensitivity *in vitro* of *C. albicans*, under various conditions, to NAIs produced by such a corona-discharge ion generator.

MATERIALS AND METHODS

Culture and preparation of *C. albicans*

Ten human isolates of *C. albicans* were used from our laboratory collection. Identification of all isolates of *C. albicans* was confirmed using both ID 32C and API 20C AUX (Bio Merieux, Basingstoke, UK). Following preliminary growth curve experiments, early stationary phase cultures of *C. albicans* were used for experiments. These were obtained by inoculating 49-ml aliquots of Sabouraud's dextrose broth (LAB M, Bury, UK) with 1-ml aliquots of overnight, stationary phase, broth cultures of *C. albicans*. A further overnight incubation was then carried out in a shaking water bath at 37 °C.

Inhibition of *C. albicans* growth by NAIs under aerobic conditions

Stationary phase cultures were diluted 10-fold in 0·01 M phosphate buffered saline of pH 7·4 at 25 °C. Aliquots (0·4 ml) of each suspension were inoculated as a lawn onto Sabouraud's Dextrose Agar plates (LAB M) in which a nickel silver wire had been placed as a ground electrode. Inocula were allowed to dry, then exposed to a stream of NAIs from a commercially available generator (Biogun, Dentron Ltd, Ashley, UK). Three separate quarters of each plate were exposed to NAIs whilst the fourth quarter acted as an unexposed control. The techniques used were a modification of those employed by Kingdon (1960).

Plates were subsequently incubated aerobically at 37 °C overnight and the resultant colonies were photographed. Where zones of inhibition were present, their areas were measured on the photograph using a planimeter (W.F. Stanley & Co. Ltd, London, UK). Each photograph incorporated a scale to facilitate measurement.

Influence of relative humidity

Relative humidity was tested for its ability to influence the inhibition of *C. albicans* by NAIs by performing the above procedure in a closed chamber (Fig. 1) at 37 °C using an emitter distance of 3 mm for an exposure time of 2 min. Relative humidities of 0%, 50% and 100% were employed. In all of the above experiments both current (mA) and voltage (kV) were recorded.

Influence of emitter distance and exposure time

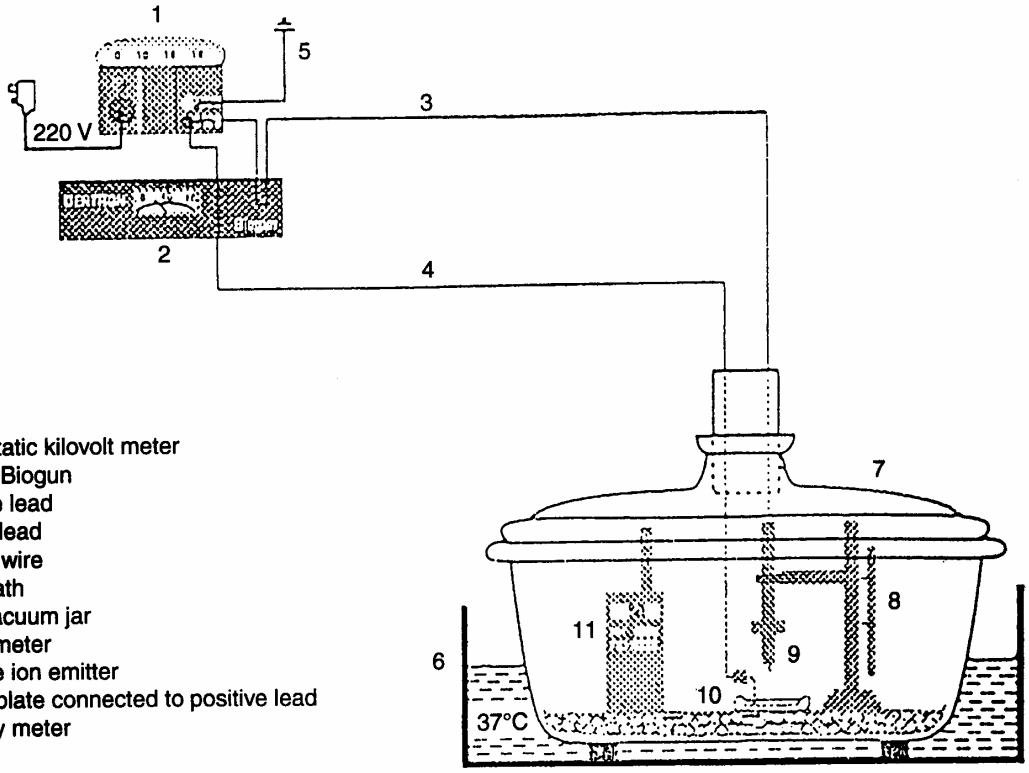
The influence of emitter distance (the distance between the surface of the agar plate and the tip of emitter) and exposure time on the efficacy of NAIs were assessed at 37 °C in 0% humidity. Emitter distances of 2, 6 and 12 mm were employed. For each emitter distance the effects of exposure times of 2 min, 3 min and 4 min were assessed. In all the above experiments, both current (mA) and voltage (kV) were recorded, so that the number of ions emitted per second could be calculated.

Determination of ozone levels at different exposure times, humidities and emitter distances

It has been postulated that inhibition of *C. albicans* growth by NAIs occurs, at least in part, as a result of the generation of ozone and nitrogen oxides. The experimental procedure described above was therefore repeated, at a relative humidity of 50%, with emitter distances of 2 mm, 6 mm and 12 mm, and at relative humidities of 0%, 50% or 100%, with a fixed emitter distance of 6 mm. Under each of the above conditions, ozone levels were determined using an ozone analyser model 8810, designed by the United States Environment Protection Agency (US–EPA designated). Ozone readings were taken at one-minute intervals over a period of 10 minutes.

The influence of an oxygen-free atmosphere

The purpose of these experiments was to exclude lethal effects due to ozone and nitrogen oxides by using an oxygen-free atmosphere. Eight isolates of *C. albicans* were cultured



1. Electrostatic kilovolt meter
2. Dantron Biogun
3. Negative lead
4. Positive lead
5. Earthed wire
6. Water bath
7. Glass vacuum jar
8. Thermometer
9. Negative ion emitter
10. Culture plate connected to positive lead
11. Humidity meter

Fig. 1 The apparatus used to study NAIs

to stationary phase and the previously described experimental standard conditions were used. The inoculum in one of four quadrants was exposed to NAIs for 2 min at a 6-mm emitter distance in the presence of air, as a positive control. Then, in a second quadrant, the inoculum was exposed to NAIs in the presence of nitrogen (oxygen-free atmosphere). This was achieved by flushing out the air present in the chamber with oxygen-free nitrogen (OFN, white spot, Grade N 4·8, purity 99·999%; supplied by BOC, Guildford, Surrey). This wash-out of air and its replacement with pure nitrogen was repeated three times, prior to exposure to NAIs. Air replacement was achieved by the use of active suction. Cultures were incubated aerobically at 37 °C overnight. The resultant zones of inhibition were photographed and measured as described previously.

Nature of the effect of NAIs on *C. albicans*

This experiment determined whether NAIs killed, inhibited or physically displaced cells of *C. albicans*. Electrically earthed plates were lawn-inoculated, allowed to dry and exposed to NAIs at an emitter distance of 3 mm for 2 min, prior to incubation as above. After overnight incubation, an inoculum of *C. albicans* was placed over the clear zone of inhibition; this was again followed by overnight incubation. Any subsequent growth in the previous clear zone area was recorded.

Influence of pre-exposure of culture medium to NAIs

To determine whether NAIs rendered Sabouraud's Dextrose Agar toxic, or lacking an essential nutrient, additional experiments were conducted. Plates were exposed to NAIs using an emitter distance of 3 mm for 15 min prior to inoculation with *C. albicans*. After overnight incubation aerobically at 37 °C, they were examined for zones of inhibition. In addition, the number of colony-forming units was recorded for both control and NAI-exposed Sabouraud's Dextrose Agar plates.

Determination of ozone dispersion in the room atmosphere

To measure ozone dispersion in an open room atmosphere, experiments were carried out in a draught-free laboratory.

Readings were recorded only when a steady state had been reached, after 2–3 min, and this was confirmed by multiple area measurements. The multiple sampling positions (46) used were defined on 3 axes around the emitter. The emitter distance was set at 2 mm to ensure maximum ozone production. At this setting, a 5-cm distance was set between each reading position. The tip of the tube of the ozone analyser, which collected the air sample, was fixed at each position using a holder.

Statistical analysis

Statistical analysis of results used the Friedmen test for several related samples and Wilcoxon test for two related samples. The SPSS/PC statistical package (SPSS Inc.) was used to analyse results.

RESULTS

Influence of relative humidity

Relative humidity was found to have a significant influence ($P < 0{\cdot}05$) on inhibition of *C. albicans* growth by NAIs. The zone of inhibition increased from 47·26 mm^2 at a relative humidity 0% to 72·99 mm^2 at 50% and to 113·71 mm^2 at 100%.

Influence of emitter distance and exposure time

Results shown in Fig. 2 demonstrate the influence of both emitter distance and exposure time on the inhibition of *C. albicans* growth by NAIs. These influences are statistically significant ($P < 0{\cdot}05$) when comparisons are made between results obtained at minimum and maximum emitter distance for all three exposure times and between minimum and maximum exposure times for all three emitter distances. The number of ions per second reaching the target area was greatest at an emitter distance of 2 mm ($10{\cdot}1 \times 10^{14}$ ions per second) compared with $6{\cdot}4 \times 10^{14}$ ions per second at 6 mm and $4{\cdot}0 \times 10^{14}$ ions per second at 12 mm.

Determination of ozone levels at different exposure times, humidities and emitter distances

Results shown in Fig. 3 demonstrate the influence of emitter distance and exposure time on ozone levels. Differences in emitter distance had highly significant effects on ozone production ($P = 0{\cdot}004$). An inverse relationship was demonstrated between emitter distance and ozone levels; the latter were greatest at an emitter distance of 2 mm ($P = 0{\cdot}004$). The results of the measurements of ozone formation inside the chamber at different r.h. and exposure times are shown in Fig. 4. Ozone levels were significantly higher at 50% r.h.

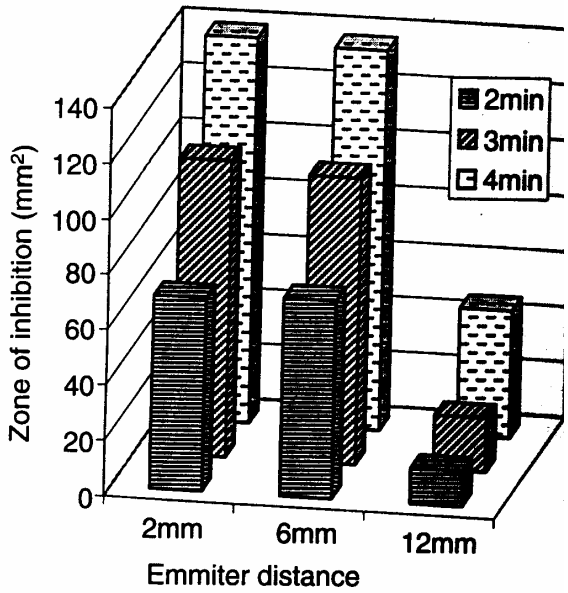


Fig. 2 The influence of emitter distance and exposure time on inhibition of *C. albicans* growth by NAIs

compared with those achieved at 0% and 100% r.h. ($P = 0{\cdot}003$). Under all experimental conditions, ozone levels increased with exposure time, so that maximum levels were achieved by 7 min.

The influence of an oxygen-free atmosphere

Results for each of 8 strains of *C. albicans*, are shown in Fig. 5. Comparisons made between the areas of zones of inhibition in air and in nitrogen demonstrated that NAIs were significantly more effective in inhibiting the growth of *C. albicans* under aerobic conditions ($P = 0{\cdot}012$).

The influence of exposure of culture medium or *C. albicans* cultures to NAIs

Zones of inhibition were not observed in those areas of the plates which had been exposed to NAIs prior to inoculation with *C. albicans*. In addition, there was no significant difference, in terms of the number of colony-forming units of *C. albicans*, when comparisons were made between Sabouraud's Dextrose Agar plates which had been exposed to NAIs for 15 min prior to inoculation (60 ± 48 cfu) and nonexposed control plates (84 ± 78 cfu). Following exposure to NAIs of plates which had been lawn-inoculated with *C. albicans*, and subsequent re-inoculation, no growth of *C. albicans* was

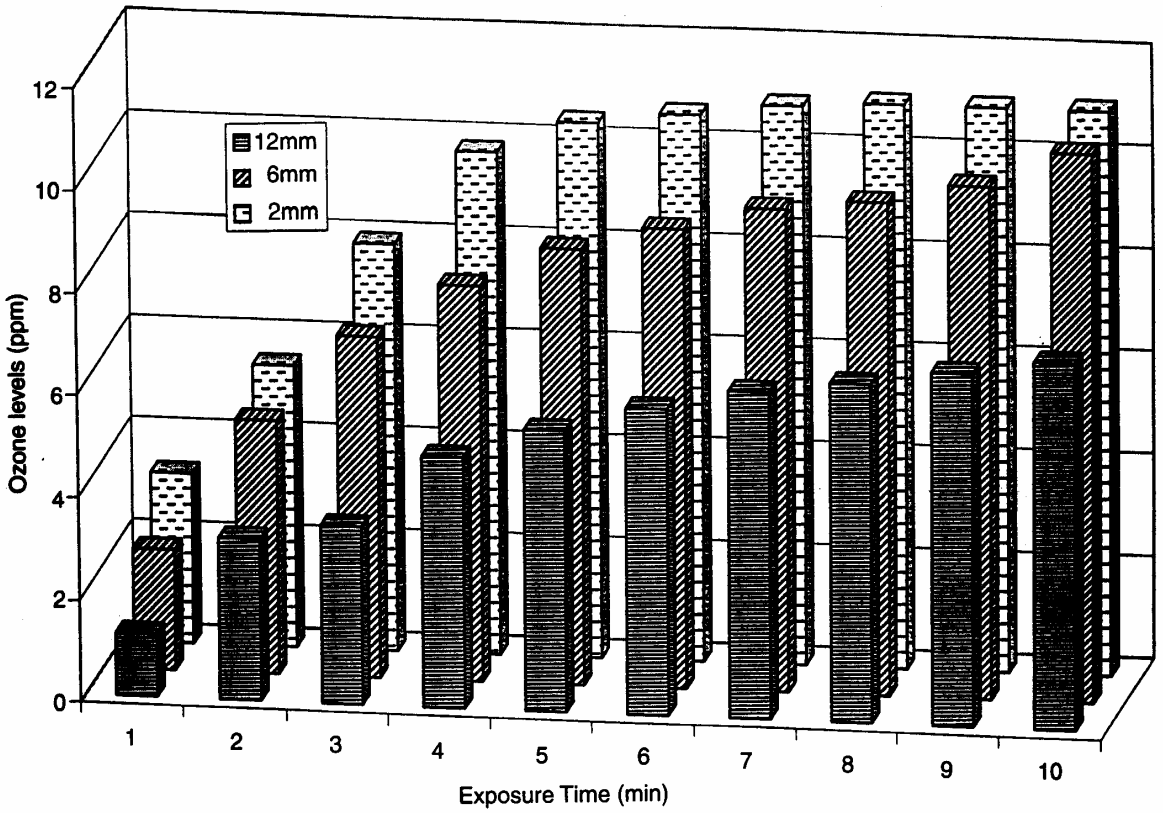


Fig. 3 The influence of emitter distance and exposure time on ozone levels

observed within the zones of inhibition up to 48 h after re-inoculation of these areas.

Determination of ozone dispersion in an open room atmosphere

The measurement of ozone in an open room atmosphere showed that the highest recorded levels were at the emitter tip (2·05 p.p.m.) and the lowest were 25 cm from emitter tip (0·002–0·08 p.p.m.) (Fig. 6).

DISCUSSION

This study measured the effectiveness of NAIs in inhibition of *C. albicans in vitro* under specified conditions designed to avoid the pitfalls (*vide supra*) described by Krueger and Reed (1976). Relative humidity has been shown in our experiments to have a significant influence on the efficacy of NAIs in inhibiting the growth of *C. albicans* and could therefore affect the outcome of experiments, even when these are carried out in the same laboratory. Dolezalek (1985) observed that an increase in r.h. resulted in both a reduction in air electrical conductivity and a decrease in the field of exposure when the same current density was used. This might explain the significant reduction in size of the zone of inhibition observed in our experiments at higher r.h. values. Clearly this parameter must be controlled to achieve reproducible results. Both emitter distance and exposure time significantly affected the lethality of NAIs with respect to *C. albicans*. In agreement with Pethig (1984), we demonstrated that the number of ions generated by corona discharge reduced as distance from the emitter increased. Thus it is not valid to make comparisons between the outcomes of studies in which dissimilar exposure times and/or emitter distances have been employed.

The mechanisms by which NAIs affect *C. albicans* are a matter of debate. In this study the growth of *C. albicans* was not affected when the agar surface had been pre-exposed to NAIs. Viable counts did not differ significantly between control plates and plates exposed to NAIs prior to inoculation with *C. albicans*. This indicates that NAIs do not exert their

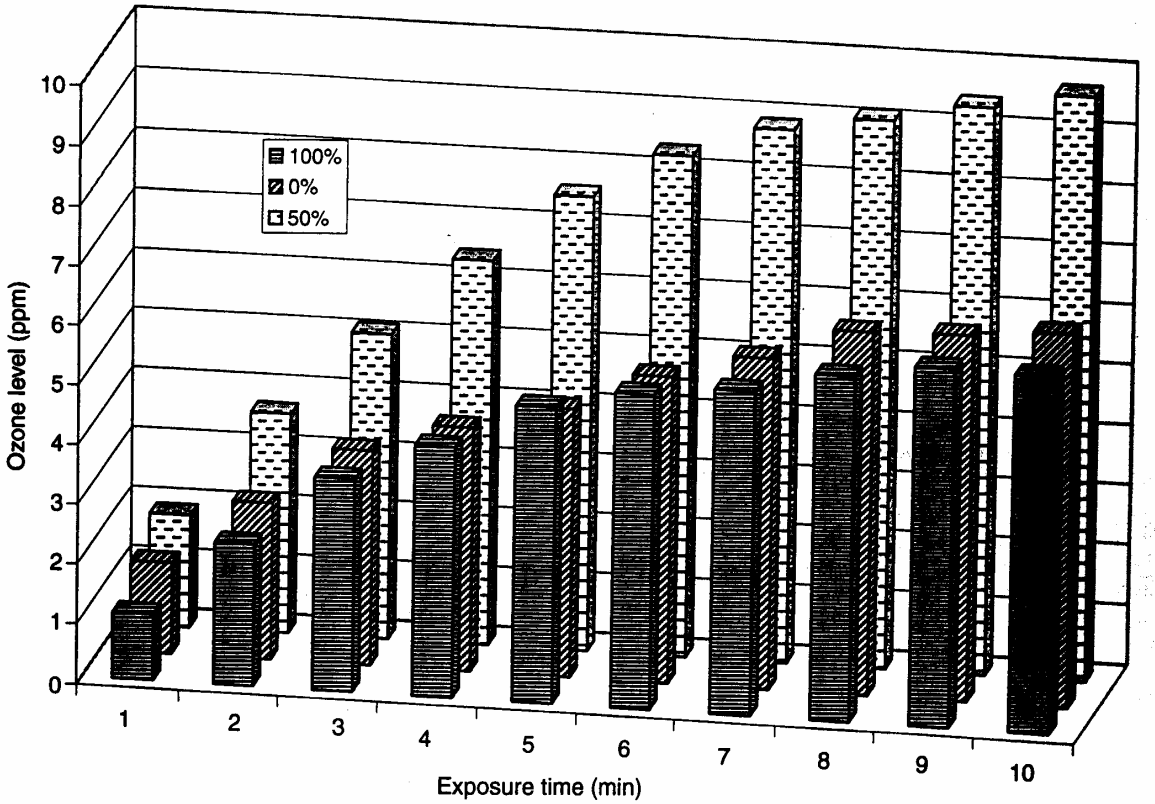


Fig. 4 The influence of relative humidity and exposure time on ozone levels

effect on *C. albicans* growth by bringing about a change in the culture medium itself and contradicts the hypothesis of Schley (1972) 'that the reduction in germs on agar gel is due to air currents caused by ions which lead to an accelerated dessication of the agar'. In our experiments, growth was not increased at the edge of the zones of inhibition, therefore migration was probably not involved in the production of these clear zones, as some have suggested (Rosenthal *et al.* 1979). In addition, no growth was achieved when the clear zones were re-inoculated with *C. albicans*. This inhibition of growth may be the result of accumulation of toxic compounds released from *C. albicans* killed previously by NAIs; an effect upon the medium can be excluded (*vide supra*).

The current study showed that the anticandidal effects of negative air ions were significantly greater under aerobic conditions than in a nitrogen (oxygen-free) atmosphere. Rosenthal *et al.* (1979) have stated that molecular oxygen is the only constituent of air that can scavenge the free electrons and that the electron configuration of molecular nitrogen prevents the formation of air ions. Therefore, in an oxygen-free atmosphere, NAIs cannnot be generated. However, the lethal effect of NAIs on *C. albicans*, although significantly reduced, was not completely abolished in the oxygen-free atmosphere. Possibly a very low percentage of molecular oxygen remained. Alternatively, it is possible, as suggested by Rosenthal *et al.* (1979), that the residual zones of inhibition observed under such conditions could be explained by the direct action of the electric field (produced by the ion generator) on *C. albicans*. The significant difference between inhibition zone sizes in air and oxygen-free atmospheres ($P = 0{\cdot}012$) supports the proposed major role of oxidants and/or superoxide radical anions produced during air ionization in the microbicidal action of negative air ionizers.

The amount of gaseous ions generated by artificial air ionization depends on the construction of the ionizer (Reiter 1978). Corona discharge ionizers deliver, as a by-product, relatively large amounts of ozone (Kroling 1985), which is always present in the vicinity of the instrument at levels that exceed the olfactory threshold (about 0·01 p.p.m.). In addition, small amounts of nitric oxide are formed (Reiter

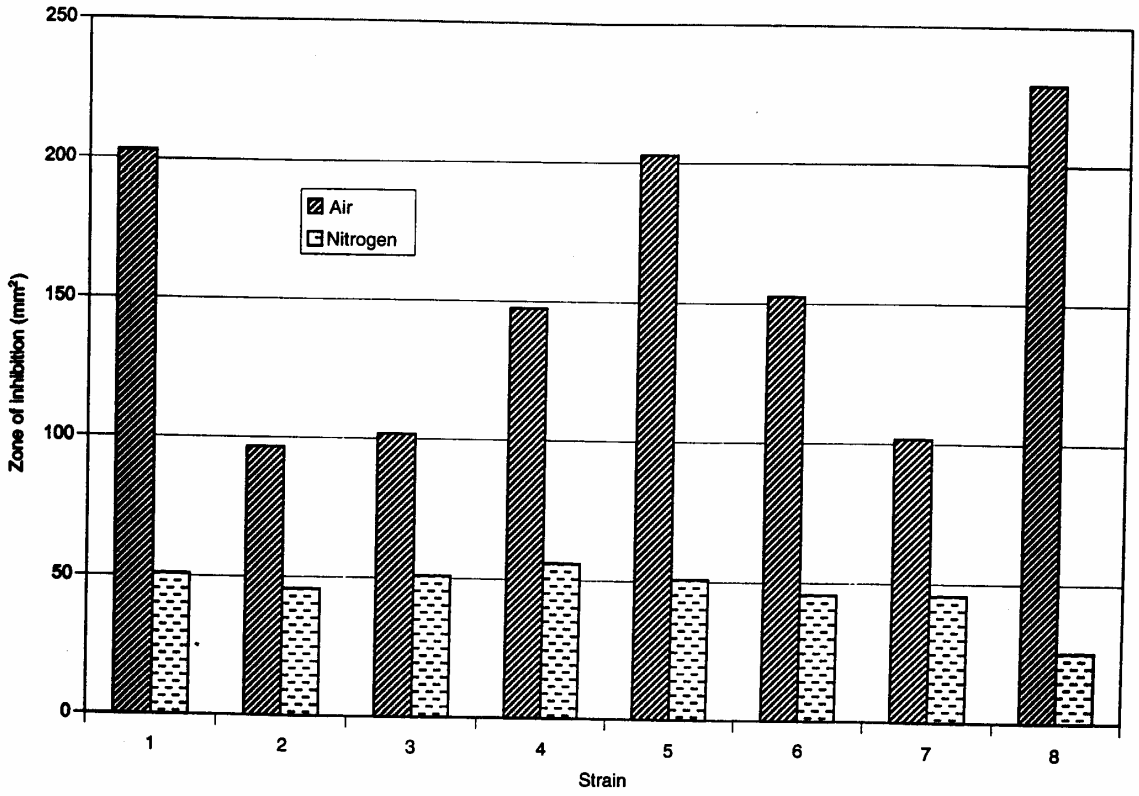


Fig. 5 The influence of an oxygen-free atmosphere on inhibition of *C. albicans* growth by NAIs

1978). However, levels of ozone generated by ionizers are rarely measured or estimated in experiments studying the microbicidal actions of NAIs, despite the fact that the presence of ozone could account for some of their toxic actions (Charry 1984; Dominique *et al.* 1988). The levels of ozone production in this study, in a closed chamber, were found to be directly related to exposure time and inversely related to emitter distance, so that NAI concentration does affect ozone production. These results parallel the relationship of exposure time and emitter distance to size of zone of inhibition. The maximum level of ozone (11 p.p.m.) was achieved after 5 min exposure at 2 mm distance and 50% r.h., although this result was limited by the maximum value that the ozone analyser could measure. In the current study, the US National Ambient Air Quality Standard for ozone (0·12 p.p.m.) was exceeded after one minute, even by the lowest levels produced (at 12 mm emitter distance) when measurements were performed in a closed chamber. Inhalation of ozone at such concentrations has been demonstrated to cause extensive pulmonary changes in rats, including epithelial injury (Barry *et al.* 1985) and fibrosis (Boorman *et al.* 1980; Hesterberg and Last 1981), and to have an adverse effect on respiratory function in humans (Tosteson *et al.* 1992). However, in a draught-free room atmosphere, ozone levels were demonstrated to be considerably lower, approaching zero at 25 cm from the emitter tip (in all directions) and 2 p.p.m. at the emitter tip. This indicates that there is a rapid diffusion of ozone into the atmosphere when corona-discharge ionizers are used in well ventilated surroundings. However, for the patient, a hazard may remain if such devices are used in the oral cavity without regard for safety, which might require good ventilation, proper protection for eyes and ensuring ozone (and other gases) do not enter the lungs by mouth or nose breathing by using powerful suction around the emitter and a mask over the nose.

The results of the above study demonstrate the *in vitro* sensitivity of *C. albicans* to NAIs produced by a corona-discharge ion generator designed for use in the oral cavity. Such a generator may thus be of value not only in the removal of bacteria from carious lesions and periodontal pockets (Cousins *et al.* 1991; Burke *et al.* 1995) but also in the treatment of oral candidosis.

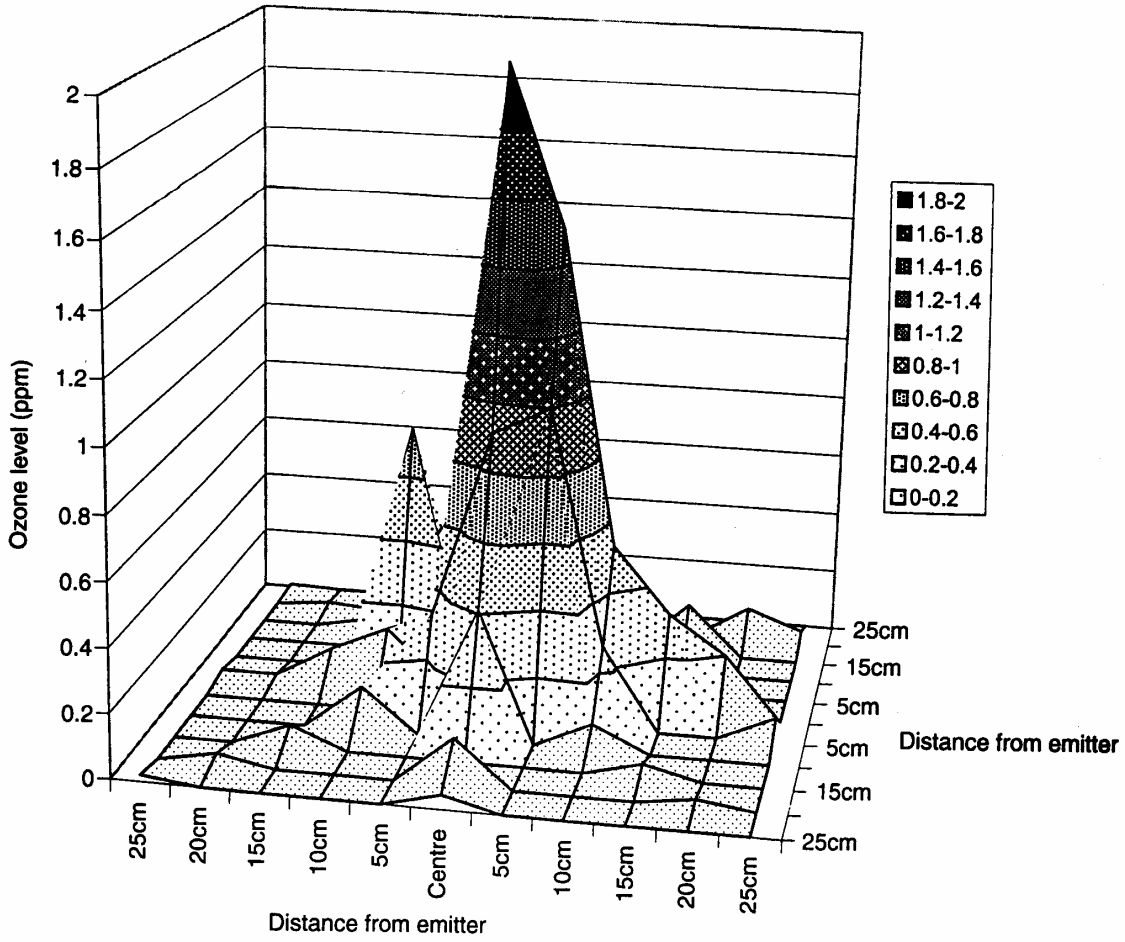


Fig. 6 Ozone dispersion in an open room atmosphere

ACKNOWLEDGEMENTS

The authors would like to thank Dentron Ltd (Ashley, UK), for loaning us the Biogun corona-discharge negative air ion generator employed in this study.

REFERENCES

Barry, B.E., Miller, F.J. and Crapo, J.D. (1985) Effects of inhalation of 0·12 and 0·25 p.p.m. ozone on the proximal alveolar region of juvenile and adult rats. *Laboratory Investigation* 53, 692–704.

Boorman, G.A., Schwartz, L.W. and Dungworth, D.L. (1980) Pulmonary effects of prolonged ozone insult in rats: morphometric evaluation of the central acinus. *Laboratory Investigation* 43, 108.

Burke. F.M., Samarawickrama, D.Y., Johnson, N.D., Beighton, D. and Lynch, E. (1995) Use of negative air ion treatment on carious microflora. *Journal of Dental Research* 74, 952.

Charry, J.M. (1984) Biological effects of small air ions: a review of finding and methods. *Environmental Research* 34, 351–389.

Cousins, D., Copus, J. and Wilson, M. (1991) Microbicidal effect of negative air ions. *Journal of Dental Research* 70, 709.

Dolezalek, H. (1985) Remarks on the physics of atmospheric ions (natural or artificial). *International Journal of Biometeorology* 29, 211–221.

Dominique, E.L., Berardi, A., Jacone, A. and Perlman, P. (1988) Effect of three oxidizing biocides on *Legionella pneumophilia* serogroup 1. *Applied and Environmental Microbiology* 3, 741–746.

Hesterberg, T.W. and Last, J.A. (1981) Ozone-induced acute pulmonary fibrosis in rats: prevention of increased rates of collagen synthesis by methylprednisolone. *American Review of Respiratory Disease* 123, 47–52.

Kellog, E.W. III, Yost, M.G., Barthakur, N. and Kreuger, A.P. (1979) Superoxide involvement in the bactericidal effects of negative air ions on *Staphylococcus albus*. *Nature* 281, 400–401.

Kingdon, K.H. (1960) Interaction of atmospheric ions with biological material. *Physics in Medicine and Biology* **5**, 1–10.

Kroling, P. (1985) Natural and artificially produced air ions: a biologically relevant climate factor? *International Journal of Biometeorology* **29**, 233–242.

Krueger, A.P. and Reed, E.J. (1976) Biological impact of small air ions. *Science* **193**, 1209–1213.

Krueger, A.P. and Smith, R.F. (1957) Effects of air ions on isolated rabbit trachea. *Proceedings of the Society for Experimental Biology and Medicine* **96**, 807–809.

Krueger, A.P. and Smith, R.F. (1958) The effects of air ions on the living mammalian trachea. *Journal of General Physiology* **42**, 69–82.

Krueger, A.P., Smith, R.F., Hildebrand, G.J. and Meyers, C.E. (1959) Further studies of gaseous ions. *Nature* **183**, 1322–1333.

Marin, V., Moretti, G. and Rassu, M. (1989) Effects of ionization of the air on some bacterial strains. *Annali Di Igiene* **1**, 1491–1500.

Pethig, R. (1984) Survey of the methodology for evaluating negative air ions relevance to biological studies. *Methods in Enzymology* **105**, 238–248.

Phillips, G., Harris, G.J. and Jones, M.W. (1964) Effect of air ions on bacterial aerosols. *International Journal of Biometeorology* **8**, 27–37.

Reiter, R. (1978) *Luftelektrisches Raumklima Forschungbericht* F 1: 468, Bonn: Bundesministerium Raumordnung, Bauwesen und Stadtebau.

Rosenthal, I. (1981) A working hypothesis for negative ion generators. *Experimentia* **39**, 718–719.

Rosenthal, B., Juven, J. and Gordin, S. (1979) Effects of negative-charged atmosphere on micro-organisms. *Journal of Applied Bacteriology* **46**, 451–454.

Schley, P. (1972) Zum Einfluß künstlich erzeugter Ionendichten auf Nutztiere (ein Beitrag zur Frage der keimhemmenden Wirkung negativ geladener Luftionen). *Deustche Tierärztlich Wochenschrift* **79**, 622–625.

Shahin, M.M. (1969) Nature of charge carriers in positive and negative corona discharges. *Applied Optics* **Suppl. 3**, 106.

Tchijevski, A.L. (1933) Transaction of Central Laboratory Scientific Research on Ionification. Voronej: The Commune.

Tosteson, T., Baum, T. and Speizer, F.E. (1992). Effects of ambient ozone on respiratory function and symptoms in Mexico City school children. *American Review of Respiratory Disease* **145**, 276–282.