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Effect of Hyperbaric Oxygen on Aerobic Bacteria *in vitro* and *in vivo*.*† (31932)

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Hyperbaric oxygen has been used for therapy of osteomyelitis(1) and has been suggested as possible therapy for other infections due to aerobic bacteria(2,3). There are no controlled studies demonstrating efficacy of hyperbaric oxygen in therapy of aerobic infections, and in fact it is possible that oxygen therapy may have a deleterious effect in some instances. In addition, the possibility exists that hyperbaric oxygen used for treatment of conditions other than infection may increase susceptibility to infection by altering the delicate host-parasite relationship and allowing bacteria which are a part of the normal flora to produce infection.

The purpose of this research was to study by quantitative techniques the effect of oxygen under pressure on aerobic microorganisms *in vitro* and to investigate the influence of oxygen therapy on aerobic infections in animals.

Materials and methods. Clinical isolates of one strain each of *Escherichia coli, Aerobacter aerogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus mirabilis, Diplococcus pneumoniae* Type 6, *Staphylococcus aureus* (coagulase positive) and *Streptococcus pyogenes* were studied. Stock cultures were maintained by storing aliquots of a 24-hour broth culture at −20°C. The broth for *D. pneumoniae* was beef heart infusion broth with 5% sheep blood; trypticase soy broth without blood was used for the other bacteria. For each experiment an aliquot of stock culture was subcultured to broth and incubated at 37°C for 24 hours (24-hour culture). Four-hour cultures were prepared by diluting a 24-hour culture 1:10,000 in broth and incubating for 4 hours at 37°C.

The number of *D. pneumoniae* in broth was determined by serial dilution in beef heart infusion broth and streaking 0.1 ml aliquots on the surfaces of sheep blood agar plates (trypticase soy agar with 5% sheep blood). Numbers of bacteria other than *D. pneumoniae* were determined by serially diluting in trypticase soy broth and making pour plates with trypticase soy agar. All plates were read after incubation for 48 hours at 37°C.

In vitro *experiments. In vitro* experiments were performed in Torbal-B.T.L. Anaerobic Jars (Torsion Balance Co.) which were fitted with pressure gauges and could be pressurized to 2 atmospheres absolute or 30 lb per square inch absolute (30 p.s.i.a.). Air was eliminated from the jars by alternately evacuating with negative pressure and filling with oxygen under pressure. Suspensions of bacteria in broth in covered petri dishes were incubated in air at 15 p.s.i.a. or oxygen at 30 p.s.i.a. The broth was only 1.5-2.5 mm deep to allow diffusion of gas. Trypticase soy broth was used for all bacteria except *D. pneumoniae* for which beef heart infusion broth with 5% sheep blood was used. Ex-

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periments were performed at 37°C. Periodically aliquots of culture medium were removed and the numbers of viable microorganisms determined.

In vivo *studies.* Male Swiss mice of the CF1 strain (Carworth Farms) weighing 20 to 24 g were used in all experiments and were allowed food and water *ad lib* when not in the hyperbaric chamber. To produce infection, bacteria from a 24-hour culture were washed and suspended in saline solution and injected intravenously in volumes of 0.2 ml. Following infection, half of the mice in each group were repetitively exposed to 100% oxygen at 45 p.s.i.a. in a Vickers Tank Type Hyperbaric Oxygen Chamber; the other half of each group was not exposed to oxygen and served as controls. Mice were removed from the chamber between exposures. The first exposure to oxygen was always started within one hour of initiation of infection. With each exposure 10 minutes were allowed for compression and 10 minutes for decompression. At operating pressure the exchange rate of oxygen in the chamber was about 114 liters per minute (12 standard cubic feet per minute).

Results. In vitro *experiments.* Four-hour and 24-hour broth cultures of strains of *E. coli, A. aerogenes, P. aeruginosa, S. typhimurium, P. mirabilis, D. pneumoniae, S. aureus,* and *Strep. pyogenes* were incubated for 24 hours at 37°C in oxygen at 30 p.s.i.a. or air at atmospheric pressure (15 p.s.i.a.). All of these bacteria multiplied in oxygen and air. In repeated experiments multiplication was never more rapid in oxygen than in air. However, the rate of growth and the peak titer achieved was frequently inhibited in oxygen as compared with air. The inhibitory effect of oxygen was inconsistent and minimal with *P. mirabilis, A. aerogenes,* and *E. coli;* more consistent with *P. aeruginosa* and *Strep. pyogenes;* and consistent and marked with *S. typhimurium, D. pneumoniae* and *S. aureus.* In general, the 24-hour cultures were inhibited by oxygen more often than the 4-hour cultures. Fig. 1 demonstrates typical experiments with *E. coli, S. typhimurium, D. pneumoniae,* and *S. aureus.*

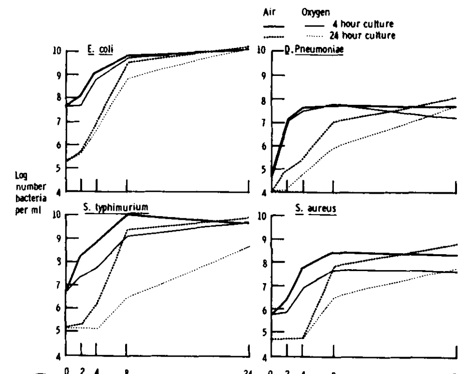
The amount of oxygen utilization or carbon dioxide production was minimal during the course of an experiment. Torbal Jars containing broth cultures of each of the strains of aerobic bacteria were filled with oxygen at 30 p.s.i.a. and incubated at 37°C. After 24 hours the jars contained 99.8% oxygen and 0.2% carbon dioxide.

Growth characteristics of these organisms in oxygen were also studied on the surfaces of agar plates. Ten-fold serial dilutions of each bacterial strain studied were made in trypticase soy broth and 0.1 ml of each dilution was plated in duplicate on the surfaces of agar plates (trypticase soy agar plates for *E. coli, A. aerogenes, P. aeruginosa, S. typhimurium, P. mirabilis,* and *S. aureus,* and sheep blood agar plates for *D. pneumoniae* and *Strep pyogenes*). Duplicate plates were incubated for 24 hours at 37°C in air at 15 p.s.i.a. or oxygen at 30 p.s.i.a. For each strain of bacteria there were equivalent numbers of colonies on plates incubated in air and oxygen. However, on the plates incubated in oxygen colonies of proteus were small and did not swarm. Similarly colonies of *S. aureus* and *D. pneumoniae* developing in oxygen were very small and the colonies of staphylococci were chalk white. (Colonies of *S. aureus* developing in air had a yellow pigmentation.) Colonies of *E. coli, A. aerogenes, P. aeruginosa, S. typhimurium* and *Strep. pyogenes* appeared the same after incubation in air or oxygen and oxygen did not alter the hemolysis produced by *Strep. pyogenes.*

In vivo *experiments.* Mice were injected intravenously with varying inocula of *D. pneumoniae, S. aureus* or *S. typhimurium* and exposed to oxygen for 30, 60 or 90 minutes. Exposures were repeated at intervals ranging from 2 to 16 hours for a total of 4 to 8 exposures. Fig. 2-4 demonstrate typical experiments in which groups of mice were injected intravenously with 10^2 *D. pneumoniae* (Fig. 2), 4×10^7 *S. aureus* (Fig. 3) or 1×10^6 *S. typhimumium* (Fig. 4) and exposed to oxygen 8 times for periods of 30 minutes each at 8- to 16-hour intervals. In these and all other experiments there was no consistent difference in rate of mortality between groups exposed to oxygen and control groups. Although there were no deaths in normal mice treated with 8 oxygen exposures of 30 minutes each, there was a 5% mortality in normal mice treated with four 90-minute exposures.

Discussion. Some studies(4-6) have indicated that oxygen at 30 p.s.i.a. inhibits the growth of many aerobic organisms on the surfaces of agar plates and often changes the appearance of the colonies. However, quantitative data is not given in these studies. Ollodart and Blair(2) found decreased growth of *E. coli, Pseudomonas* and *S. aureus* in broth cultures during 3 hours of incubation in oxygen at 45 p.s.i.a. In all instances there was never more than a 3-fold difference in growth in air at 15 p.s.i.a. as compared with growth in oxygen. In the present study differences between cultures in oxygen and air were most marked after 4 to 8 hours of incubation and were often 10-fold or more. Some of the differences in results may be related to differences in the strain of bacteria and to variations in experimental methods and in the bacteriological growth media.

The aerobic bacteria inhibited most *in vitro* by oxygen (*S. typhimurium, D. pneumoniae* and *S. aureus*) were injected in mice and the protective effect of hyperbaric oxygen was evaluated. Mortality rates in mice infected intravenously with these organisms were not



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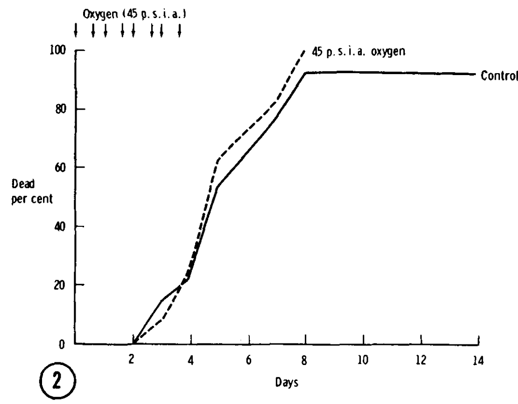

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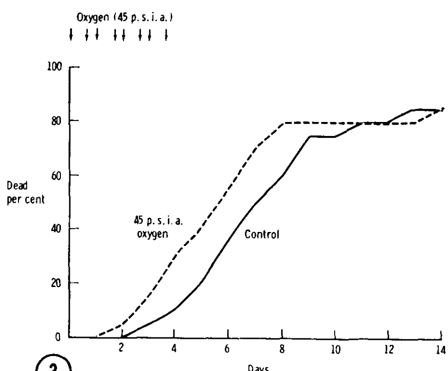

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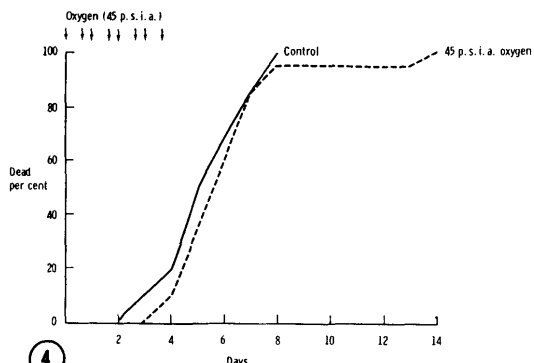

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FIG. 1. Effect of oxygen on *Escherichia coli, Salmonella typhimurium, Diplococcus pneumoniae* and *Staphylococcus aureus.* Number of *E. coli, S. typhimurium, D. pneumoniae* and *S. aureus* per ml of trypticase soy broth following incubation at 37°C of 4-or 24-hour cultures in air at 15 p.s.i.a. or oxygen at 30 p.s.i.a.

FIG. 2. Effect of hyperbaric oxygen on infection with *Diplococcus pneumoniae.* Cummulative mortality after intravenous inoculation of 10^2 viable units of *D. pneumoniae* in 13 control mice and 12 mice treated with exposure to oxygen at 45 p.s.i.a. for 8 treatment periods of 30 min each.

FIG. 3. Effect of hyperbaric oxygen on infection with *Staphylococcus aureus.* Cumulative mortality after intravenous inoculation of 4×10^7 viable units of *S. aureus* in 20 control mice and 20 mice treated with exposure to oxygen at 45 p.s.i.a. for 8 treatment periods of 30 min each.

FIG. 4. Effect of hyperbaric oxygen on infection with *Salmonella typhimurium.* Cumulative mortality after intravenous inoculation of 1×10^6 viable units of *S. typhimurium* in 20 control mice and 20 mice treated with exposure to oxygen at 45 p.s.i.a. for 8 treatment periods of 30 min. each.

altered by exposure of the mice to oxygen at 45 p.s.i.a.

Ross and McAllister(3) concluded that oxygen therapy significantly prolonged life in 2 out of 8 experiments with mice injected with *D. pneumoniae* intraperitoneally. It is difficult to evaluate protection in their experiments; with the inoculum used, 90% of mice were usually dead within 24 hours after infection. In the present study following intravenous infection with much smaller inocula and delayed death, no protection was observed in mice treated with hyperbaric oxygen.

Grogan(7) found that oxygen at 45 p.s.i.a. did not protect mice from death following infection with *S. aureus* intraperitoneally. In fact, he concluded that oxygen therapy increased mortality. In his studies exposures to oxygen lasted 2 to 3 hours which in the present study caused deaths in normal mice. Although in the present study no protection was afforded by oxygen therapy of aerobic infections, it is also of note that no increase in mortality from infection occurred in mice exposed to oxygen.

Summary. Strains of *E. coli, A. aerogenes, P. aeruginosa, S. typhimurium, P. mirabilis, D. pneumoniae, S. aureus*, and *Strep. pyogenes* in broth multiply in oxygen at 30 pounds per square inch absolute (p.s.i.a.). The rate of growth and the peak titer achieved were frequently inhibited in oxygen as compared with air at 15 p.s.i.a. The inhibitory effect of oxygen was most marked with *S. typhimurium, D. pneumoniae* and *S. aureus* and was minimal or absent with *P. mirabilis, A. aerogenes* and *E. coli*. In general, 24-hour cultures were more susceptible to the inhibitory activity of oxygen than were 4-hour cultures. Each of the strains of aerobic bacteria grew on the surfaces of agar plates when incubated in oxygen or air. Although equal numbers of colonies developed in oxygen and air, differences in colonial morphology were noted in some of the strains incubated in oxygen. Exposure to oxygen at 45 p.s.i.a. did not protect mice against death from infection produced by intravenous administration of *D. pneumoniae, S. aureus*, or *S. typhimurium*.

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Food Intake, Weight Gain and Amino Acid Pools of Rats Receiving 1-Aminocyclopentane-1-Carboxylic Acid.* (31933)

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Christensen and Jones(1) observed that the cyclic amino acid, 1-aminocyclopentane-1-carboxylic acid (ACPC) when injected into the rat is not metabolized and maintains a steady-state concentration in the blood and tissues for several days. These biological characteristics led Akedo and Christensen(2) to propose the use of ACPC as a model for the study of amino acid transport. In experiments with rats fed diets containing ACPC, Ross *et al*(3) reported a decreased growth rate and suggested that the compound might function as an amino acid antagonist. Berlinguet *et al*

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