Bactericidal Effect of Hyperbaric Oxygen Determined by Direct Exposure* (33744)

GEORGE H. BORNSIDE

Department of Surgery, Louisiana State University, School of Medicine, New Orleans, Louisiana 70112

During exposure of aerobic bacteria on the surface of agar media to pure oxygen at pressures up to 4 atmospheres absolute (4 ATA), colonial growth may be absent or retarded; upon subsequent incubation of the agar medium in normobaric air, colonial growth resumes and after overnight incubation appears comparable to that of controls not exposed to oxygen under pressure (1, 2). This phenomenon has been observed with pure oxygen at pressures up to 10 ATA (3). It is well known that air at these pressures has no inhibitory effect on the growth of aerobes, and that oxygen under pressure, but not pressure alone, has antibacterial effects (4). ZoBell and Hittle (5) demonstrated that hydrostatic pressures of 400–600 atm were necessary to inhibit bacterial reproduction and that antibacterial effects of pure oxygen were intensified by hydrostatic pressure.

Although there has been considerable interest in the antibacterial effect of high pressure oxygen (HPO) on both aerobic and facultative anaerobic bacteria, the bactericidal effect of oxygen at 2 and 3 ATA has been well documented with strict anaerobes (6), but not with aerobes. Hopkinson and Towers (2) reported that 2 of 6 strains of *Escherichia coli* and 1 of 4 strains of *Pseudomonas aeruginosa* did not recover from exposure to pure oxygen at 4 ATA. Gottlieb and associates (7) were unable to distinguish between bacteriostatic and bactericidal effects of HPO at 3 ATA on mycobacteria, but observed a bactericidal effect of HPO on *Vibrio comma* and only a bacteriostatic effect on strains of *Salmonella* and *Shigella* (8). Brown *et al.* (9) exposed *Ps. aeruginosa* on the surface of membrane filters to 70% pure oxygen and observed a bactericidal effect on 1 of 3 strains. Subsequently, they reported that inocula of a few hundred cells were more sensitive to oxygen at 1 ATA than had generally been realized (10).

This paper reports a bactericidal effect of HPO on a variety of aerobic and facultative anaerobic microorganisms. A simple quantitative method employing direct exposure of small inocula to HPO on the surface of blood agar plates is utilized.

Materials and Methods. The effect of HPO on colonial growth was assayed on the surface of blood agar plates. Organisms were grown overnight in broth, and serially diluted in broth. One-tenth ml from each of 2 appropriate tubes in the dilution series was seeded upon agar surfaces and spread with a wire loop. Aluminum culture tube closures were placed at the circumference on the surface of each plate to raise the covers of the petri dishes 5 mm above the rim of the bottom to facilitate exchange of gases within the covered dish. Three of the 6 plates inoculated with each dilution were routinely incubated at 37° in a small hyperbaric chamber (11), and exposed to flowing 100% oxygen at 3 ATA for 24 hr. The chamber was humidified with water vapor. The remaining 3 plates were incubated in air at 37° and served as controls. All plates were examined after incubation for 24 hr, when those from the air incubator were usually counted. Those removed from the hyperbaric chamber were incubated in normobaric air for an additional 24 hrs, and then counted. The percentage change was calculated by comparing mean colony counts on control plates with those on plates exposed to HPO and then incubated in air. A constant area of each surface was removed from the experiment by the aluminum culture tube closures. Although the inoculum trapped within the closure cap was protected from oxygen, pressure changes during

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TABLE I. Effect of Exposure of Microorganisms on the Surface of Blood Agar Plates to Oxygen at 3 ATA.

Species	No. of strains	No. of colonies[a]		Kill[b] (%)
		Control in air	HPO	
Streptococcus pyogenes	11	365 ± 315	0	100
Diplococcus pneumoniae	2	125 ± 75	0	100
Candida albicans	1	93 ± 28	0	100
Paracolobactrum sp.	1	42 ± 18	0	100
Herellea vaginicola	13	231 ± 142	6 ± 7	97.5 ± 2.9
Bacillus sp.[c]	6	116 ± 85	3 ± 4	97.1 ± 3.7
Chlorella pyrenoidosa	1	73 ± 6	4 ± 5	94.5
Mima polymorpha	10	146 ± 152	20 ± 58	91.5 ± 23.0
Sarcina lutea	6	87 ± 43	18 ± 27	85.1 ± 18.6
Proteus rettgeri	3	107 ± 78	26 ± 34	81.4 ± 12.4
Proteus morganii	4	89 ± 22	57 ± 18	35.3 ± 17.7
Proteus vulgaris	4	56 ± 13	39 ± 10	28.2 ± 3.0
Mima polymorpha var. *oxidans*	2	146 ± 46	122 ± 60	20.1 ± 26.9
Aerobacter aerogenes	2	55 ± 24	50 ± 28	12.1 ± 7.7
Serratia marcescens	2	41 ± 20	34 ± 12	11.7 ± 26.2
Staphylococcus albus	7	94 ± 63	85 ± 56	8.8 ± 5.3
Proteus mirabilis	4	50 ± 15	48 ± 14	8.3 ± 14.0
Escherichia coli	8	97 ± 60	99 ± 67	4.5 ± 8.0
Pseudomonas aeruginosa	5	147 ± 31	144 ± 27	−1.2 ± 21.8
Staphylococcus aureus	10	183 ± 79	183 ± 72	−2.3 ± 15.5

[a] Values are the mean ± SD.

[b] Calculated from counts of 3 control plates and 3 exposed plates with each strain according to the relationship $(\bar{X}_{Air} - \bar{X}_{HPO}/\bar{X}_{Air}) \times 100$, and expressed here as the mean and standard deviation of the individual percentage kill.

[c] *B. subtilis, B. sphaericus, B. laterosporus, B. licheniformis.*

decompression frequently caused the agar at the periphery of a closure cap to become seeded. A zone of microbial growth appearing around a closure cap following reincubation in air was a minor disadvantage that was offset by the overall simplicity and convenience of the technique. A 50–100% reduction in the number of colonies was scored as a bactericidal effect. Lesser changes were scored as a bacteriostatic effect. If each of the dilutions employed did not yield consistent colony counts, the experiment was repeated.

Results and Discussion. Pure oxygen administered at 3 ATA for 24 hr was bactericidal for some species of aerobes and facultative anaerobes and bacteriostatic for others. Table I lists the collection in descending order of the mean percentage kill for each species, and summarized the mean colony counts. The percentage kill for each strain was calculated from the colony counts for that strain. Although HPO was bactericidal for species in the upper half of the list, there was wide variation in the response of strains within a given species and of species within a genus (such as the genus *Proteus*). The bactericidal effect was independent of common taxonomical characteristics—cellular morphology, gram-staining reaction, and a strict or facultative growth requirement for oxygen in normobaric air.

Colonial growth was not usually visible upon plates removed from the hyperbaric chamber. However, staphylococci and some strains of *Proteus morganii* and *P. mirabilis* exhibited pinpoint colonies which were not counted until after normobaric incubation. Colonies of *Serratia marcescens* were countable and nonpigmented when plates were removed from the hyperbaric chamber, but developed the characteristic red pigment, prodi-

TABLE II. Influence of Pressure upon Bactericidal Effect of Pure Oxygen on *Herellea vaginicola.*

Strain no.	Kill following 24 hr exposure to HPO (%)	
	2 ATA	3 ATA
2	100	94
10	0	95
12	0	100
14	0	93
15	100	100
18	95	94
28	76	100
31	0	100
37	0	100

giosin, only during subsequent normobaric incubation.

Some microorganisms killed by exposure to pure oxygen at 3 ATA were also killed by exposure at 2 ATA; other were not. For example, HPO at 2 ATA was bactericidal for all strains of *Streptococcus pyogenes* and *Diplococcus pnuemoniae,* but for only 4 of 9 strains of *Herellea vaginicola* (Table II). These results suggested that the bactericidal effect of HPO might also vary according to the duration of exposure. The results of a preliminary experiment (Table III) indicated that exposure for longer than 24 hr was necessary for a bactericidal effect where only a bacteriostatic effect had regularly been demonstrated.

This relatively simple and direct method has provided quantitative measurements of the antibacterial effect of HPO. The bactericidal effect of HPO was dependent directly upon the duration and intensity of exposure. In the present studies, human blood agar medium was employed as a nutritionally complete medium suitable for the growth of the spectrum of microorganisms studied. However, with the use of defined media, this method should facilitate the study of cellular mechanisms of oxygen toxicity by allowing the screening of nutrients which potentiate the response of microorganisms to HPO as well as those which reverse oxygen toxicity (12, 13).

Summary. Exposure of small inocula of microorganisms on the surface of blood agar medium to high pressure oxygen (HPO; 100% O_2 at 3 atmospheres absolute) has provided a simple direct method for quantitating its antibacterial effect upon 102 strains from among 20 microbial species. The bactericidal effect was dependent upon the duration and intensity of exposure to HPO, and was independent of common taxonomical characteristics such as cellular morphology, gram-staining reaction, and a strict or facultative growth requirement for normobaric oxygen.

TABLE III. Influence of Exposure to HPO at 3 ATA upon Bactericidal Effect.

Species	Kill following exposure (%)				
	6 hr	12 hr	1 day	2 days	4 days
Sarcina lutea ATCC no. 9341	86	100	100	—	—
Strep. pyogenes ATCC no. 12,203	19	91	100	—	—
Ps. aeruginosa no. 283	—	—	21	40	97

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