Antimicrobial agents of mixed aerobic-anaerobic agents.

We have tested the ability of hyperoxia (98% O₂−2% CO₂ at 2.8 atmospheres absolute [ca. 284.6 kPa]) to enhance killing of Escherichia coli (serotype O18 or ATCC 25922) by nitrofurantoin, sulfamethoxazole, trimethoprim, gentamicin, and tobramycin. We have also looked for interactions between hyperoxia and the aminoglycosides against Pseudomonas aeruginosa ATCC 27853. Hyperoxia significantly enhanced bacteriostatic activity of nitrofurantoin and trimethoprim as measured by MIC testing. The possibility exists that these effects might be due to the method required to test MICs under hyperoxic conditions rather than to the effect of hyperoxia itself. In addition, hyperoxia enhanced killing of bacteria by trimethoprim as measured by MBC testing. Hyperoxia decreased numbers of E. coli by 1.3 log₁₀ and P. aeruginosa by 2.7 log₁₀ in cation-supplemented Mueller-Hinton broth medium. The bacteriostatic effects of hyperoxia did not affect MICs of gentamicin or tobramycin. The lack of interaction between hyperoxia and gentamicin or tobramycin was confirmed by determining the number of viable bacteria remaining after 24 h of exposure to hyperoxia by using a pour plate method. We conclude that hyperoxia potentiates the antimicrobial activity of the reduction-oxidation-cycling antibiotic tested (nitrofurantoin) and of one of the antimetabolites tested (trimethoprim). Hyperoxia does not enhance the bactericidal effects of gentamicin and tobramycin, which require oxidative metabolism for transport into bacterial cells.

Hypoxic tissues are particularly susceptible to the establishment of mixed aerobic-anaerobic bacterial infections. These types of infections cause localized abscesses or may spread along fascial planes to cause necrosis of muscle and skin (7). Standard treatment consists of incision and drainage of infected tissues and intravenous administration of antimicrobial agents (6). The rationale for using hyperoxia in these infections is to restore normal O₂ tensions in ischemic tissue, improve oxidative cellular metabolism (13), and enhance phagocytic killing of bacteria (12, 18).

It has also been suggested that hyperoxia may be beneficial by increasing the activity of specific antimicrobial agents. The best evidence in favor of this proposal was presented by Gottlieb and co-workers (8, 15). In a series of in vitro and in vivo experiments, they reported synergy between sulfonamides and hyperoxia against Vibrio anguillarum (15) and Moraxella (Branhamella) catarrhalis (8). Evidence of decreased bacteriostatic and bactericidal activity of aminoglycosides in anaerobic environments has been presented by several laboratories (24, 27–29); hyperoxia can restore the bactericidal activity of tobramycin (1). However, it is not known whether hyperoxia potentiates the bactericidal activity of aminoglycosides in comparison with normal oxygen conditions. Hyperoxia may also increase bactericidal activity of antimicrobial agents, such as nitrofurantoin and nifurtimox, by increasing the generation of superoxide anion (O₂⁻). These antimicrobial agents undergo one-electron reduction to free radicals (nitrofurantoin + e⁻ → nitrofurantoin⁻). In the presence of oxygen, the antimicrobial agent free radical undergoes oxidation to generate O₂− (5) (nitrofurantoin⁻ + O₂ → nitrofurantoin + O₂⁻). The sum of these reactions is termed reduction-oxidation cycling.

The generation of superoxide anion from the drug free radical follows first-order kinetics with respect to oxygen (14). Adriamycin, for example, generates fourfold more oxygen-derived free radicals at 100% O₂ compared with 21% O₂ (19).

Finally, hyperoxia may be lethal to bacteria (17) and parasites (5) that lack adequate antioxidant defenses by increasing the generation of O₂⁻ and other toxic oxygen species by cellular metabolic reactions.

In this work, we have tested representative compounds from three classes of antimicrobial agents for interactions with hyperoxia in bacterial killing. The following interactions may exist between hyperoxia and antimicrobial agents: hyperoxia alone has no effect but potentiates the mode of action of the antimicrobial agent, and hyperoxia does not potentiate the mode of action of the antimicrobial agent but has an effect which is either additive or synergistic with the antimicrobial agent. Synergy exists when oxygen and an antimicrobial agent combine to produce an effect which is greater than the sum of their activities taken separately. Additive effects result when the actions of oxygen and an antimicrobial agent are equal to the sum of their activities taken separately. The use of a bacterial strain which is sensitive to hyperoxia maximized the chances of seeing an effect, whether it was additive or synergistic. Endpoint colony counting of bacteria by a pour plate method was used in addition to MIC and MBC testing for the detection of interactions between oxygen and antimicrobial agents.

(Periods of this study were presented at the Annual Scientific Meeting of the Undersea and Hyperbaric Medical Society, New Orleans, La., 1988.)

MATERIALS AND METHODS

Chemicals. Nitrofurantoin, trimethoprim, sulfamethoxazole, gentamicin, tobramycin, nitrofurazone, menadione, rifamycin SV, and NADPH were purchased from Sigma Chemical Co., St. Louis, Mo. Nitrofurantoin was solubilized in dimethylsulfoxide. Sulfamethoxazole and trimethoprim

* Corresponding author.
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were diluted in 0.1 N NaOH and 0.05 N HCl, respectively. Stock solutions of gentamicin and tobramycin were made in 0.1 M phosphate buffer (pH 7.8) and supplemented with 25 μg of Mg²⁺ and 50 μg of Ca²⁺ per ml (22). The stock solutions of antibiotics were adjusted to pH 7.4, filter sterilized (pore size, 0.20 μm), and diluted to desired concentrations.

**Bacterial strains.** A clinical isolate of *Escherichia coli* (serotype O18) was used for MIC and MBC determinations, endpoint quantitation of bacterial numbers, and polarographic measurement of O₂ consumption. Two reference strains, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, were used for MIC and MBC determinations and endpoint colony counting of bacteria.

**MIC and MBC determinations.** MICs were determined by broth macrodilution testing (22). MIC testing was performed in 12-well (22-mm-diameter) culture plates (Costar, Cambridge, Mass.). Serial twofold antimicrobial agent dilutions were made in either minimal salts-acetate broth medium (MSA) as described by Brunker and Brown (3) or Mueller-Hinton broth supplemented with 50 μg of Cu²⁺ and 25 μg of Mg²⁺ per ml. Each well contained 0.5 ml of antimicrobial agent diluted in specific medium, 0.4 ml of bacterial inoculum in the same medium, and 0.1 ml of 1 M HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid) buffer (pH 7.4). A growth control well (bacterial inoculum and HEPES buffer) and a sterility control well (growth medium and HEPES buffer) were included with each MIC test. The total volume per well was 1.0 ml, with a depth of 3 mm. This depth was selected to maximize O₂ penetration into the medium as shown by Gudewicz et al. (10). The final bacterial inoculum was approximately 5 × 10⁵ CFU/ml and was confirmed for each assay by counting colonies in pour plates of 10-fold dilutions. The number of CFU in pour plates was determined with a semiautomatic colony counter (Acculte model 133-8002; Fisher Scientific, Pittsburgh, Pa.).

The cultures were incubated at 35 to 37°C either at 1 atmosphere absolute (ATA) (101.3 kPa) in a gaseous environment of 21% O₂, 5% CO₂, and the balance N₂, or in 98% O₂-2% CO₂ at 2.8 ATA (ca. 284.6 kPa) for 24 h. The MIC tests performed in normal oxygen pressure were read as soon as the growth control showed suitable turbidity (approximately 24 h after the beginning of the experiment). The MICs determined under hyperoxic conditions were read 24 to 30 h after removal from the hyperbaric chamber (approximately 48 h after the beginning of the experiment). The MIC was the lowest antimicrobial agent concentration that produced a clear well (no growth) at the time that turbidity was apparent in the growth control well. MICs of nitrofurantoin, trimethoprim, sulfamethoxazole, and tobramycin for *E. coli* serotype O18 were determined in MSA. MICs of gentamicin and tobramycin for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were determined in Mueller-Hinton broth. The MBC was the lowest antimicrobial agent concentration that decreased the original inoculum by >99.9%. To determine MBCs, 10-fold dilutions of all antimicrobial agent-containing wells lacking turbidity were plated onto Mueller-Hinton agar medium with 5% sheep blood added. After 24 h of incubation, MBC break points were determined by using rejection values as described by Pearson et al. (23). MBCs were determined for the same bacteria and antimicrobial agent combinations as described above.

**Endpoint enumeration of bacteria.** In order to compare MICs of aminoglycosides for bacteria exposed to hyperoxia with MICs determined in a normal oxygen environment, bacteria in the culture wells were enumerated by making pour plates of serial 10-fold dilutions at 24 h of incubation. The growth control well and the wells containing concentrations of antibiotics two and four times the MIC for each organism were chosen for quantification of bacterial numbers.

**Exposure of bacterial cultures to hyperoxia.** Bacterial cultures were treated in a small (27 by 50 cm) Plexiglas hyperbaric chamber (model 1224, Bethlehem Corporation, Bethlehem, Pa.). Cultures were exposed to 98% O₂-2% CO₂ at a pressure of 2.8 ATA for 24 h. The pressure at which the O₂ was administered (2.8 ATA) is used clinically and does not induce toxicity in human patients. Before compression, the chamber was thoroughly flushed with 98% O₂-2% CO₂. The temperature in the chamber was maintained at 35 to 37°C by circulating heated water through coils of copper tubing located in the bottom of the chamber.

**Polarographic measurement of antimicrobial agent reduction-oxidation cycling.** The ability of nitrofurantoin, nitrofurazone, menadione, and rifampycin SV to generate superoxide anion was measured by oxygen consumption induced in rat liver microsomes and in *E. coli* serotype O18. Oxygen consumption was measured with a YSI model 53 biological oxygen monitor equipped with a Clark-type electrode, standard bath stirrer assembly (Yellow Springs Instrument Co., Yellow Springs, Ohio), and strip chart recorder (Beckman Instruments Co., Irvine, Calif.) by using a modification of the polarographic assay described by Hassan (11).

A microsomal fraction was prepared from rat liver (2) to test for reduction of nitrofurantoin, nitrofurazone, menadione, and rifampycin SV by cytochrome P-450 reductase. For microsomal assays, each antimicrobial agent (0.7 mM) and NADP (0.4 mM) were added to 50 mM potassium phosphate buffer (pH 7.0) to achieve a final volume of 3.0 ml. Oxygen consumption began after the addition of 10 to 20 mg of microsomes per ml. In bacterial assays, *E. coli* was grown overnight in tryptic soy broth, centrifuged, and suspended in 50 mM potassium phosphate buffer containing 2 mM MgCl₂ (pH 7.0) at 4°C. The final concentration of the bacterial cell suspension was 100 to 200 μg/ml. The O₂ uptake reaction was started by adding glucose (0.17%) to the reaction mixture as a carbon source. NaCN (2.0 mM) was added after 1 to 2 min of O₂ consumption to inhibit bacterial cellular respiration. Cyanide was used to inhibit cytochrome oxidase, which transfers electrons to oxygen. Cyanide-insensitive respiration continued and resulted in the formation of hydroperoxides. After a steady state was established, the antimicrobial agent was added (0.7 mM) and O₂ consumption was measured. The net rate of O₂ consumption was determined by subtracting O₂ consumption rates before addition of the antimicrobial agent from rates after addition of the antimicrobial agent.

**Data analysis.** Statistical evaluation of the data was done by using Student’s t test (paired comparison).

RESULTS

**Hyperoxia and a reduction-oxidation-cycling antimicrobial agent (nitrofurantoin).** We hypothesized that hyperoxia would increase antimicrobial activity through increased O₂⁻-production by reduction-oxidation-cycling antibiotics such as nitrofurantoin. Reduction of these compounds can occur through NADH- and NADPH-dependent reductases. Autooxidation of the reduced compound results in O₂⁻-production. In order to investigate this hypothesis, we first tested the ability of oxygen to increase O₂ consumption after reduction of nitrofurantoin, nitrofurazone, menadione, and...
rifamycin SV by *E. coli* serotype O18. Oxygen consumption was measured with a Clark-type electrode. Figure 1 is a tracing from a typical polarographic experiment. Cellular respiration of *E. coli* was induced by the addition of glucose (0.17%). In the presence of NaCN, nitrofurantoin caused an increase in the rate of O₂ consumption. This is indirect evidence for the production of O₂⁻. Cyanide only slowed the rate of cellular respiration, because it does not inhibit hydroperoxide respiration of the bacterial cells. O₂ consumption by nitrofurantoin and nitrofurazone with endogenous *E. coli* reductases was somewhat reduced compared with values obtained with microsomal NADPH reductase (Table 1). Menadione generated similar quantities of O₂⁻ with both endogenous *E. coli* reductases and microsomal NADPH reductases. Rifamycin SV failed to consume O₂ with either *E. coli* or microsomal NADPH reductase. O₂ consumption with concomitant generation of O₂⁻ was shown to be linearly correlated to the concentration of nitrofurantoin in the range of 0.2 to 3.3 mM (data not shown). These data demonstrate that nitrofurantoin is capable of reduction-oxidation cycling when reducing equivalents, reducing enzymes, and molecular oxygen (21% O₂) are available. It has been shown that the generation of O₂⁻ by reduction-oxidation cycling is potentiated by increasing the oxygen concentration (19). Hyperoxia (98% O₂) would thus be expected to increase bacterial killing by nitrofurantoin through increased production of O₂⁻. This is supported by the data shown in Table 2. The MIC of nitrofurantoin in hyperoxic conditions was significantly decreased (*P* = 0.01) to one-third of the MIC in normal oxygen conditions. The MBC of nitrofurantoin for *E. coli* serotype O18 was also decreased, but this decrease did not achieve statistical significance.

In summary, under normal oxygen conditions, nitrofurantoin undergoes reduction-oxidation-cycling reactions. Hyperoxia increases bacteriostatic and bactericidal effects of nitrofurantoin, probably through increased production of O₂⁻.

**Hyperoxia and antimetabolites (sulfamethoxazole and trimethoprim).** It has also been hypothesized that hyperoxia may increase antibacterial effects of sulfamethoxazole and trimethoprim by maintaining folate synthetic enzymes in an oxidized state (8). Sulfamethoxazole and trimethoprim act synergistically to inhibit bacterial growth by blocking sequential steps in the production of folic acid. Hyperoxia significantly reduced the MICs and MBCs of trimethoprim for *E. coli* serotype O18, as shown in Table 2. The MIC of trimethoprim for *E. coli* under hyperoxic conditions was 0.31 µg/ml, and the MIC determined in normal oxygen conditions was 0.58 µg/ml. MBCs of trimethoprim were significantly decreased (*P* = 0.005) from 0.86 µg/ml in normal oxygen conditions to 0.40 µg/ml in hyperoxia. MICs were determined in MSA medium to maximize the possibility of finding interactions between hyperoxia and antimicrobial agents. MSA medium with acetate added as a carbon source is sufficient for growth of bacteria but lacks nutrients such as thymidine which would otherwise allow bacteria to bypass blocks in folate metabolism.

**Hyperoxia and antimicrobial agents with oxygen-dependent uptake (gentamicin and tobramycin).** Aminoglycosides require oxidative metabolism for transport into bacterial cells. Normal oxygen conditions restore the bactericidal activity of aminoglycosides, which is diminished in anaerobic environments. We wished to test the ability of hyperoxia to increase bacterial killing over that seen in normal oxygen conditions. MIC and MBC testing of tobramycin for *E. coli* serotype O18 performed in MSA medium showed no significant differ-

![Graph](https://example.com/graph.png)

**FIG. 1.** Tracing of a polarographic recording of oxygen consumption by nitrofurantoin in the presence of *E. coli* serotype O18. After equilibration of *E. coli* in phosphate buffer (pH 7.0) in ambient air at 37°C, the recorder was calibrated. Oxygen consumption began upon addition of glucose (0.17%). NaCN was then added to inhibit O₂ consumption. Addition of nitrofurantoin resulted in increased O₂ consumption. Net O₂ consumed was derived by subtracting the rate of O₂ consumption before the addition of nitrofurantoin from the rate of O₂ consumption in the presence of nitrofurantoin.

**TABLE 1.** Oxygen consumption by reduction-oxidation-cycling compounds in the presence of microsomal NADPH reductase or *E. coli* serotype O18

<table>
<thead>
<tr>
<th>Compound</th>
<th>O₂ consumption (nmol of O₂/min per µmol of compound) by:</th>
<th>Microsomes</th>
<th><em>E. coli</em> serotype O18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>103</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>72</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Menadione</td>
<td>81</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Rifamycin SV</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Results are means of three determinations tested in triplicate.

**TABLE 2.** Effects of hyperoxia on MICs and MBCs of selected antimicrobial agents for *E. coli* serotype O18

<table>
<thead>
<tr>
<th>Antimicrobial agent (n)</th>
<th>Oxygen exposure</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21% O₂⁸</td>
<td>98% O₂⁹</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin (10)</td>
<td></td>
<td>2.94</td>
<td>5.28</td>
</tr>
<tr>
<td>Sulfamethoxazole (3)</td>
<td></td>
<td>2.08</td>
<td>608</td>
</tr>
<tr>
<td>Trimethoprim (9)</td>
<td></td>
<td>0.58</td>
<td>0.86</td>
</tr>
<tr>
<td>Tobramycin (6)</td>
<td></td>
<td>5.57</td>
<td>12.8</td>
</tr>
</tbody>
</table>

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⁸ Results are geometric means of MICs or MBCs calculated by taking the nth root of the product of n determinations. Determinations were performed in duplicate.

⁹ 21% O₂−5% CO₂−74% N₂ at 1 ATA for 24 h.

⁹ 98% O₂−2% CO₂ at 2.8 ATA for 24 h.

* Significantly different (*P* = 0.01) from the value found with normal oxygen conditions.

* Significantly different (*P* = 0.005) from values found with normal oxygen conditions.
ences between normal oxygen conditions and hyperoxia (Table 2). No significant differences were seen in MICs or MBCs of gentamicin or tobramycin in MHB for either *E. coli* 25922 or *P. aeruginosa* 27853 (data not shown). Endpoint colony counting did not reveal increased bactericidal effects of gentamicin or tobramycin against *E. coli* serotype O18 under hyperoxic conditions, as shown in Table 3. In addition, no enhanced bactericidal effects of gentamicin or tobramycin were demonstrated against *E. coli* strain ATCC 25922 or *P. aeruginosa* ATCC 27853 under hyperoxic conditions (data not shown).

**TABLE 3. Effects of hyperoxia on colony counts of *E. coli* serotype O18 in the presence of gentamicin or tobramycin**

<table>
<thead>
<tr>
<th>O2</th>
<th>Gentamicin (μg/ml)</th>
<th>CFU* of <em>E. coli</em> serotype O18 with:</th>
<th>Tobramycin (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>21%b</td>
<td>8.9 ± 0.3</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>98%c</td>
<td>7.8 ± 0.5d</td>
<td>6.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8.9 ± 0.3</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.8 ± 0.5d</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data are means of log CFU ± standard error, n = 12 determinations; determinations were performed in duplicate.
* 21% O₂—5% CO₂—74% N₂, at 1 ATA for 24 h.
* 98% O₂—2% CO₂ at 2.8 ATA for 24 h.
* Significantly different (P = 0.0001) from the value found in normal oxygen conditions.

**Bacteriostatic effects of hyperoxia as shown by endpoint colony counting.** During MIC testing, marked bacteriostatic effects were seen in growth control wells of all bacteria exposed to hyperoxia. Typically, turbidity was seen in the culture wells at 24 to 30 h after the multiwell plates were removed from the hyperoxic environment. To rule out the loss of effects of hyperoxia on aminoglycosides caused by the delay in appearance of turbidity in growth controls, endpoint colony counts were performed. A 1-log₁₀ difference (P = 0.0001) was seen between growth controls exposed to normal oxygen conditions and growth controls exposed to hyperoxia for *E. coli* serotype O18 (Table 3). A 1.5-log₁₀ difference (P = 0.0001) was seen between growth controls of *E. coli* (ATCC 25922) exposed to normal oxygen conditions and hyperoxia. A 2.7-log₁₀ difference (P = 0.006) was seen between growth controls of the reference *P. aeruginosa* strain (ATCC 27853) exposed to normal oxygen conditions and hyperoxia (data not shown). These findings are important, since bacteriostatic effects of hyperoxia have been shown to be strain specific (9). When looking for additive or synergistic effects of hyperoxia and antimicrobial agents, one needs to use a bacterium for which hyperoxia is at least bacteriostatic. Endpoint enumeration of bacteria revealed that all three bacterial strains were sensitive to the bacteriostatic effects of hyperoxia.

**DISCUSSION**

There are several possible mechanisms by which hyperoxia may influence the actions of antimicrobial agents. We explored the possibility that hyperoxia could interact with reduction-oxidation-cycling compounds such as nitrofurantoin to increase bacterial killing. Nitrofurantoin [N-(5-nitro-2-furanylidenem)-1-amino hydantoin] is a nitro compound which is toxic for a broad spectrum of bacteria known to cause acute urinary tract infections. The exact mode of action of nitrofurantoin is not known. However, like other nitro compounds, nitrofurantoin produces O₃ in the presence of molecular oxygen. The first step in the reduction-oxidation-cycling reaction of nitrofurantoin is a one-electron reduction to the nitro anion (NO₂⁻). When molecular oxygen is present, NO₂⁻ is oxidized to NO₂ and O₂ is concomitantly reduced to O₂⁻ (20). This oxygen-derived free radical may undergo further reduction to form H₂O₂, which is toxic for bacteria. Hydrogen peroxide may take part in the Fenton reaction, where it is converted to the highly reactive hydroxyl radical (16). These toxic oxygen species may inactivate bacterial enzymes, promote lipid peroxidation of bacterial cell membranes, and alter bacterial DNA. Bacteria lacking antioxidant enzymes are particularly susceptible to the effects of oxygen-derived free radicals. Moreover, nitrofurantoin or hyperoxia (100% O₂ at 4.2 ATA [425.5 kPa]) can each inhibit the growth of *E. coli* by inducing stringency (25, 26). The stringency response is defined as intracellular accumulation of guanosine 5'-diphosphate 3'-diphosphate, which is a regulatory inhibitor of biosynthetic processes in bacteria (25). Nitrofurantoin blocks synthesis of branched-chain amino acids such as valine and leucine (26). The observed decrease in the MIC of nitrofurantoin for a clinical bacterial isolate (*E. coli* serotype O18) during hyperoxic exposure may be due to a combination of these mechanisms. However, there remains a small possibility that this difference could be due to the method required to test MICs under hyperoxic conditions and not to the effect of hyperoxia itself.

We next examined the effects of hyperoxia and the antimetabolites sulfamethoxazole and trimethoprim against *E. coli* serotype O18. Sulfonamides such as sulfamethoxazole block the conversion of para-aminobenzoic acid into dihydrofolic acid by competitive inhibition. It is thought that sulfonamides may have a higher affinity for bacterial tetrahydrofolate acid synthetase than the naturally occurring substrate para-aminobenzoic acid. Trimethoprim blocks the next step in bacterial folic acid synthesis, the conversion of dihydrofolate to tetrahydrofolate, by inhibition of the enzyme dihydrofolate reductase. The sequential blockage in the folate biosynthetic pathway results in synergy between these antimicrobial agents against a number of pathogenic bacteria. Gottlieb (8) suggested that oxidation of metabolic intermediates by hyperoxia might alter the kinetics of the folate synthetic pathway. In the studies with *V. anguillarum* (15), hyperoxia increased bacterial killing when combined with sulfisoxazole and trimethoprim. In the present study, we saw a significant decrease in MICs and MBCs of trimethoprim for *E. coli* serotype O18. These findings are in agreement with our previous work (21), which showed that hyperbaric oxygen combined with sulfamethoxazole–trimethoprim decreases bacterial numbers in the peritoneal cavity of rats with *E. coli* serotype O18-induced sepsis. Similar interactions between hyperoxia and sulfamethoxazole were not seen. *Vibrio* spp. are known to be more highly susceptible to effects of hyperoxia than are other gram-negative bacteria, such as the ones that we have examined.
Uptake of aminoglycoside antibiotics such as gentamicin and tobramycin is an energy-dependent process. Electron transport involving quinone oxidation-reduction cycles is an important source of energy for aminoglycoside entry into bacteria (4). This may account for the resistance of anaerobic and facultative anaerobic bacteria to aminoglycosides in anaerobic environments. Our results indicate that no enhanced activity of aminoglycosides is achieved under hypoxic conditions in vitro. These results do not exclude the possibility that hyperoxia could restore oxygen tensions in tissues to levels required for aminoglycoside uptake by bacteria. A similar restoration of O₂ tensions allows polymorphonuclear leukocytes to kill bacteria in previously hypoxic tissues (12, 18). Finally, we have used endpoint enumeration of viable bacteria to look for interactions between hyperoxia and aminoglycosides. This was necessary because the cultures exposed to hyperoxia had not grown to visual turbidity in growth control wells at the time they were removed from the hyperbaric chamber. Endpoint colony counts confirmed the bacteriostatic effects of hyperoxia in a quantitative manner but did not show any interactions with aminoglycosides. Endpoint colony counting may be more suitable than MIC and MBC testing for examining the interactions between hyperoxia and antimicrobial agents, because bacteria are quantified at the same standard time point that MICs in air are normally read (24 h).

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LITERATURE CITED