show a peak energy at 1,000-1,100 cycles/second for the entire duration of the swallowing sound; "wet" swallows, on the other hand, began at a lower frequency (400-600 cycles/second), and increased in frequency to reach peak intensity at 1,000 cycles/second at the end of swallowing; (3) the delta or third component of the swallowing complex tended to be absent in "dry" swallows. The origin of this component is not known, but the nature of the temporal sequence suggests two possibilities: motion of the epiglottis at the end of deglutition or activity of lower esophageal areas as the swallowed fluid approaches the cardio-esophageal sphincter.

Transient apnea during deglutition was a feature of both "wet" and "dry" swallows. In the vast majority of cases, swallowing occurred during expiration or at the end of inspiration(2). No differences in the time of respiratory inhibition were noted between "dry" and "wet" swallows(3).

Conclusions. Sound produced by the act of swallowing was used to indicate the occurrence of this event. Minimal artifact was produced by this method which imposed no restriction on motion of the neck. A distinct and reproducible acoustic profile of swallowing was recognized. The nature of the swallowed material was found to have specific effects on the configuration of the swallowing complex. Further work requires the simultaneous use of cineradiographic equipment to delineate the physiologic events responsible for the generated acoustic energy. Transient apnea during the expiratory phase of respiration was seen with deglutition. This phenomenon was unrelated to the nature of the swallowed material, and was seen in both "wet" and "dry" swallows.

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Exposure of *Pseudomonas aeruginosa* to Hyperbaric Oxygen: Inhibited Growth and Enhanced Activity of Polymyxin B.* (32301)

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There has been new interest in the inhibitory effect of high pressure oxygen (HPO) upon the *in vitro* growth of a variety of pathogenic aerobic bacteria(1). Wiseman *et al*(2) found striking differences when the effect of HPO on the growth of aerobic bacteria on solid media was contrasted with that in liquid media. They reported that the results of counts of viable bacteria did not correlate with the size and appearance of colonies, and were unable to demonstrate any effect of HPO upon the activity of several antibiotics. Ollodart and Blair(3) reported the growth of *Escherichia coli, Staphylococcus aureus* and a pseudomonad to be enhanced in liquid cultures under oxygen at 1 atmosphere absolute (1 ATA) but to be inhibited at 3 ATA. Stark and Orr(4) found that growth in broth cultures aerated with 100% oxygen at 3 ATA was greatly retarded; the generation time for *E. coli* was 90 minutes compared to 20 minutes in controls.

The potentiality for growth inhibition on surface cultures and growth enhancement in deeper cultures suggests restrictions in the use of HPO therapy for bacterial infections with aerobes. Irvin *et al*(5) applied oxygen at 2 ATA to guinea pigs with experimental surface wounds infected with *Pseudomonas aeruginosa* and *Staph. aureus*, and observed inhibited bacterial growth during treatment with

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oxygen followed by increased infection when treatment was stopped. Subsequently, these authors demonstrated a maximal inhibitory effect of HPO on the growth of *E. coli* in layers of broth 0.9 mm deep(6). Schreiner(7) evaluated the effect of HPO upon the activity of several antibiotics with a disc technique, and showed enhanced antibiotic activity against *Staph. aureus*.

Quantitation of the combined effect of HPO and antibiotics may ultimately permit the rational and efficient employment of these combined agents in the treatment of bacterial infections. *P. aeruginosa* is commonly found in infected wounds and burns, and may invade the blood stream and cause a fatal generalized infection. A high incidence of *P. aeruginosa* has been found in the upper respiratory tract of patients with cystic fibrosis of the pancreas (8). Polymyxin B continues to be one of the most effective antibiotics against *P. aeruginosa*. The bactericidal activity of this antibiotic has been explained in terms of disorganization of a cellular membrane controlling the osmotic equilibrium of the cell(9). Accordingly, experiments have been conducted to evaluate the combined effect of HPO and polymyxin B upon the *in vitro* growth of *P. aeruginosa*.

Materials and methods. Six strains of *P. aeruginosa* were used. Strains #66 and #151 were isolated from postoperative infections. Strains CF 29, CF 60, CF 31, and CF 193 were isolated from the respiratory tract of children with cystic fibrosis of the pancreas. Strains CF 31 and CF 193 were atypical mucoid colonial variants associated with cystic fibrosis(8). All strains were oxidase positive, produced pyocyanine, grew at 42 C, oxidized potassium gluconate, and produced slime in potassium gluconate medium(10).

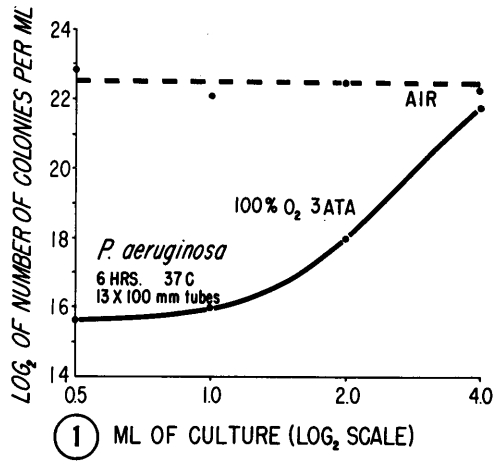
Polymyxin B sulfate was obtained from Pfizer Laboratories, New York, as a sterile buffered diagnostic preparation. Minimal inhibitory concentrations were determined in 13 × 100 mm culture tubes loosely plugged with cotton. An overnight culture of *P. aeruginosa* was diluted 10^{-5} in trypticase soy broth, and 0.5 ml of culture was added to 0.5 ml volumes of decreasing concentrations of antibiotic diluted in trypticase soy broth. The concentration of polymyxin B decreased from 16 units/ml to 1 unit/ml in steps of 1 unit. One unit of polymyxin B equals 0.5 μg. The minimal inhibitory concentration of antibiotic was read as the lowest concentration inhibiting the development of visible growth. All tube dilution tests were performed at least 3 times and on separate days. End points were averaged.

The chamber for hyperbaric oxygenation consisted of a table-top autoclave that had been stripped down to the stainless steel compartment and door (21.6 cm × 21.6 cm × 42.5 cm). It was fitted with a pressure release valve, pressure gauge, thermometer, and thermoregulator, and was wrapped with heat conducting wire and then with insulating material. Compressed oxygen (U.S.P.) from cylinders was utilized and flowed continuously through the 19 liter chamber at the rate of 4 to 6 liters/min. All studies were conducted in flowing oxygen at 3 ATA (gauge pressure of 30 lb/sq in.). This pressure is most commonly used in clinical work. The temperature within the chamber was maintained at 37 C.

For studies of the effect of HPO on antibiotic activity, 4 tube-dilution series of polymyxin B were prepared. A control containing no added antibiotic complemented each. Three complete dilution series were placed in the hyperbaric chamber. After 3, 6, and 12 hours exposure to HPO, one set was withdrawn from the chamber and incubated in air at 37 C along with the fourth series which had been incubated at the start of each experiment. Tubes were read after incubation for 48 hours.

For studies of the effect of HPO on bacterial growth, one ml samples of a 10^{-5} dilution in trypticase soy broth of an overnight culture of strain #66 were added to 13 × 100 mm tubes covered with aluminum caps. At intervals of 3 hours after starting an experiment, one tube was withdrawn from the hyperbaric chamber and a control tube was withdrawn from the air incubator. Each was serially diluted in sterile saline solution. A fresh pipette was used for each transfer, and all dilutions were dispersed with the aid of a vortex-mixer. Three trypticase soy agar pour plates were prepared from each of at least two appropriate dilution tubes.

Results. The effect of HPO on the growth



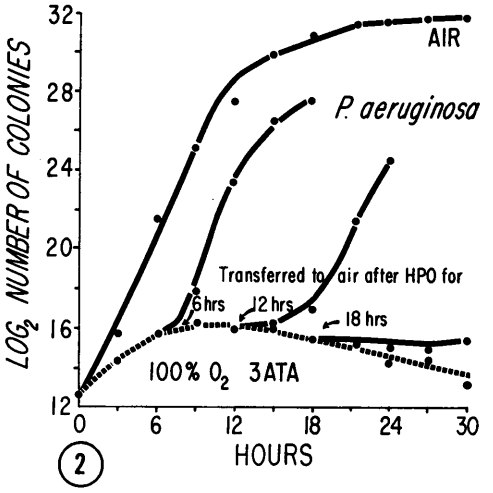


FIG. 1. Effect of volume of culture on growth. Initial count in these cultures was 7,470 colonies per ml ($\log_2 = 12.87$).

FIG. 2. Growth curves for one ml cultures exposed to hyperbaric oxygen and normobaric air.

of *P. aeruginosa* #66 was evaluated in the presence of increasing volumes of trypticase soy broth. A 10^{-5} dilution of an overnight culture was dispensed in volumes of 0.5, 1.0, 2.0, and 4.0 ml into the 13 × 100 mm tubes routinely used. The average depth of these cultures was 6, 11, 22.5, and 45 mm. During exposure to 100% oxygen at 3 ATA, the greatest inhibition of growth occurred in the shallower cultures where the barriers to diffusion of oxygen were smallest (Fig. 1). Multiplication in the 4.0 ml culture exposed to HPO was comparable to that occurring in air, where multiplication was independent of volume.

Curves for the growth of *P. aeruginosa* #66 during exposure to HPO were plotted. Exposure to HPO greatly inhibited multiplication (Fig. 2). Maximal growth during exposure to HPO obtained at about 12 hours and was comparable to that after only 3 hours in an air incubator. However, when cultures were transferred to the air incubator after being exposed to HPO for 6 and 12 hours, the rate of logarithmic growth was characteristic of that in cultures exposed to air. With increased exposure to HPO before being incubated in air the lag phase of the growth curve increased, and after 18 hours exposure to HPO, the lag period was more than 12 hours.

The effect of HPO at 3 ATA on antibiotic activity was assayed (Table I). With all strains of *P. aeruginosa,* the longer the period of exposure to HPO, the lower the minimal inhibitory concentration (MIC) of polymyxin B. Mean MIC's following any given exposure period were similar regardless of the strain of *P. aeruginosa* studied.

The MIC of polymyxin B following exposure to compressed air instead of 100% oxygen at 3 ATA was unchanged, and averaged 12.3 units/ml after 3 hours exposure, 12.3 after 6 hours, 11.0 after 12 hours, and 11.7 for unexposed controls.

Discussion. The greater multiplication of *P. aeruginosa* in increasing volumes of broth during exposure to HPO demonstrates the gradient in oxygen tension between the surface and the depth of the medium. The one ml stationary cultures routinely employed in this study allow an inhibitory effect of HPO to be measured although not the total in-

TABLE I. Mean Minimal Inhibitory Concentration of Polymyxin B Following Exposure to HPO.

Strain of *P. aeruginosa*	Exposure to HPO			
	None	3 hr	6 hr	12 hr
	Units/ml			
# 66	11.3	10.0	6.7	4.0
#151	11.7	9.0	6.3	3.3
CF 29	11.3	7.3	5.7	3.5
CF 60	12.0	8.0	5.0	3.0
CF 31	10.5	8.0	5.8	3.8
CF 193	12.3	8.7	5.3	3.3

hibition reported in shallower cultures. Although the growth of *P. aeruginosa* is greatly inhibited during exposure to HPO, growth in air eventually resumes at the rate characterizing unexposed cultures. When bacteria initiate rapid and luxuriant growth upon incubation after exposure to HPO, it is unlikely that oxygen has reacted with components of the medium to produce significant concentrations of toxic substances(11). The greatly exaggerated lag periods demonstrated in Fig. 2 following increased exposure to HPO may have resulted from either production of a toxic substance during exposure or from a direct effect of HPO on cellular constituents necessary for growth and replication. Further studies of the effect of HPO on bacterial growth are needed to elucidate the mechanism by which the lag period is inhibited. HPO appears to be harmful at the cellular level because it inactivates enzymes essential to the normal function of oxidative cycles and electron-transport pathways(12,13).

Regardless of the strain of *P. aeruginosa* used, the enhanced activity of polymyxin B is similar for a given exposure to HPO. The minimal inhibitory concentration of antibiotic decreases with increased exposure to HPO. Although the mean MIC's shown in Table I for each strain of *P. aeruginosa* are not independent values for a single strain, it is, nevertheless, convenient to summarize the effect of HPO on the activity of polymyxin B by averaging the values shown for a given exposure. Accordingly, it can be calculated that after 3 hours exposure to HPO, 74% of the amount of polymyxin B required for the MIC in unexposed cultures is needed to achieve the MIC; after 6 hours exposure, 50%; and after 12 hours exposure, only 31%.

Schreiner(7) reported enhanced antibiotic activity against *Staph. aureus* during exposure to oxygen at 3 ATA, but not at 1 or 2 ATA. He used a disc technique with pour plates of microorganism 1.2 mm deep to demonstrate various degrees of enhanced activity with penicillin, chlortetracycline, tetracycline, oxytetracycline, erythromycin, neomycin, kanamycin, dihydrostreptomycin, and nitrofurantoin. On the other hand, Wiseman and colleagues(2) were unable to demonstrate any effect of exposure to HPO on the growth response of broth cultures of *P. aeruginosa* in the presence of polymyxin B and of *Staph. aureus* in the presence of penicillin and erythromycin. This discrepancy is probably due to their use of oxygen at no more than 2 ATA. Our data show that oxygen at 3 ATA enhances or potentiates the activity of polymyxin B to a similar extent against several different isolates of *P. aeruginosa*. This effect is not due to pressure alone since exposure of *P. aeruginosa* to air at 3 ATA does not alter antibiotic activity.

The term *synergism* signifies that the combined effect of two antimicrobial agents exceeds the sum of the effects of each agent separately(14). Schreiner(7) reported that growth was inhibited in shallow layers of broth under partial pressures of oxygen as low as 1 ATA, but that the minimal effective dose of penicillin was not influenced by the partial pressure of oxygen, and concluded that the combined antibacterial activities were additive and not synergistic. Under the conditions of our experiments, the bacterial inoculum is exposed to antibiotic during the entire 48 hours period of incubation, but is exposed to HPO for not longer than the first 12 hours. Qualitatively, the control tubes (containing no antibiotic) of dilution series exposed to HPO show visibly lesser turbidity after 24 hours, but equally luxuriant growth after 48 hours. Thus, the longer the combined action of HPO and antibiotic, the greater the combined antibacterial effect. If this end result actually reflects a greater rate of early bactericidal action of the combined agents as compared to the sum of the rates of kill for each agent alone, then HPO and polymyxin B qualify as a synergistic combination against *P. aeruginosa*. We have not yet determined if the combined effect is actually synergistic or only a simple addition of the effect of each agent. Until the results of further studies clarify this point, the combined effect is best described as an enhancement or a potentiation of antibiotic activity.

Studies of experimental infections in laboratory animals must confirm these *in vitro* findings before the therapeutic use of HPO combined with antibiotic therapy can be

considered. Here the considerable problems of oxygen toxicity resulting from prolonged exposure to HPO may be an insurmountable obstacle(1). Nevertheless, the possibility of being able to increase the therapeutic effectiveness of a maximum dosage of an antibiotic is attractive. This could be possible if exposure of the patient to HPO could also bathe the infecting microorganisms with oxygen. Pennock(15) has expressed concern that although therapy with HPO might inhibit pathogenic aerobes at the surface of a lesion it might also stimulate their multiplication in deep infections where the gradient in oxygen tensions might actually provide optimal conditions for multiplication. Combined therapy may be of value in the treatment of subacute bacterial endocarditis, superficial wounds, and burns(16). Currently, there are valid clinical indications for the use of combinations of antibiotics only in streptococcal bacterial endocarditis and tuberculosis(17).

Summary. The growth of *Pseudomonas aeruginosa* was studied in stationary broth cultures (11 mm deep) exposed to 100% oxygen at 3 atmospheres absolute (3 ATA). During exposure, growth was greatly inhibited. Cultures transferred to air after high pressure oxygen (HPO) resumed logarithmic growth at rates similar to unexposed cultures, but lag periods increased. The minimal inhibitory concentration (MIC) of polymyxin B was determined for 6 strains of *P. aeruginosa* after exposure to HPO for 3, 6 and 12 hours. The longer the exposure, the lower the MIC. Regardless of the strain, after 3 hours exposure to HPO about 75% of the amount of antibiotic required for the MIC of unexposed cultures was needed; after 6 hours exposure, about 50%; and after 12 hours exposure, about 30%. This suggests that it may be possible to increase the therapeutic effectiveness of an antibiotic administered at its maximum dosage if exposure of the patient to HPO can also bathe the infecting microorganisms with oxygen.

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