

Microbial Growth Modification by Compressed Gases and Hydrostatic Pressure

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Studies of the growth-modifying actions for *Escherichia coli*, *Saccharomyces cerevisiae*, and *Tetrahymena thermophila* of helium, nitrogen, argon, krypton, xenon, and nitrous oxide led to the conclusion that there are two definable classes of gases. Class 1 gases, including He, N₂, and Ar, are not growth inhibitors; in fact, they can reverse the growth inhibitory action of hydrostatic pressures. Class 2 gases, including Kr, Xe, and N₂O, are potent growth inhibitors at low pressures. For example, at 24°C, 50% growth-inhibitory pressures of N₂O were found to be ca. 1.7 MPa for *E. coli*, 1.0 MPa for *S. cerevisiae*, and 0.5 MPa for *T. thermophila*. Class 1 gases could act as potentiators for growth inhibition by N₂O, O₂, Kr, or Xe. Hydrostatic pressure alone is known to reverse N₂O inhibition of growth, but we found that it did not greatly alter oxygen toxicity. Therefore, potentiation by class 1 gases appeared to be a gas effect rather than a pressure effect. The temperature profile for growth inhibition of *S. cerevisiae* by N₂O revealed an optimal temperature for cell resistance of ca. 24°C, with lower resistance at higher and lower temperatures. Overall, it appeared that microbial growth modification by hyperbaric gases could not be related to their narcotic actions but reflected definably different physiological actions.

Growth inhibition by anesthetics, including compressed gases, was demonstrated by Claude Bernard before the turn of this century. Inhibition appears to be universal among procaryotes and eucaryotes and can be demonstrated by the use of single-celled organisms: fungi, protozoa, bacteria, and HeLa cells in culture. Compressed gases can also affect microbial differentiation. Enfors and Molin (6) found that gases were potent inhibitors of chemically induced germination of *Bacillus* spores, and on the basis of their experimental data they constructed a potency series with CO₂ > N₂O > Xe > Kr > Ar > N₂ > H₂. Helium was not inhibitory even at a pressure of 10 MPa (1 MPa = 10 atmospheres). The CO₂ effect on germination appeared to be a gas effect rather than one due to bicarbonate (7). This inhibitory series for germination is similar to the series for narcotic potency and also to the series reported for growth inhibition. For example, Buchheit et al. (3) presented a series for inhibition of growth of *Neurospora crassa* with Xe > Kr > Ar >> Ne >> He > N₂; the partial pressures required for 50% inhibition of growth were, respectively: 0.08, 0.16, 0.38, 3.5, and ca. 30 MPa, with no value determined for N₂ because it was not possible to achieve 50% growth inhibition with N₂. Generally, in narcotic series, N₂ falls between Ar and Ne, and the basis for its unusual position in the series for inhibition of fungal growth is not known. For experiments on narcotic action, N₂O is commonly used rather than Xe because the latter is very expensive. N₂O and Xe have approximately the same potency, and in fact, Xe has been used experimentally as a surgical anesthetic (5). N₂O and Xe also have approximately the same potency for inhibition of bacterial growth (9).

Careful examination of the various potency series presented by various authors for growth inhibition and of the data on which the series were based revealed to us a number of inconsistencies. Moreover, although both the growth-inhibitory and narcotic actions of gases are often considered together, it is difficult to assess whether the two actions are

related. Many authors have avoided the question; a few have considered that growth inhibition and narcosis may be related (9, 18).

In this paper, we present some of the results of an extensive set of experiments, mainly with *Escherichia coli*, *Saccharomyces cerevisiae*, and *Tetrahymena thermophila*, which led us to conclude that there are two identifiable classes of gases with respect to growth inhibition and that inhibition should not be considered as due to narcotic action, at least not under the most widely accepted definitions of narcosis. In a recently published review (1), a distinction has been made between so-called group A responses to inert gases, which include narcosis, hyperbaric bradycardia, and high-pressure convulsions, and non-group A responses, which include modification of growth.

MATERIALS AND METHODS

Microorganisms and media. *E. coli* B was routinely grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) with 0.1% (wt/vol) KNO₃. *S. cerevisiae* CC836 was obtained from R. Christensen of this institution and was grown in tryptone-glucose-Marmite medium (16). *T. thermophila* was obtained from J. Wilhelm of this institution and was grown in a modified medium containing, per liter, 20 g of glucose, 1 g of Marmite, 10 g of Difco tryptone, and 0.035 g of EDTA. Ampicillin (162 µg/ml) was added to the growth media for *S. cerevisiae* and *T. thermophila*.

Growth was assessed turbidometrically with light of 700 nm wavelength or by direct counting with a standard hemacytometer.

Compression of cultures. Cultures to be compressed hydrostatically without compressed gas were placed in plastic syringes, and all air was expelled. Then, each syringe end was sealed with a hypodermic needle and stopper. The syringes were placed in standard pressure chambers with O-ring seals (15) and pressurized by use of a hydraulic hand pump. Collapse of the rubber tips of syringe plungers under pressure was avoided by drilling small holes in the plastic backings for the tips.

For experiments on the combined effects of hydrostatic

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pressure and gases, cultures were placed in gastight glass syringes (Glenco Scientific, Inc., Houston, Tex.) with measured volumes of gas.

All cultures contained glass beads or magnetic stirring bars to allow for mixing under pressure or after decompression. In general, fully grown cultures were used as inocula, and initial cell numbers for bacteria, *S. cerevisiae*, and *T. thermophila* were, respectively, 10^7 , 10^5 , and 10^4 cells per ml. Growth curves were obtained by repeated sampling of single cultures in most instances rather than by use of multiple replicate cultures. Sampling involved decompression, mixing of the culture, sampling, and repressurization. The operation could be completed in only a little over a minute when cultures did not contain a gas phase.

Cultures exposed to hyperbaric gases without additional hydraulic compression were prepared with 50 ml of culture in cotton-plugged 250-ml Erlenmeyer flasks. A sterile, Teflon-coated stirring bar was placed in each culture, and the flask was placed in an oversized standard pressure chamber with a capacity of some 3,200 ml. Chambers were connected by means of high-pressure tubing through a two-way valve (American Instruments Co., Silver Spring, Md.) to tanks of compressed gas. Unless indicated otherwise, the air initially in the chambers was not flushed out. The chambers were pressurized with the desired gas, or mixture of gases, and then the two-way valves were closed to seal the chambers. The high-pressure tubing was disconnected, and a pressure gauge was attached to each valve so that the actual pressure within the chambers could be checked. Control 1-atm (0.1-MPa) cultures were incubated on the shelf next to the pressurized chambers. Magnetic stirrers were used only to achieve initial gas transfer, but not for long periods, so that the cultures did not become heated by the stirrer. The chambers were made of nonmagnetic steel.

The chambers were decompressed for sampling over a period of approximately 5 min. A portion of each culture was removed, and the remaining portions were recompressed immediately. Measured temperature changes in cultures after decompression from, for example, 40 atm (4 MPa) of helium were at most only ca 5°C. Obviously, this cooling would be reversed on recompression of the culture. Previous work (17) has indicated that cycles of compression and decompression, even with compressed gases, do not affect microbial growth and viability. Presumably, compression-decompression involving Xe or Kr also would not be damaging. Intracellular bubbles do not form in these cells (11).

Compressed gases were obtained from Air Products & Chemicals, Inc., of Allentown, Pa. Gas purities were 99.995% for He, 99.998% for N₂, 99.995% for Ar, 99.6% for O₂, 99.995% for Kr, 99.995% for Xe, and 98% for N₂O, with air as the major impurity.

RESULTS

Growth inhibition by individual gases. Examples of the inhibitory effects of N₂O on growth of *E. coli* B are shown by the sample growth curves in Fig. 1. The gas at a pressure of 1.36 MPa (in the presence of air) slightly extended the lag phase, slowed exponential growth, and reduced the final growth yield, given here in terms of light absorbance at 700 nm (*A*₇₀₀). A pressure of 2.72 MPa nearly completely stopped growth. This same pattern of growth effects was observed for cultures of *S. cerevisiae*, *T. thermophila*, and a variety of bacteria. A reasonable, comparative measure of the inhibitory effectiveness of N₂O or other gases is the percent change in culture yield relative to unpressurized

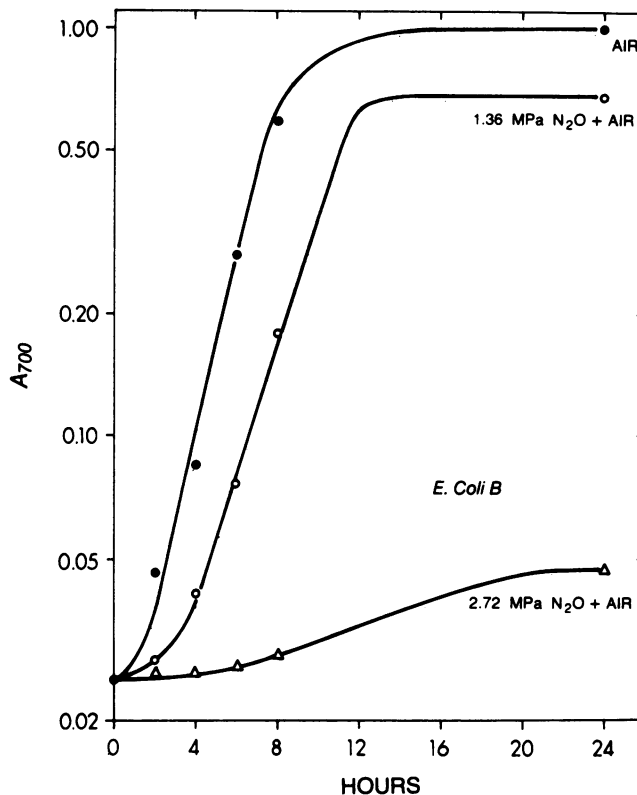


FIG. 1. Inhibition of growth of *E. coli* by 1.36 and 2.72 MPa of nitrous oxide at 24°C.

control cultures. Yields were determined as light absorbances assessed early in the stationary phase of growth or as direct counts for cultures of *T. thermophila*.

Figure 2 shows comparative data from a large number of individual experiments on the effects of He, N₂, Ar, and N₂O on growth yields of *E. coli*, *S. cerevisiae*, and *T. thermophila*. The data show that N₂O is an effective growth inhibitor with 50% inhibitory doses of ca. 1.7 MPa for *E. coli*, 1.0 MPa for *S. cerevisiae*, and 0.5 MPa for *T. thermophila*. The data indicated the usual sigmoidal dose-response curves. In contrast, the data for He, N₂, and Ar at pressures up to nearly 6 MPa indicate that these gases were not growth inhibitory. In fact, there is indication that they may, on average, have been stimulatory for growth. The data given for *T. thermophila* show mean direct counts, standard deviations in the counts, and the number of experiments on which each mean value was based. The variance shown is related partly to variations among experiments and partly to the high counting errors of hemacytometer direct counts. The assessment of light absorbance does not have such a large error, and the variations shown in the points presented for *E. coli* and *S. cerevisiae* indicate mainly experiment-to-experiment variation. Clearly, there is no sign of an inhibitory effect on growth for He, N₂, or Ar.

Because of their high cost, Xe and Kr were used for only a few assessments of growth inhibition. The 50% growth-inhibitory pressures of Xe were ca. 1.8 MPa for *E. coli* and 1.3 MPa for *S. cerevisiae*; Kr pressures were ca. 1.3 MPa for *E. coli* and 1.0 MPa for *S. cerevisiae*. Thus, Xe and Kr appeared to have approximately the same potency as N₂O for inhibition of growth. Examples of inhibition of *S. cerevisiae*

iae by Kr and Xe are shown in Fig. 3, and the data indicate also the nearly equal potencies of Xe and N₂O. Attempts were not made to assess effects on growth rate here, but only on yield. Because Xe and Kr are noble gases, their capacities to inhibit growth indicate that inhibition does not require formation of covalent bonds or metabolism of the gases. Of course, simply because they have similar potencies, one should not conclude that N₂O and Xe or Kr have exactly the same biochemical actions affecting growth.

Overall, the data given indicate that there were two classes of gases. One consisted of N₂O, Xe, and Kr, which were potent growth inhibitors for a variety of microbes. The other consisted of He, N₂, and Ar, which did not appear to be growth inhibitors. The latter gases will be referred to as class 1 gases, and the former as class 2 gases.

Reversal of pressure inhibition of growth by He, N₂, and Ar.

Class 1 gases can inhibit growth at very high pressures, but at these higher pressures it is necessary to distinguish between effects of the gases themselves and the effects of hydrostatic pressure. *S. cerevisiae* is relatively sensitive to pressure, and hydrostatic pressures of ca. 5 MPa and greater at 24°C inhibit growth (Fig. 4). The data also indicate clearly that growth inhibition was significantly less severe when pressure was applied with compressed He, N₂, or Ar than when it was applied hydraulically without a gas phase. In fact, the gases at pressures less than ca. 7.5 MPa were actually stimulatory for growth. The effects of pressure were

not affected by inclusion of 0.02 MPa of O₂ in the gas phase or in oxygenated fluorocarbon liquids (FC-80 of 3M Corporation, St. Paul, Minn.), which can be used as oxygen reservoirs for cultures under pressure. Hydrostatic pressures greater than 20 MPa were found to suppress growth completely, but the same pressure applied with the gases allowed for approximately half of the growth seen in an unpressurized control culture.

The data show clearly that Ne, N₂, and Ar antagonized the inhibitory effects of hydrostatic pressure and led to the conclusion that class 1 gases were actually stimulatory for yeast growth under pressure. Similar antagonistic actions of class 1 gases and pressures are evident in the data of Macdonald (13) for *Tetrahymena*, those of Taylor (20) for a marine pseudomonad, and those of MacNaughtan and Macdonald (14) for *Acholeplasma laidlawii*.

Potentialization of the growth-inhibitory actions of N₂O and O₂ by class 1 gases. The antagonism of the growth-inhibitory actions of hydrostatic pressure by class 1 gases indicates clearly that these gases interact in physiologically important ways with microbial cells. Physiological interactions are reflected also in the dramatic potentiation by class 1 gases of the inhibitory actions of N₂O and O₂. Data are given in Table 1 for *E. coli*, *S. cerevisiae*, and *T. thermophila*. For each organism, a pressure of N₂O, which alone had only a minimally inhibitory effect on growth, became highly inhibitory in combination with a class 1 gas. Growth curves for *E.*

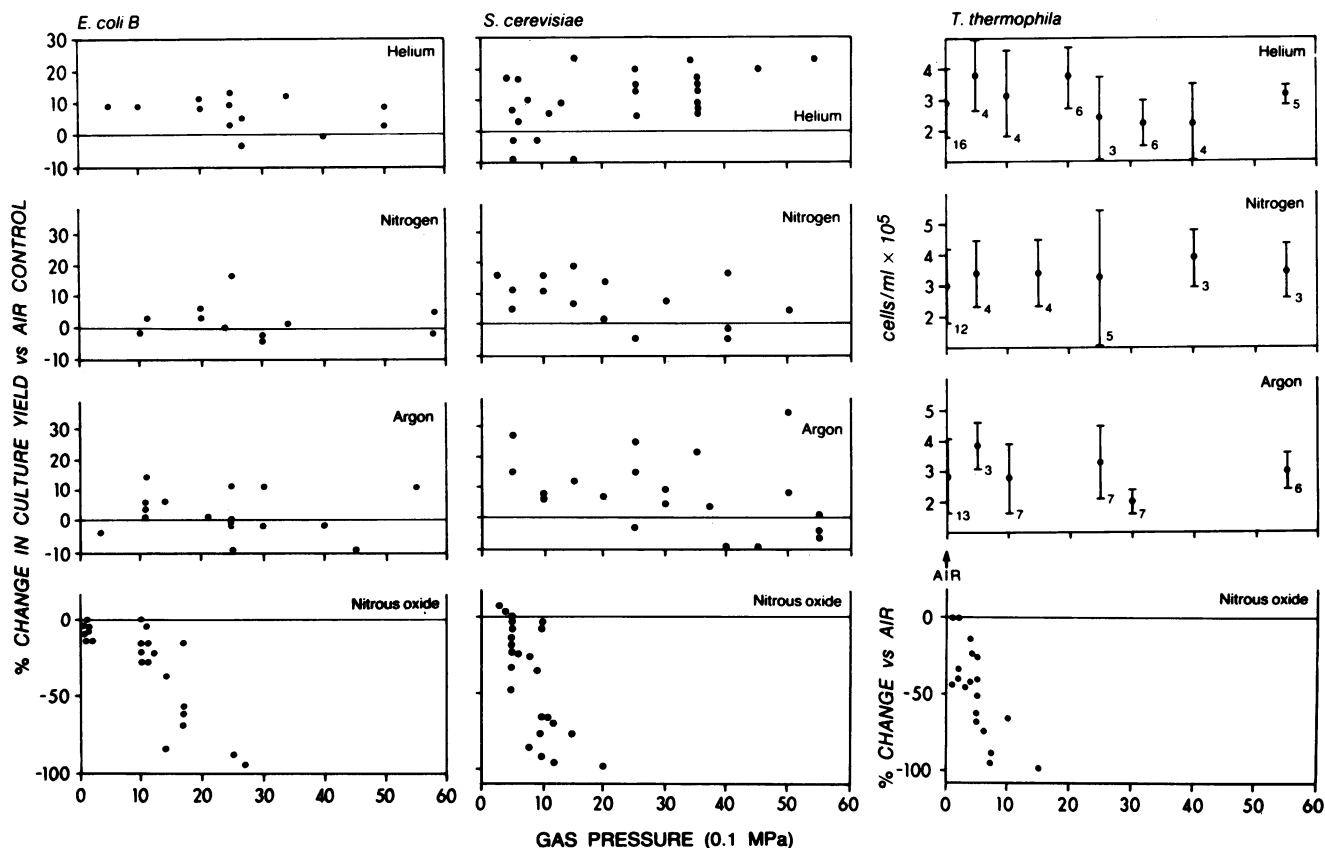


FIG. 2. Effects of helium, nitrogen, argon, and nitrous oxide on growth of *E. coli*, *S. cerevisiae*, and *T. thermophila* at 24°C. Gas pressures are given in terms of 0.1 MPa (atmospheres). Single points represent growth yields, in terms of maximal A₇₀₀, compared with yields of unpressurized control cultures. Data for the effects of helium, nitrogen, and argon on *T. thermophila* show average hemacytometer counts and standard deviations, as well as the numbers of experiments carried out to obtain the average values shown.

coli indicated also that the combination of N_2O and Ar could induce a lytic response in stationary-phase cultures, a response seen also in some experiments with *T. thermophila*. Care was taken in obtaining the data for Table 1 to be sure that cultures had not entered a lytic phase before determinations of final growth yields. It appears (Table 1) that the gases did not vary greatly in potency. Even larger numbers of experiments might have revealed differences in potency, but presumably they would have been small differences. The potentiation of O_2 or N_2O toxicity by He, N_2 , or Ar was not the result of hydrostatic pressure, as shown, for example, by the data for O_2 (see Table 3) or by the data for N_2O given previously (21). In fact, hydrostatic pressure established without the use of compressed He, N_2 , or Ar markedly antagonized the inhibitory actions of N_2O .

Again, because of the expense, only a few experiments were carried out with Xe and Kr. However, He significantly potentiated the inhibitory actions of these noble class 2 gases, here for *S. cerevisiae* (Table 2).

Range of sensitivities to nitrous oxide. The differences in sensitivity to N_2O among the three main test organisms in this study represent only part of the spectrum of sensitivity among microbes. The sensitivity range in terms of doses required for 50% inhibition of growth (ID_{50} s) at 30°C was from ca. 0.2 MPa for *Tetrahymena* to nearly 2.6 MPa for *Streptococcus faecalis* ATCC 9790 (Fig. 5). The high resistance of *S. faecalis* to N_2O is accompanied by high resistance to O_2 . However, in general, there does not seem to be a close correlation between oxygen sensitivity and sensitivity to N_2O , although both gases have been shown to be involved in chemical radical metabolism. Presumably, the major resistance mechanisms for oxygen tolerance have to do with

protective enzymes and low metabolic capacity for the gas. The same mechanisms do not appear to confer tolerance to N_2O .

Hydrostatic pressure effects on oxygen toxicity. We have described elsewhere (21) the reversing action of hydrostatic pressure on N_2O toxicity. Previously, ZoBell and Hittle (23) had reported that, in contrast, hydrostatic pressure greatly increased sensitivities of a range of microbes to oxygen. However, no such sensitization by hydrostatic pressure occurred with *E. coli* or *S. cerevisiae*, even at growth-inhibitory pressures (Table 3). The enhancement of oxygen toxicity by He was readily demonstrated in the same experiments. Moreover, there may be some antagonism since the combined effects of oxygen and hydrostatic pressure were equal to or less than the effects of the individual agents.

Temperature and growth inhibition by nitrous oxide. As mentioned above, increases in hydrostatic pressure antagonize the growth-inhibitory action of N_2O . Experiments on the effects of temperature on the growth-inhibitory potential of N_2O for *S. cerevisiae* revealed (Fig. 6) a profile with an optimum temperature for microbial resistance to the gas and diminished resistance at higher or lower temperatures. Here, N_2O doses are expressed in terms of aqueous solubilities (millimoles per milliliter of water) rather than gas pressures because the Ostwald coefficient for N_2O in water varies from 0.705 at 18°C to 0.401 at 42°C (22). The lower panel of the figure shows the nearly linear parts of the dose-response profiles. The sensitivity of *S. cerevisiae* at 37°C was found to be nearly equal to its sensitivity at 18°C, and a single line was drawn for the points at the two temperatures. Of course, the pressures of N_2O required to achieve the doses indicated were higher at 37°C than at 18°C because of the lower water

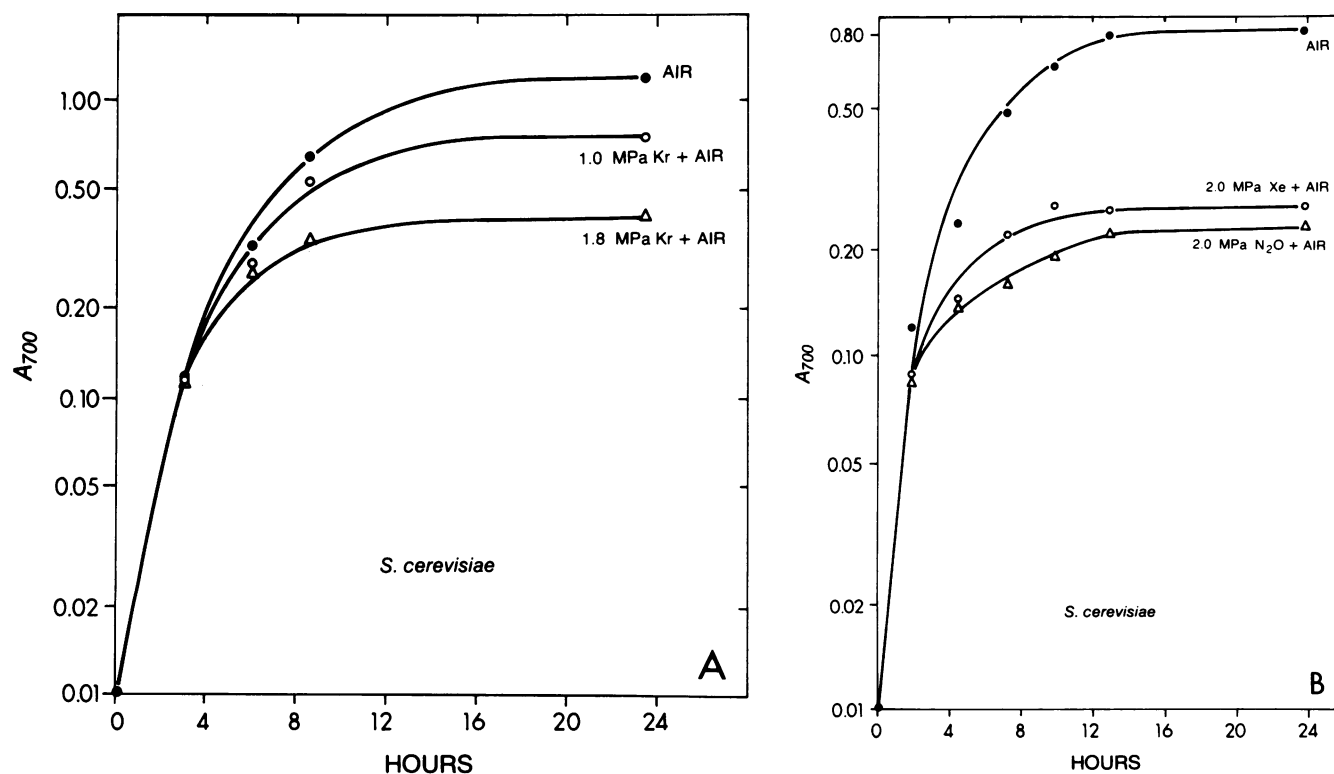


FIG. 3. Inhibition of growth of *S. cerevisiae* at 24°C by krypton (A) and xenon or nitrous oxide (B).

solubility of the gas at higher temperature. The ID_{50} at 18 or 37°C was approximately 0.28 mmol of N_2O per ml. Resistance was increased at 24°C with an ID_{50} of ca. 0.41 mmol/ml, and decreased at 42°C with an ID_{50} of 0.16 mmol/ml.

The data in the lower panel of Fig. 6 also show the enhancing effects of 2 MPa of He for N_2O inhibition. The enhancement appeared greatest at low temperatures, at which He was more water soluble.

DISCUSSION

The results given in this paper lead to a view of growth inhibition by hyperbaric gases significantly different from those proposed previously. Basically, it appears that the gases tested are best considered as members of two classes. Members of one class are outright growth inhibitors; members of the other class do not inhibit growth but can affect growth by antagonizing the inhibitory effects of hydrostatic pressure and by potentiating the actions of class 1 gases.

He, H_2 , and Ar were not growth inhibitory for any of our test organisms. Our results are at least partly at variance with results obtained previously. Buchheit et al. (3) assessed growth of *N. crassa* in terms of hyphal extension. The unpressurized control rate was 4.8 mm/h. Exposure to ca. 3 MPa of N_2 or He reduced this rate to ca. 3.5 mm/h, but higher gas pressures, up to ca. 12 MPa, had little or no additional inhibitory activity. Ar was potent and could stop growth almost completely at pressures of only 2 MPa. Kr and Xe were still more potent and stopped growth at pressures of only ca. 0.35 MPa. Fenn and Marquis (9) found that 4.1 MPa of He could reduce culture yields of *S. faecalis* up to 34%; 4.1 MPa of N_2 produced up to 22% reduction, and 4.1 MPa of Ar produced up to 42% reduction. However, 4.1 MPa of hydrostatic pressure produced up to 40% reduction, and so it seems that He, N_2 , and Ar had little net inhibitory effect on growth not due to hydrostatic pressure exerted by the gases. N_2O and Xe at pressures of 0.68 to 0.86 MPa completely stopped growth in this series of experiments. Subsequently, Fenn (8) reported that 4.8 MPa of N_2 or Ar was without effect on *Paramecium caudatum*, and as reviewed previously, Macdonald (13) found that compressed He or H_2 actually was less inhibitory for growth of *Tetrahymena* than was an equal hydrostatic pressure. Similar results to those for *Tetrahymena* were reported by MacNaughtan and Macdonald (14) for *A. laidlawii*. Taylor (20) found that even highly compressed He at 50 MPa was able to reduce the inhibitory effect of 50 MPa of hydrostatic pressure for growth of a marine pseudomonad. The studies of Bruemmer et al. (2) with HeLa cells in monolayer culture indicated little inhibitory effect of He, Ne, or N_2 except at pressures above ca 5 MPa. Ar caused a 25% reduction in cell count at a pressure ca. 3.5 MPa. Xe, N_2O , and Kr were potent growth inhibitors, and they sharply reduced growth at pressures less than 2 MPa.

Overall, these past findings together with our findings indicate clearly that He is not inhibitory for growth. It seems reasonable to conclude also that N_2 is not a growth inhibitor, except perhaps for *N. crassa*. N_2 inhibition of growth of the mold was only partial and may have been the result of experimental manipulation rather than due to the gas. There is clear variance in the results obtained with Ar. We find no inhibitory action against *E. coli*, *S. cerevisiae*, and *T. thermophila* at pressures up to nearly 6 MPa. In contrast, Ar does seem to inhibit growth of *N. crassa*, HeLa cells, and possibly *S. faecalis*, although we subsequently have not been able to demonstrate an inhibitory effect of Ar for *S.*

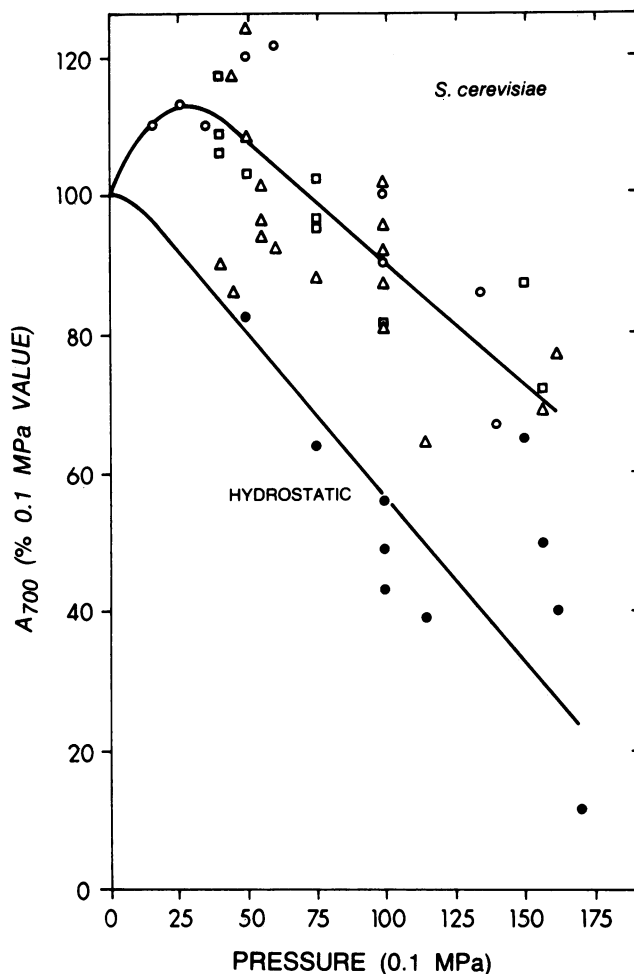


FIG. 4. Comparative effects of hydrostatic pressure (●) and helium (○), nitrogen (□), or argon (△) under pressure on growth of *S. cerevisiae* at 24°C.

faecalis. Our preliminary experiments with HeLa cells indicate that Ar can inhibit growth, but pressures greater than 4 MPa were required under conditions in which less than 1 MPa of N_2O completely stopped growth. He at pressures greater than 8 MPa also inhibited growth, but of course, at these high pressures the major effect is probably one of hydrostatic pressure rather than a specific gas effect (Technical Report no. 9, March 1981, Office of Naval Research). The conclusion that there are two classes of gases in regard to growth inhibition remains. Nitrogen narcosis or "the rapture of the deep" does not seem to have an analog in hyperbaric effects on growth. In fact, growth modification by hyperbaric gases appears not to be a narcotic effect. The membrane perturbations or other structural changes caused by gases that result in narcosis apparently do not necessarily also result in growth inhibition. However, inhibition of spore germination by compressed gases does appear to be more closely related to narcosis, at least in terms of a gas potency series. He is the only noneffective gas, even when used at pressures above 10 MPa. N_2 and Ar both were effective inhibitors at pressures well below 10 MPa, and a direct correlation between anesthetic doses of the gases and germination-inhibiting doses were found (6). We have repeated

TABLE 1. Potentiation by He, N₂, and Ar of the growth-inhibitory actions of N₂O and O₂

Organism	Gas pressure (MPa)					Avg % unpressurized control value		No. of experiments
	He	N ₂	Ar	O ₂	N ₂ O	Growth yield ^a	Growth rate ^b	
<i>E. coli</i>	3.0	3.0	3.0	0.2	1.0	91	73	7
					1.0	31	38	2
					1.0	28	34	5
					1.0	15	35	3
	1.0	1.0	1.0	0.2	0.2	94	79	6
					0.2	30	44	2
					0.2	46	48	2
					0.2	49	53	4
					0.5	84	96	4
					0.5	66	52	2
<i>S. cerevisiae</i>	3.0	3.0	3.0	0.03	0.5	60	53	4
					0.5	66	52	2
					0.5	60	53	4
					0.5	52	44	3
	0.8	0.8	0.8	0.03	0.03	100	100	3
					0.03	16	38	1
					0.03	26	43	4
					0.03	17	50	4
					0.5	68	90	3
					0.5	65	50	1
<i>T. thermophila</i>	1.5	1.5	1.5	0.05	0.5	8		1
					0.5	8		1
					0.5	8		5
					0.5	23	68	3
	1.5	1.5	1.5	0.05	0.05	15	52	2
					0.05	13	58	1
					0.05	13	58	1
					0.05	11		2

^a The average variance between replicate values was 6%, with a maximum of 8%.
^b The average variance between replicate values was 13%, with a maximum of 17%.

parts of the work of Enfors and Molin with spores of *Bacillus cereus terminalis* and of *Bacillus megaterium* ATCC 19213 and found that N₂ and Ar do indeed inhibit chemically induced germination.

The effects of hyperbaric gases on growth differ from narcotic or anesthetic effects in other respects. Both types of effects can be antagonized by hydrostatic pressure, and this antagonism forms one of the major bases for the critical volume hypothesis of narcosis. However, the mechanism of the antagonism is not clearly defined at present, even for narcosis. The antagonism is dramatic. Initially, Johnson and Flagler (12) showed that tadpoles anesthetized with ethanol could be made to reawaken and swim by compressing them to pressures of 15 to 30 MPa. We found (21) that 1.64 MPa of N₂O nearly stopped growth of *S. cerevisiae*, but compression of the arrested culture to 20 MPa allowed growth to

TABLE 2. Potentiation by He of the growth-inhibitory actions of Kr and Xe for *S. cerevisiae*

He	Gas pressure (MPa)		Growth yield: avg % unpressurized control value
	Kr	Xe	
6.0	0.4		105
	0.4		44
6.0	0.5		185
	0.9		26
	1.0		94
	1.0		60
6.0	0.9		10
		0.6	108
6.0		0.6	28

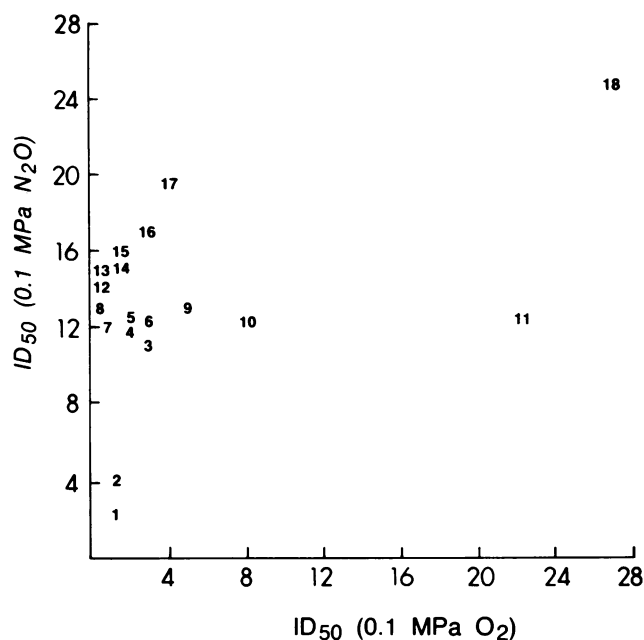


FIG. 5. Comparative survey of the sensitivities of various microorganisms to oxygen and to N₂O. Numbers refer to the following organisms: 1, *T. thermophila*; 2, *Rothia dentocariosa*; 3, *S. cerevisiae*; 4, *Lactobacillus plantarum*; 5, *Bacillus licheniformis*; 6, *B. megaterium* KM; 7, *Streptococcus mutans* LM-7; 8, *Streptococcus sanguis*; 9, *E. coli*; 10, *Serratia marcescens*; 11, *S. faecalis* 10Cl, 12, *Streptococcus mutans* GS-5; 13, *Lactobacillus casei*; 14, *Micrococcus* sp.; 15, *S. mutans* SL-1; 16, *Bacillus subtilis* BR151; 17, *Staphylococcus aureus* H; 18, *S. faecalis* 9790.

proceed at nearly control rates. Alone, a hydrostatic pressure of 20 MPa completely stopped growth.

The potentiation of the inhibitory effects of class 2 gases on growth by class 1 gases also was dramatic. This potentiation applies also to oxygen. Again, the mechanism is unknown, although it is possible that He, N₂, and Ar act to expose sensitive target sites to N₂O, and O₂. The mechanism seems not to involve stabilization of free radicals since it applies also to growth inhibition by Kr and Xe, which presumably cannot be converted to radical form in biological systems.

The finding of an optimal temperature for resistance of *S. cerevisiae* growth to N₂O is at variance with previous findings for narcotic effects (4, 10), which generally show simple decreases in resistance (increases in potencies of the narcotics) with decreasing temperature. However, none of the previous studies has involved gaseous anesthetics, although the study of Flook et al (10) did focus on changes in light emission by the microbe *Photobacterium phosphoreum*. Indeed, the technical problems of working with compressed gases have limited experimentation and made it difficult to carry out large numbers of experiments in any one study. However, it seems that the growth-modifying effects of hyperbaric gases are of considerable fundamental and practical importance. The agents can be removed readily from biological systems simply by decompression, without the need to add neutralizing substances. Moreover, there is a fascination in considering mechanisms of action for agents such as He, Ar, Kr, and Xe which cannot be involved in formation of covalent bonds. Generally, it is considered that the major sites of action for these gases are in the hydrophobic regions of the cell membrane. The gases are highly hydrophobic and could dissolve in and distort these regions. However, they could also act by upsetting hydrophobic

TABLE 3. Comparison of the effects of helium pressure and hydrostatic pressure on sensitivities to oxygen

Organism	Oxygen concn (μg/ml) ^a	Helium pressure (MPa)	Additional hydrostatic pressure (MPa)	$A_{700}^{\max b}$	
				A_{700}^{\max}	% of 1-atm (0.1-MPa) control
<i>E. coli</i>	8	8	0	0.644	100
	8	2	0	0.647	100
	8	0	20	0.638	99
	48	0	0	0.240	37
	48	2	0	0.030	5
	48	0	20	0.268	42
	8	0	40	0.224	34
<i>S. cerevisiae</i>	48	0	40	0.245	38
	8	0	0	0.404	100
	8	4	0	0.408	101
	48	0	0	0.354	88
	48	2	0	0.140	35
	48	4	0	0.030	7
	8	0	5	0.258	64
	8	0	10	0.226	56
	48	0	5	0.250	62
48	0	10	0.259	64	

^a It was assumed that the culture medium equilibrated with air at 24°C contained 8 μg of oxygen per ml and that the concentration of oxygen increased in direct response to increased amounts of the gas added to the compressed system, as indicated by Taylor (19).

^b A_{700}^{\max} refers to the maximum absorbance of the culture assessed with light of 700 nm wavelength.

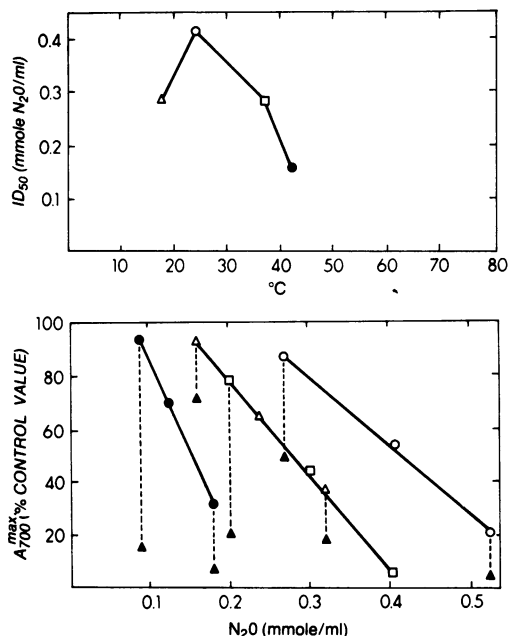


FIG. 6. Temperature profile for growth inhibition of *S. cerevisiae* by nitrous oxide. The symbols indicate data obtained for growth at 18°C (Δ), 24°C (○), 37°C (□), or 42°C (●). The dashed lines indicate data obtained when 2 MPa of helium was included with the N₂O. Aqueous concentrations of N₂O at various N₂O pressures and temperatures were calculated by use of the Ostwald coefficients given by Wilhelm et al (22). Doses of N₂O required for 50% reduction in growth yield (ID₅₀) were estimated from the results shown in the lower panel.

interactions in proteins. What clearly is needed now is study of the biochemistry of gas effects. Possibly, these studies will be spurred by the growing interest in the microbiology of deep oil reservoirs where microbes are exposed to hyperbaric hydrocarbon gases, especially methane. Moreover, it seems that in view of recent developments in hyperbaric medicine and physiology, extensive work on the growth-modifying actions of compressed gases on animal cells is needed.

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