

Growth Responses of *Neurospora crassa* to Increased Partial Pressures of the Noble Gases and Nitrogen

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ABSTRACT

BUCHHEIT, R. G. (Union Carbide Corp., Tonawanda, N.Y.), H. R. SCHREINER, AND G. F. DOEBBLER. Growth responses of *Neurospora crassa* to increased partial pressures of the noble gases and nitrogen. *J. Bacteriol.* 91:622-627. 1966.—Growth rate of the fungus *Neurospora crassa* depends in part on the nature of metabolically "inert gas" present in its environment. At high partial pressures, the noble gas elements (helium, neon, argon, krypton, and xenon) inhibit growth in the order: Xe > Kr > Ar >> Ne >> He. Nitrogen (N₂) closely resembles He in inhibitory effectiveness. Partial pressures required for 50% inhibition of growth were: Xe (0.8 atm), Kr (1.6 atm), Ar (3.8 atm), Ne (35 atm), and He (~300 atm). With respect to inhibition of growth, the noble gases and N₂ differ qualitatively and quantitatively from the order of effectiveness found with other biological effects, i.e., narcosis, inhibition of insect development, depression of O₂-dependent radiation sensitivity, and effects on tissue-slice glycolysis and respiration. Partial pressures giving 50% inhibition of *N. crassa* growth parallel various physical properties (i.e., solubilities, solubility ratios, etc.) of the noble gases. Linear correlation of 50% inhibition pressures to the polarizability and of the logarithm of pressure to the first and second ionization potentials suggests the involvement of weak intermolecular interactions or charge-transfer in the biological activity of the noble gases.

The noble gases, helium, neon, argon, krypton, xenon, and radon, although apparently nonessential to life, do exhibit physiological activities when applied to living systems in sufficient concentration. The stable isotopes of the noble gases act upon intact organisms, cellular systems, or isolated tissues, producing narcosis (8, 10, 14, 15), decreased responses to stimuli (1, 2, 12), alteration of metabolism (4, 24), decreased oxygen-dependent sensitivity to radiation (6), and altered rate of development (4, 11). Except in the case of metabolic effects, activity parallels the periodic classification of the elements, i.e., varies in the order of atomic number and molecular weight (21).

Using the filamentous fungus, *Neurospora crassa*, Schreiner et al. (23) observed a dependence of linear growth rate on the nature of the diluent noble gas in environments containing 0.95 atm of "inert gas" and 0.05 atm of oxygen. In the present study, we have extended these observations to include partial pressures of the noble gases and nitrogen from 0.8 to 120 atm. In terms of increasing partial pressures required for 50% inhibition of growth, the order of effectiveness of

the gases was:

Xe > Kr > Ar >> Ne >> He > N₂

MATERIALS AND METHODS

N. crassa NTCC 5297a was used as the test organism. Stock and experimental cultures were grown on the medium described by Nicholas et al. (19) containing, in addition, biotin (6 μg/ml) and agar to a concentration of 3%. Growth rates were measured by observing the linear progression of the mycelial frontier on the surface of 15 ml of the above solid medium contained in modified Tatum tubes (cylindrical glass tubes 36 cm long and 1.3 cm in outside diameter). Techniques and growth tubes were essentially those described by Ryan et al. (22).

Cultures were grown in air (1 atm) for 50 to 55 hr at 30.0 ± 0.5 C after inoculation by a dense suspension of spores in liquid medium applied as a droplet at one end of each tube. Spore germination had occurred, and linear growth was taking place at a constant rate of 4 to 5 mm/hr after the above air incubation period. Tubes were placed in glass cylinders or a special steel pressure cylinder of 900-ml volumes and were further incubated at 30 C for 17 hr in various gaseous environments contained at known pressures within the cylin-

ders. Water-soaked sponges provided moisture sufficient to saturate the atmosphere.

For experiments at 1 atm, the glass cylinders were flushed for 10 min with mixtures of inert gases and oxygen (80:20, v/v) and were sealed with rubber stoppers.

For pressures above 1 atm, use was made of a stainless-steel pressure cylinder capable of confining a gas at pressures up to 120 atm. The cylinder design is shown in Fig. 1. The assembly was completed by an internal thermocouple for monitoring temperature during compression and decompression and by interchangeable gauges recording pressure to 300 psi in 2-psi increments, and to 3,000 psi in 20-psi increments. Both were calibrated to 0.25% (i.e., 0.05 and 0.5 atm, respectively). Compression was done directly from compressed-gas cylinders of dry high-purity xenon, krypton, argon, neon, helium, or nitrogen (Union Carbide Corp., Linde Div., Tonawanda, N.Y.). Partial pressures of gases above 0.8 atm were superimposed upon 1 atm of air.

Variations of initial oxygen and carbon dioxide partial pressures were attained by suitable mixing of pure argon or xenon with argon-oxygen, argon-carbon dioxide, or xenon-oxygen.

Final oxygen and carbon dioxide partial pressures were measured in representative experiments by gas chromatography.

RESULTS

Growth rates at low total pressure. *N. crassa* cultures which had grown for 50 to 55 hr in air were allowed to grow for 17 hr in glass cylinders containing environments of oxygen (20% by volume) plus "inert" gases (80% by volume). Initial compositions of each gaseous environment were: $P_{\text{inert gas}} = 0.77$ atm, $P_{\text{O}_2} = 0.19$ atm, and $p_{\text{H}_2\text{O}} = 0.04$ atm. Helium, neon, argon, krypton, xenon, and nitrogen (N_2) were studied in two to five experiments each. Linear growth

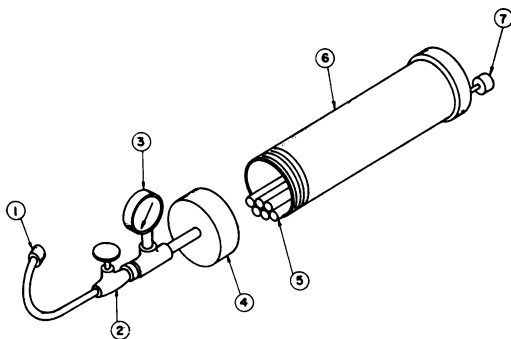


FIG. 1. High pressure culture vessel assembly. Parts include: (1) Nipple for attachment to compressed gas cylinder, (2) needle valve, (3) pressure gauge, (4) stainless-steel threaded 2-inch pipe cap, (5) culture tubes, (6) stainless-steel threaded 2 × 16-inch pipe, and (7) thermocouple (Aminco).

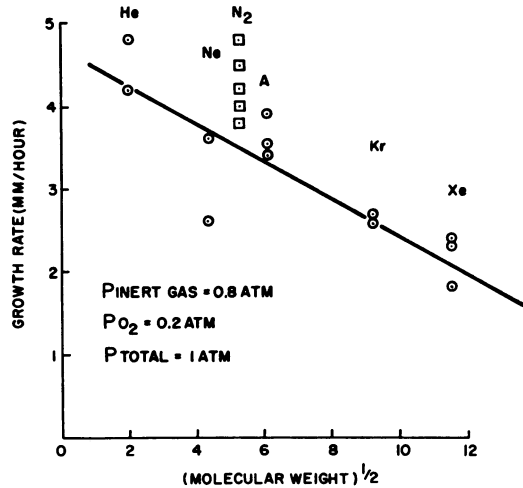


FIG. 2. Relation between growth rate of *Neurospora crassa* and molecular weight of inert gas present at 1 atm.

rates were measured on six tubes in each experiment, the averages of which are shown in Fig. 2 against an abscissa of molecular weight to the half-power. This gives an approximate linear relationship, as described previously (23). A linear regression curve was computed from the data and is shown in Fig. 2. Within each experiment, growth rates among the six tubes varied as expressed by ± 1 SD by 0.05 to 0.3 mm/hr.

From the regression curve, the theoretical growth rate (i.e., for a dilute inert gas of molecular weight zero) was read as 4.72 mm/hr. This value was used in computing 50% inhibition of growth levels in experiments performed at pressures above 1 atm. At 1 atm, growth rates for N_2 consistently were above those expected for a noble gas of equivalent molecular weight. Radon, which cannot be studied because of radiobiological phenomena, would give theoretically a growth rate two-thirds that of Xe. For total inhibition, a molecular weight of over 400 should be required.

Growth rates at high pressures. Environments of nitrogen, helium, neon, and argon at partial pressures of 3 to 120 atm were superimposed on 1 atm of air. Initially, the environments contained partial pressures of oxygen, 0.19 atm; nitrogen, 0.77 atm; and water, 0.04 atm. After 17 hr of growth, the P_{O_2} varied between 0.07 and 0.14 atm, and p_{CO_2} varied between 0.08 and 0.16 atm. Separate experiments are reported below which demonstrate that growth rates were not sensitive to oxygen or carbon dioxide partial pressure changes over these ranges.

Growth rates obtained in the presence of increasing partial pressures of N_2 , He, Ne, and Ar

are shown in Fig. 3. Complete suppression of growth was obtained with argon at a partial pressure of about 15 atm. Complete suppression of growth was not observed with N₂, He, or Ne at any pressure up to 120 atm, the working limit of the pressure vessel used. Nitrogen was a less effective growth inhibitor than any of the noble gases. At 30 and 60 atm, differences between N₂ and He were not statistically significant ($P > 0.05$). At 120 atm, N₂ produced significantly less inhibition than He ($P < 0.005$); inhibition averaged 28% for N₂ and 38% for He.

Fifty per cent inhibition of growth (relative to the theoretical 100% growth rate) occurred at partial pressures of 300 (extrapolated value), 35, and 3.8 atm for He, Ne, and Ar, respectively.

Growth rates at intermediate pressures. Growth rates of *N. crassa* were measured in argon, krypton, and xenon environments of 1 and 3 atm partial pressures superimposed on 1 atm of air. Essentially, complete inhibition of growth occurred at 3 atm in Kr or Xe, and marked inhibition occurred in Ar. Results are shown in Fig. 4. Fifty per cent inhibition of growth occurred at partial pressures of 1 atm (approximately 0.8 atm) for Xe and 1.6 atm for Kr. Reversibility of inhibition was examined for the 3-atm Xe system. Complete reversibility was found. Pre-exposure growth rates were obtained in all cases upon removal of cultures from Xe and further incubation in air at 1 atm.

Effects of variation in initial oxygen and carbon dioxide partial pressures. Growth rates were measured as a function of initially imposed oxygen partial pressures between 0.2 and 1.0 atm in environments of xenon at 1.0 atm and argon at 10 atm. Results are shown in Fig. 5. No reversibility of Xe or Ar inhibition occurred as oxygen

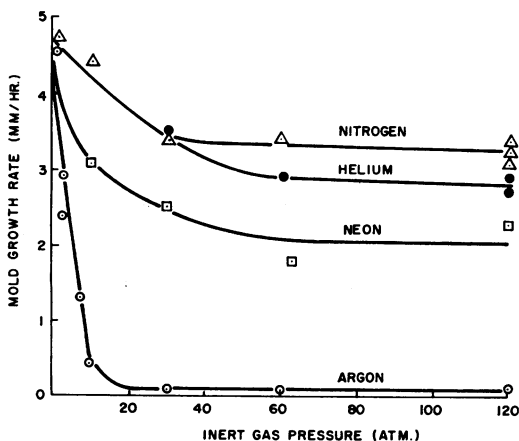


FIG. 3. Relation between inert gas partial pressure and growth rate of *Neurospora crassa*.

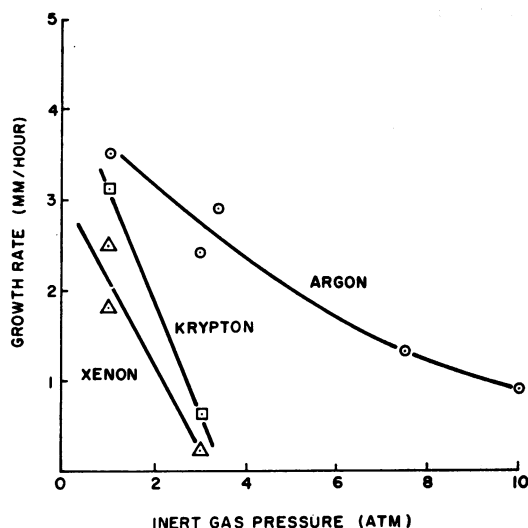


FIG. 4. Relation between xenon, krypton, and argon partial pressures and growth rate of *Neurospora crassa*.

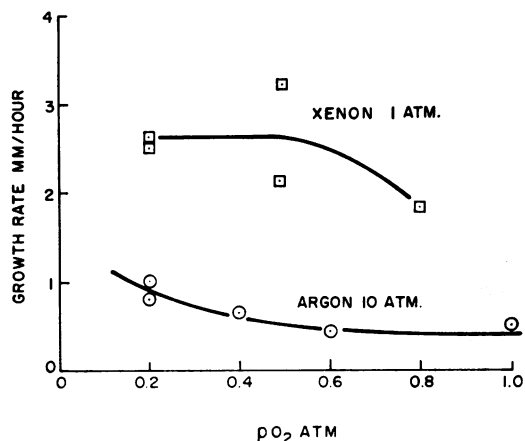


FIG. 5. Effect of oxygen partial pressure on inhibition of *Neurospora crassa* growth rate by xenon or argon.

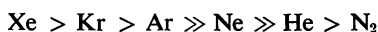
partial pressure was increased. Decreased growth rates at the higher O₂ pressures may reflect onset of oxygen toxicity effects. Fenn (8) reported synergism by argon in toxic effects of high O₂ pressures on *Drosophila*.

As described above, O₂ partial pressure decreases and CO₂ partial pressure increases significantly in our closed system during partially inhibited growth over the experimental period of time as a result of cellular respiration. It was considered necessary to examine experimentally the possibility that observed growth rate effects were influenced by the altered O₂ and CO₂ levels prevailing during our noble gas experiments. In the

presence of a fixed partial pressure of argon (3.4 atm), CO₂ partial pressure was held very low or increased to relatively high levels in the presence of normal (0.2 atm) or decreased partial pressures of O₂. Results shown in Table 1 indicate no significant influence on growth rate inhibition by argon caused by the altered CO₂ and O₂ levels.

DISCUSSION

At low partial pressures (<1 atm), *N. crassa* exhibits a growth rate dependent in part on the nature of "inert gas" present in its environment. Growth rate is a linear function of the half-power of molecular weight, as observed previously in low-oxygen systems (23). At high partial pressures, the noble gas elements suppress markedly or totally cellular growth. The order of biological effectiveness of the gases studied was:



Nitrogen could not be distinguished from He except at 120 atm. Partial pressures required for equivalent biological response (i.e., 50% inhibition of growth) were: Xe (0.8 atm), Kr (1.6 atm), Ar (3.8 atm), Ne (35 atm), and He (~300 atm). These stand in the approximate ratios Xe-Kr-Ar-Ne-He of 1:2:4:40:400.

With respect to inhibition of growth, the noble gases and N₂ differ qualitatively and quantitatively from the order of effectiveness found with other biological effects. In producing narcosis (17, 18) and inhibiting development of certain insects (11), N₂ resembles Ar, and both are more potent than Ne or He. In depressing glycolysis and increasing O₂ uptake by tissue slices (24), the order was: He = Xe > Ar > N₂. Inert gas depression of O₂-dependent radiosensitivity has been reported (6) with the order of effectiveness being: Xe > Kr = Ar >> N₂ >> He.

Interpretations of the biological action of the noble gases and other nonpolar gases have considered (i) a lipid phase as the site of action with correlation between activity and lipid solubility

TABLE 1. *Effects of variation in initial partial pressures of O₂ and CO₂ on growth rates of Neurospora crassa obtained in agron at 3.4 atm*

Partial pressures (initial)			Growth rate
Ar	CO ₂	O ₂	
atm	atm	atm	mm/hr
3.4	0.0004	0.20	2.9±0.16
3.4	0.10	0.18	2.7±0.14
3.4	0.0002	0.10	2.7±0.08
3.4	0.05	0.09	2.6±0.30

or oil-water partition coefficients (16, 17); (ii) an aqueous site of action with correlation between activity and dissociation pressures of gas hydrates (18, 20); (iii) association of inert gases with proteins (7); (iv) competition between inert gas and O₂ for intracellular binding sites (6, 23); or (v) nonspecific attainment of some critical chemical potential of the inert gas solute (9).

Solubilities and solubility ratios for the noble gases parallel, but are not directly proportional to, their biological effectiveness (Fig. 6 and 7). Hydrates appear only to be formed by Ar and heavier molecular weight gases (3). Over a five-fold range in partial pressure of O₂, we observed no competition between noble gas and O₂ (Fig. 5). At partial pressures for 50% inhibition of growth, respective aqueous concentrations of the series Xe to He are shown in Table 2. Corresponding concentrations would be approximately 10 times greater for each gas at equilibrium in

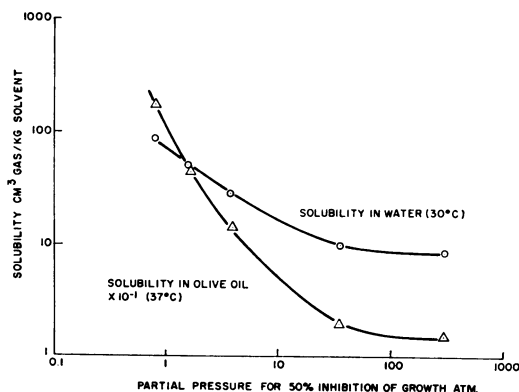


FIG. 6. *Solubilities in water and olive oil of noble gases and partial pressures for 50% inhibition of growth of Neurospora crassa.*

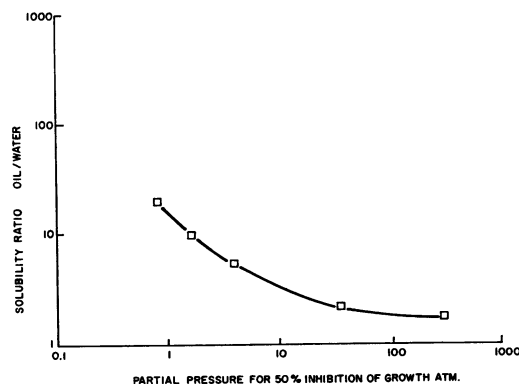


FIG. 7. *Solubility ratios (oil-water) of noble gases and partial pressures for 50% inhibition of growth of Neurospora crassa.*

TABLE 2. Concentrations and calculated volumes and surface areas occupied by the noble gases in aqueous solution at partial pressures for 50% inhibition of growth

Gas	Concn in water for 50% inhibition (moles/liter $\times 10^3$)	Van der Waals "b" (cm ³ /mole)	Molecular diameter (A)	Calculated	
				Volume (A ³ $\times 10^{-22}$)	Area (A ² $\times 10^{-20}$)
Xe	3.07	51.0	4.9	157	332
Kr	3.53	39.8	4.2	140	293
Ar	4.86	32.3	3.7	156	297
Ne	15.3	17.1	2.6	262	460
He	113	23.7	2.2	2,678	2,584

typical lipids. This suggests at once that molar concentrations of the noble gases are not directly related to biological effectiveness in *N. crassa*. In Table 2 we have calculated also approximate space-filling parameters for aqueous solutions at partial pressures for 50% inhibition of growth. Xe, Kr, and Ar are equivalent in volume occupied or area covered if all molecules were adsorbed at an interface. Markedly higher values are found for He and Ne.

The intracellular concentration and distribution of a noble gas would be determined by its lipo-hydrophilic character and partition coefficient. Within a cell, the dissolved gas could exert a "biological effect" through involvement in a critical physical or biochemical process in a local region as proposed by Hansch and Fujita (13) for other pharmacologically active agents.

In addition to nonspecific occupation of space and interference with some process of transport or partially specific adsorption competition at an interface with some essential cellular constituent, specific weak intermolecular interactions (i.e., dipole-induced dipole or transient dipole-induced dipole interactions) or charge-transfer complexes might well be involved.

Stability of the products of intermolecular interactions would be proportional to polarizability (α) of the noble gas atoms, and indeed an approximate linear relation exists between α and partial pressures for 50% inhibition (Fig. 8). Ionization potentials would in part determine the likelihood of formation and stability of noble gas associations with other molecules, including charge-transfer complexes. Approximate linear relations exist between first and second ionization potentials and the logarithms of partial pressures for 50% inhibition (Fig. 9). The noble gas elements may, therefore, derive their biological activities from relative capacities to form intermolecular associations with essential constituents

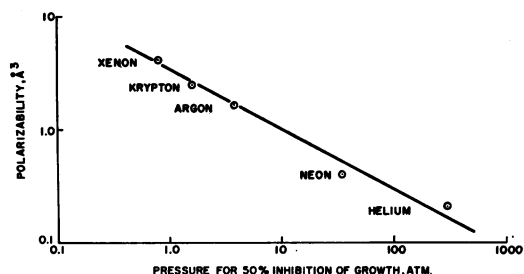


FIG. 8. Polarizabilities of noble gases and partial pressures for 50% inhibition of growth of *Neurospora crassa*.

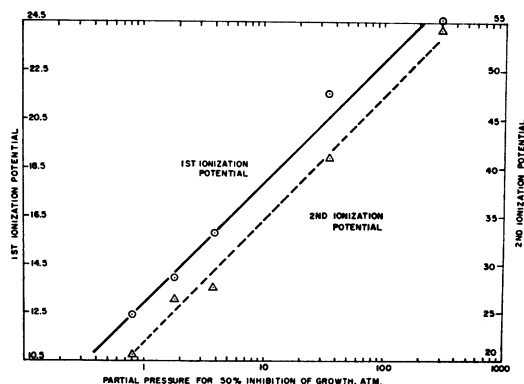


FIG. 9. Ionization potentials of noble gases and partial pressures for 50% inhibition of growth of *Neurospora crassa*.

of biological systems and may provide useful means of probing the biochemical significance and mechanisms of such weak interactions.

ACKNOWLEDGMENT

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