

is dependent upon the presence of ubiquitous surfactants. This can be shown by aspirating all existing surface film from a system in which countercurrent streaming is occurring; the circulations cease and are not re-established until the clean surface has aged for some time or surfactants are introduced.

Several observations indicate that surface film is subject to a surface pressure originating downstream: (i) separation of flow is maintained at slow speeds over long distances; (ii) countercurrent streaming does not begin until the stream flows into a static body of water; (iii) the entire surface film of a loop travels counter to the flow in the interior; and (iv) when relatively rapid flow from the nozzle is cut off (or slowed), the film circulating at some distance downstream (Fig. 1, *e*) spreads to the upstream limits like an expanding monolayer.

A film pressure develops because of inequalities in surface tension in different regions of the system. Interfacial tension is high at the freshly formed surface near the nozzle but becomes progressively lower as surfactants accumulate during the downstream flow. As the surface tension decreases, the film pressure increases, providing motive force for the backflow. Experimental manipulations that destroy the gradient also stop the vortical circulations.

Resemblances between countercurrent streaming and protoplasmic movements suggest that the same forces operate in both. Since the cell is essentially a bag of interfaces, in which new surfaces are often produced at a great rate, these forces could play a major role. Continuous production of interfaces as a result of protoplasmic syntheses probably leads to sustained gradients of interfacial tension at many points within the cell. Acting in consort, these gradients could give rise to the observed multifarious streaming movements. Local changes in interfacial tension also might account for such phenomena as the gyrating contortions of mitochondria which are, so to speak, "struggling to put forth new interfaces." Since on this basis energy for movement would be an additional debt to be met by energy of formation of membranes (taking into account both synthesis and structural ordering), correlation of extent of protoplasmic movements with amount of synthetic activity suggests itself.

Energy of formation of membranes might be transformed into energy of movement by at least three processes: (i) movement of existing films of low interfacial tension (at old interfaces) along gradients of interfacial tension at new interfaces; (ii) gross hydrodynamic flow of protoplasm into spaces between newly separating membranes (possibly responsible for nuclear rotation); and (iii) osmotic movement of water out of channels between membranes as a result of gradients set up by attraction of water molecules away from new interfaces. The metabolites of smallest intrinsic free surface energy, which accumulate concomitantly by positive adsorption, probably provide building blocks for further membrane formation. Thus one consequence of membrane formation might be the assemblage of the next generation of building blocks.

Counter movement of surface film caused by film pressure gradients also could explain the otherwise enigmatic phenomenon of filament streaming (4) in the long slender reticulopodia of *Allogromia*; on this basis filament streaming is interpreted as follows. When a reticulopodium is being extended, a filament of protoplasm flows outward but its surface film (and adhering particulate matter) flows inward to the cell body. When it is being withdrawn, the central protoplasm flows inward but the surface film flows outward. Surface film reaching the outer tip of the filament converges into the interior, carrying particulate matter inward to the cell body. In this way, feeding could be carried out both in the processes of extending and withdrawing reticulopodia—the animal simply "moves its mouths." The complex movements observed in networks of anastomosing reticulopodia are understandable on the same basis. They can be simulated in models by forming a network of peripheral loops.

A remarkable demonstration of countercurrent streaming can be carried out in the domestic closet bowl. When water begins to rise after a flushing, pencil sharpener filings are deposited on its surface. Many centers of countercurrent streaming are set up around the surface of the bowl (5).

*Note added in proof.* It now has been shown that the relatively high dynamic surface tension of the stream is primarily a consequence of its motion. The phenomenon is particularly pronounced in aqueous solutions of surfactants flowing in thin sheets. The entire

surface film actually spreads upstream; the apparent failure to do so in the center is an illusion, based upon transverse velocity gradients in the stream. The surface tension of dynamic fluids has been treated theoretically by Stuke [*Z. Electrochem.* **63**, 140 (1959)].

Forces at nonequilibrium interfaces also can give rise to vigorous longlasting interior circulations in fixed fluid bodies. Some papers describing the possible significance of these processes for protoplasmic contractility and transport of cytoplasmic matrix are in preparation.

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#### References and Notes

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5. This work was supported by grant G-14533 from the National Science Foundation.

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### New Biological Effect of the Gases of the Helium Group

*Abstract.* The mold *Neurospora crassa* ATCC 5297a was grown in gaseous environments of helium, neon, argon, krypton, xenon, or nitrogen containing approximately 5 percent oxygen. A close correlation of the growth rate  $R$  (in millimeters per hour at 30°C) with the molecular weight  $MW$  of the chemically inert gas was observed. This correlation is described by the empirical equation:  $R = 3.88 - 0.1785(MW)^{1/2}$ .

Although ordinarily they do not enter chemical reactions, the gases of the helium group, if present in sufficiently high concentration, are capable of eliciting physiological responses in a number of higher biological systems (1). Very little information, however, is available concerning the responses of simple life forms to the presence of noble gases. In order to make a quantitative assessment of the environmental influence of these gases on a well-characterized biological model system, an exploratory study was carried out with the fungus *Neurospora crassa*.

A helium-tight incubator capable of confining an experimental gas mixture for a minimum of 2 weeks without significant loss was designed and con-

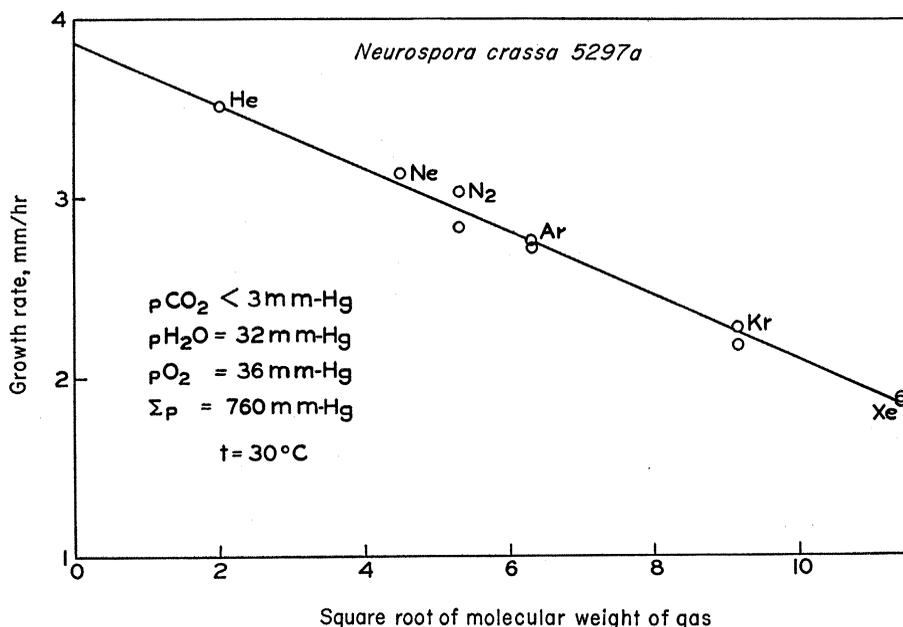


Fig. 1. Growth response of *Neurospora crassa* 5297a in the presence of gases of the helium group. The computed standard deviation of regression is  $\pm 0.0651$  mm/hr. Differences in growth rates are due to the nature of the noble gas present ( $p < .01$ ).

structured for this investigation (2). A 1600-cm<sup>3</sup> volume of the test gas was sealed in the controlled-atmosphere incubator and continuously circulated at a rate of approximately 100 cm<sup>3</sup>/min. By passing this gas stream through 1.0N aqueous K<sub>2</sub>CO<sub>3</sub> partial pressure of CO<sub>2</sub> was maintained below 3 mm-Hg. Carbon dioxide removed in this manner was replaced automatically with stoichiometric amounts of oxygen by a pressure-sensitive servomechanism. The temperature of the incubator was maintained at  $30 \pm 0.5^\circ\text{C}$ , total system pressure at  $760 \pm 2$  mm-Hg, and the relative humidity at 100 percent.

The growth rate of *N. crassa* was measured by the tube method of Ryan *et al.* (3). The organism was allowed to grow against the direction of gas flow across the surface of 15 ml of solidified mineral medium (4) containing sucrose and NaNO<sub>3</sub> as sources of carbon and nitrogen, respectively. For each growth experiment of 100 hours' duration a specimen section of eight cotton-plugged glass tubes measuring 400 by 13 mm was connected in series to complete the gas circulation system of the incubator. Growth rates were determined by recording the position of the self-regulating mycelial frontier at time intervals. For reasons not well understood, growth in the two end tubes of the specimen section was erratic in some experiments. In order to minimize the systematic error of the observations

made, readings of all end tubes were discarded. An oxygen level of approximately 5 percent was chosen because it supported adequate growth and development of the test organism. This made it possible to introduce the environmental gases to be tested at partial pressures close to 1 atmosphere.

The nature of the gaseous environment has a significant effect on the rate of linear growth of *N. crassa*. This is shown in Fig. 1.

The growth rate of the mold is linearly related to the square root of the molecular weight or, with somewhat poorer fit, to the molecular diameters and volumes of the gases studied. Only a partial correlation of the growth response of *N. crassa* to the Van der Waals constants of these gases or to their viscosities was obtained. The fungal growth rate increases nonlinearly with increasing thermal conductivity and decreases nonlinearly with increasing molecular weight, density, magnetic susceptibility, solubility in water and lipid, solubility ratio of lipid: water, refractive index, polarizability, and relative dielectric constant of the major constituent of the sealed system; except that nitrogen does not follow the trend of the last four parameters.

Sporulation of the mold was noticeably diminished in the presence of krypton and completely inhibited by xenon. Linear growth became established within 7 to 16 hours after inocula-

tion with an aqueous suspension of spores. Statistical treatment of these lag phases revealed no significant trends attributable to the nature of the gaseous environment. The rate of oxygen uptake paralleled the rate of linear growth in all experiments.

The biological manifestations of chemically unreactive gases are necessarily physical in origin. A number of correlations exist between physical properties of these gases and the magnitude of the biological effect they produce (5). The evidence obtained with *Neurospora crassa* indicates that some physical property which, for the helium group gases, varies in the same way as their molecular weights is involved in determining biological activity. The mechanism of this activity is by no means clear at this time. The term  $0.1785 (MW)^{\frac{1}{2}}$  of the empirical equation given in the abstract suggests the possibility that diffusion characteristics of the gases studied may be of importance in producing the effects noted. It is equally possible that these gases affect fungal growth by altering the physical properties of specific subcellular structures for which they show a high degree of affinity, and thus interfere with their normal functions.

The growth responses of *N. crassa* appear to provide a valuable parameter through which the mechanism of biological phenomena produced by chemically unreactive gases may be explored further.

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