Table 2. Two series of measurements of copper contents of ashed substrates.

Sample	Copper content, ash (μg/mg)	
	Series I	Series II
1	0.093	0.28
2	.073	.20
3	.088	.16
4	.010	.20
5	.074	.19
6		.30
$\bar{x}$	.086	.22

copper holdings only in a rather precarious manner, since the original source of the metal (the leaf litter) contains only about 25 percent of the concentration required to merely compensate the loss of copper by digestion.

Realization that the copper content of a well-digested fecal substrate should approach the steady-state of copper assimilation enables one to determine whether the assumption holds that assimilation of fecal copper follows the same rules as assimilation of leaf-litter copper. This I did by analyzing the copper content of substrates in culture dishes in which large populations of *P. scaber* had been fed for years entirely on carrots and poplar-leaf litter; the substrates consisted almost exclusively of accumulated droppings. I made two series of measurements from various dishes and at different times of year (Table 2).

The values of Table 2 represent copper levels lying between the concentration in the feces originally produced by *P. scaber* (after feeding on primary vegetable matter) and some unknown steady-state level. Since according to Fig. 1 the feces produced by *P. scaber*, after feeding on leaves containing copper at about 0.24 μg per milligram of ash, would contain approximately 0.7 μg Cu per milligram of ash, the expected steady state could be as low as or lower than 0.086 μg Cu per milligram of ash, the lower of the mean values in Table 2. But if one allows for individual or seasonal differences, a steady-state level of 0.15 μg is assumed to hold for assimilation from fecal material.

Thus, by reverting to coprophagy, *P. scaber* should be able to solve its problem of copper retrieval. A copper content in the new food of 0.015 percent—well below that of the original source of copper—would suffice to maintain its body concentration of copper, whereas under the conditions holding for digestion of leaf litter, the animals would have lost copper heavily at this concentration. An explanation of this new relation is afforded by the activity of microorganisms, which most likely convert so much of the copper bound to proteins into less-tightly bound forms that a larger percentage of total copper can be assimilated by the isopods. Copper in living material can exist in both easily dissociable and tightly bound states (*7*).

Figure 2 summarizes the new concept of feeding activity by *P. scaber*. It is assumed that a population of isopods starts feeding on leaf litter, from which copper can be extracted only if the copper concentration is around 1 μg per milligram of ash (Fig. 2: regression line *I*). Starting with poplar litter containing 0.25 μg (Fig. 2: *1*), the animals would lose copper heavily and the first batch of feces would contain ~ 0.7 μg Cu per milligram of ash. After the onset of microbial activity the isopods would switch to coprophagy, for which the steady-state level is assumed to be ~ 0.15 μg Cu per milligram of ash (Fig. 2: regression line *II*). Thus the starting value of feces copper (Fig. 2: *2*) would allow very high retention of this element under the new conditions. Within three more cyclings of the feces (Fig. 2: *3* to *5*) the copper level in the fecal substrate would approach the steady-state level. By alternating between consumption of primary vegetable matter and coprophagy, *P. scaber* could fill its demand for copper and perhaps for other essential food components.

This interpretation has two corollaries: isopods probably consume less primary vegetable matter and are more implicated in secondary breakdown of organic material than has been assumed.

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Nitrogen- and Helium-Induced Anoxia: Different Lethal Effects on Rye Seeds

Abstract. *Prolonged exposures to acute anoxia caused progressive reductions in the viability of hydrated seeds of Prolific rye. For equal exposures of 9 days or longer, mortality was significantly higher in helium than in nitrogen. The findings suggest that prolonged use of helium as a component of atmospheres in manned space capsules may be harmful.*

Atmospheres within manned space capsules launched by the United States have so far consisted of 100-percent oxygen at 0.34 atm. There is general agreement among space scientists that atmospheres consisting of gas mixtures are desirable, possibly essential, for prolonged missions in space. Difference of opinion exists regarding choice of a second gas—should helium replace nitrogen. Saving in weight, higher thermal conductivity, and reduced probability of embolism have been cited as advantages of helium. The opposite view is that biologic effects of helium are insufficiently understood and that its unknown physiological hazards may exceed its putative advantages (*1*).

Despite their chemically unreactive nature, noble gases can produce various responses in biologic systems (*2*). Although their mechanisms of action are unknown, their effects must reflect their physical properties. Magnitudes of response can be correlated with their molecular weights whether they are used to dilute oxygen (*3*) or as pure anoxic environments (*4*). Unfortunately, current knowledge of the biologic effects of rare gases cannot resolve the arguments concerning the relative merits of nitrogen and helium as components of gas mixtures.

Recent findings regarding effects of these gases on germinating seeds seem especially pertinent. Seeds of Prolific spring rye were hydrated anaerobically and held in anoxia for up to 15 days. Dry, resting seeds (50 per dish) were placed in petri dishes lined with moist filter paper. Samples, 100 to 200 seeds per treatment, were promptly sealed in helium-tight chambers of welded Lucite which were quickly evacuated to −76 cm-Hg and then filled and flushed for several minutes with nitrogen or helium. The chambers were thereafter kept in

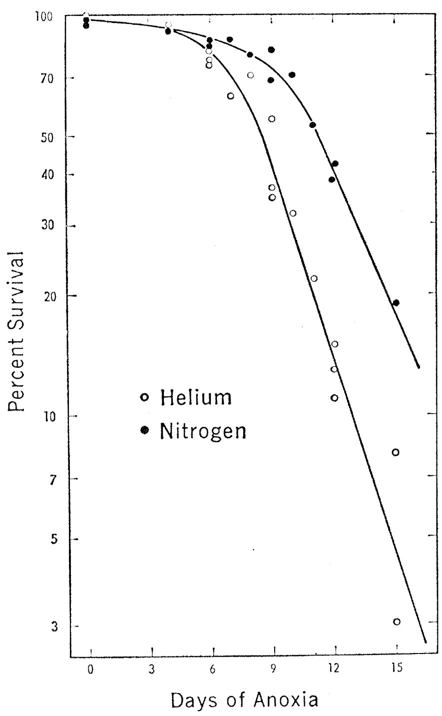

Fig. 1. Survival curves for seeds of Prolific rye subjected to prolonged anoxia. Points indicate size of surviving fractions after 7-day recovery in air after treatment.

the dark at 22°C under slight positive pressure (less than 0.07 atm).

In two of the tests for stress tolerance all treatments began on the same day. All chambers were checked for oxygen, reevacuated, and filled and flushed at 1- to 3-day intervals, and seeds from chambers showing evidence of oxygen leakage were discarded. Periodically one chamber from each series was opened, and its content of seeds were allowed to recover in air. In a third experiment, paired nitrogen and helium treatments were initiated at 3-day intervals and all treatments were terminated simultaneously. After removal from anoxia most of the sample seeds were planted in soil; a few were kept in petri dishes under light : darkness regimes of 14 : 10 hours for observation of post-treatment morphogenesis. The size of surviving fractions, the principal criterion of response to prolonged anoxia, was evidently unaffected by the variations in treatment procedures or in post-treatment handling. Active growth of shoots and roots was required to show evidence of seed viability.

Seeds hydrated and held under conditions of acute anaerobic stress underwent progressive reduction in viability. The magnitude of response was related principally to (i) the duration of exposure to anoxia and (ii) the gas used to induce anaerobiosis. Helium produced significantly greater reductions in viability than did nitrogen in samples held in anoxia for equal periods of 9 days or longer. Survival curves were characteristically biphasic, showing comparable rates of decline for the two series during both the initial interval of gradual decrease and the subsequent phase of rapid loss (Fig. 1). The main difference between the two series of treatments was in the time lapse before the apparent threshold of lethality was reached: 5 to 7 days in helium and 7 to 9 days in nitrogen. As a result of the earlier inception of rapid decline, seeds subjected to acute anoxia reached their LD_{50} level (50-percent mortality) about 2.5 days earlier in helium than in nitrogen.

Nitrogen and helium had no discernibly different effects on other aspects of seed physiology. Anaerobic germination and morphological development were relatively constant regardless of atmosphere. Post-treatment growth of survivors of prolonged anoxia was markedly less than that of air-grown controls. However, despite the lower incidence of survivors among seeds exposed to helium, their rates of post-treatment growth were comparable to those of their counterparts from nitrogen.

Since the mechanism by which chemically inert gases produce their effects on biologic systems is unknown, it is not certain whether the deleterious effects of helium, beyond those of nitrogen, result from an acceleration of events conditioned by anoxia per se or from some special property of the helium atom. However, the striking difference between helium and nitrogen in the magnitude of their effects clearly suggests that the physiologic hazards of atmospheres containing relatively large admixtures of helium may outweigh any technological advantages in its use in manned space vehicles.

These effects of pure helium on seeds may or may not provide a valid basis for prediction of its effects on other biologic systems, either alone or mixed with other gases. There are indications, however, that helium-oxygen mixtures also can be highly deleterious: Weiss *et al.* (*5*) found that viability of chick embryos incubated in 21 percent oxygen plus 79 percent helium was reduced by 50 percent. Biologic systems vary in response to helium anoxia (*6*), but seeds are probably more tolerant of anaerobic stress than the mammalian systems of critical interest to space scientists. Tests on appropriate mammalian systems are needed to fully assess the advisability of using helium as a component of life-support systems.

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Montmorillonite: Effect of pH on Its Adsorption of a Soil Humic Compound

Abstract. *The* d_{001} *spacing of sodium montmorillonite increased from 9.87 to 17.50 angstroms after interaction of the compound with fulvic acid at* pH *2.5. The magnitude of the spacing decreased with increase in* pH *between 2.5 and 6.0. At* pH *2.5 40 milligrams of Na-montmorillonite adsorbed 31 mg of fulvic acid; at* pH *6.0, only 15.8 mg.*

In many soils much of the organic matter occurs as clay–organic matter complexes (*1*) that significantly influence the physical, chemical, and biologic properties of the soils. While the literature of the last 25 years (*1*) is rich regarding interactions between clays and known organic compounds, much remains to be learned of reactions leading to formation of natural clay–organic matter complexes. We now report results of an investigation by x-ray and chemical methods of reactions between a preparation of purified organic matter extracted from soil and montmorillonite.

Methods of extracting, purifying, and drying the organic matter have been described (*2*). The extracted and purified matter (ash content, < 2 percent) was soluble in both dilute base and acid, so that, according to the definition accepted in soil science, it was fulvic acid; it was completely water soluble. Ultimate analysis, on a moisture- and ash-free basis, gave: C, 50.92