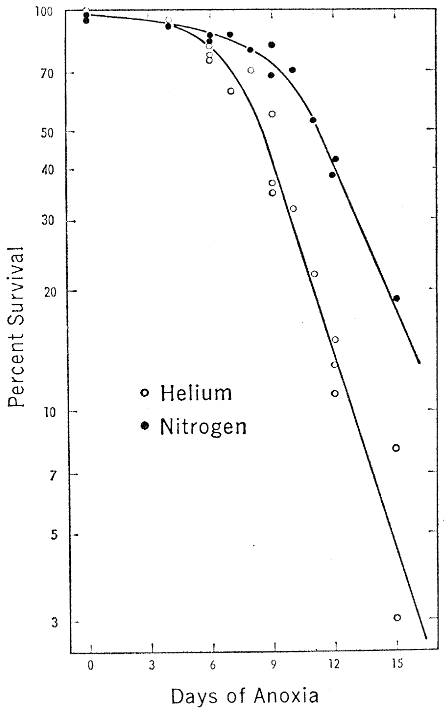

Fig. 1. Survival curves for seeds of Prolific rye subjected to prolonged anoxia. Points indicate size of surviving fractions after 7-day recovery in air after treatment.

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* p*H 2.5. The magnitude of the spacing decreased with increase in* p*H between 2.5 and 6.0. At* p*H 2.5 40 milligrams of Na-montmorillonite adsorbed 31 mg of fulvic acid; at* p*H 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

percent; H, 3.34 percent; N, 0.74 percent; S, 0.26 percent; and O (by difference), 44.74 percent. Its number-average molecular weight, measured by freezing-point depression of sulfolane and cyclohexanol, was 670 (*2*). One molecular weight of organic matter contained six COOH, two phenolic OH, three alcoholic OH, and two C=O groups (*2*). Ultraviolet, infrared, and nuclear magnetic resonance spectra of this material and of its fractions have been published (*3*).

Sodium-saturated Wyoming bentonite ($< 0.2\text{-}\mu$ equivalent spherical diameter) was prepared from the Ca form by five successive treatments with 1*N* NaCl solution. After removal of excess salt by successive washings with water and methanol, the clay was then dried in air.

For the experiment 100 mg of fulvic acid was weighed into a 35-ml graduated, ground glass–stoppered, centrifuge tube and dissolved in 8 ml of distilled water; the *p*H was adjusted with dilute sodium hydroxide solution. After addition of 40 mg of Na-saturated montmorillonite, the *p*H was rechecked and the volume was raised to 15 ml with distilled water. Tube and contents were shaken for 18 hours at room temperature, before centrifugation for 1 hour at 2000 rev/min. The supernatant was decanted and the residue was suspended in 2 ml of water. Washing of the residue was omitted because preliminary experiments had shown that four successive washings with water removed less than 6 percent of the fulvic acid from the clay–fulvic acid complex prepared at *p*H 3.

One milliliter of this suspension was pipetted onto a 37.5- by 25-mm glass slide and allowed to dry before x-ray analysis (*4*) of the oriented aggregate; passage of dry air during the analysis kept the relative humidity under 3 percent (*5*).

The supernatant was dried first on a steam bath and then for 3 hours in an oven at 100°C; the residue was cooled in desiccator and weighed. Carbon was determined in a sample of the residue by dry combustion, the sample being covered with tungstic trioxide to ensure complete combustion (*6*). Adsorption of carbon by the clay was the difference between carbon recovered from the supernatant and carbon originally added.

The d_{001} of the original Na-montmorillonite in dry air was 9.87 Å; after interaction with fulvic acid it increased to a maximum of 17.60 Å at *p*H 2.5 (Fig. 1). Interplanar spacings were clearly *p*H-dependent and decreased with increase in *p*H, the steepest decrease occurring between *p*H 4 and 5. Notably the apparent *p*K value of the fulvic acid, as determined from a *p*H-versus-log $[\alpha/(1-\alpha)]$ plot (Henderson-Hasselbach equation) (*7*), was 4.5; this suggested that the magnitude of the d_{001} was related to the degree of ionization of the functional groups (*2*) in the fulvic acid. At $p\text{H} < 4$, relatively few of these groups had ionized, so that the fulvic acid behaved like an uncharged molecule that could penetrate the interlamellar spaces and displace water molecules from between the silicate layers of montmorillonite, which is a 2 : 1-type-layer silicate mineral. As the *p*H rose, more and more functional groups ionized to result in an increased negative charge; thus, at $p\text{H} > 5$, d_{001} was less than 11 Å, indicating repulsion of negatively charged fulvic acid by negatively charged montmorillonite.

The curve depicting adsorption of C by montmorillonite at different *p*H levels (Fig. 1) essentially resembles that showing d_{001} values. Adsorption of C decreased with increase in *p*H, except for a small increase at *p*H 7. Since the original fulvic acid contained approximately 50 percent C (*2*), amounts of fulvic acid could be estimated by multiplying C (mg) by 2. Thus at *p*H 3 40 mg of montmorillonite adsorbed 31 mg of fulvic acid (4.6×10^{-5} moles) (*2*) and 1 g of montmorillonite adsorbed 1.2×10^{-3} moles of fulvic acid. Whether at this *p*H all the fulvic acid was adsorbed in the interlamellar spaces or whether a portion of it was adsorbed by the crystal edges awaits further investigation.

At $p\text{H} > 5$, amounts of fulvic acid adsorbed by 40 mg of montmorillonite were about 16 to 19 mg, but d_{001} spacings remained practically constant at < 11 Å (Fig. 1); probably most of the acid was adsorbed by the crystal edges of the montmorillonite. Additional evidence of the effect of *p*H on adsorption and desorption of fulvic acid by montmorillonite was obtained by preparing a fulvic acid–montmorillonite complex at *p*H 2.5, adjusting the *p*H of the system to 7, and shaking for 18 more hours; the *d*-spacing decreased from 17.60 to 10.47 Å.

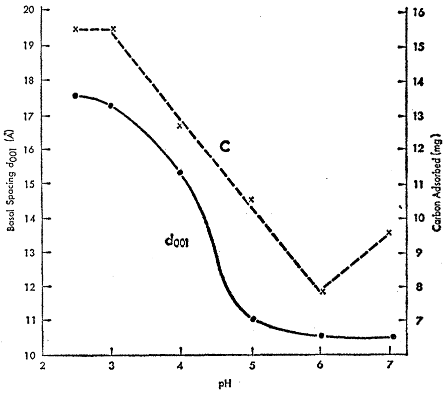

Fig. 1. Effect of *p*H on d_{001} and adsorption of carbon.

Exhaustive oxidation with H_2O_2 of the complex prepared at *p*H 2.5 destroyed 96 percent of the fulvic acid and reduced the d_{001} to 11.7 Å. The Debye-Scherrer powder pattern of untreated fulvic acid exhibited a halo with a broad maximum at 4 Å, which was similar to the basal reflection of coals (*8*).

If one assumes that the structure of fulvic acid resembles that of low-rank coals, the increase (7.73 Å) in interlamellar spacing in the complex prepared at the lowest *p*H from that in the untreated clay corresponded to approximately two layer thicknesses of fulvic acid. Calcium montmorillonite interacted with fulvic acid gave results essentially similar to those with Na-montmorillonite. Interactions similar to those described are likely to occur in nature in acid soils such as podzols, in which in surface horizons up to 50 percent of the organic matter may consist of fulvic acid and in which *p*H values between 3.5 and 4.5 are not uncommon (*9*).

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