

Biochemistry and Soil Science

Biochemical activities of microorganisms play a role in soil genesis, morphology, fertility, and physics.

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Aware of the development of modern plant and animal biochemistry, chemists realize that soil is more than a static aggregate of minerals, water, humus, and microorganisms. During the development of soil microbiology as a field, as described by Waksman (1) a generation ago, surveyors were busy classifying soils according to evolutionary concepts (2), and physical chemists were extracting soils and, with spectacular success, determining the detailed crystallographic structure of clay minerals. The basic concepts of polymer chemistry were just being worked out by Staudinger and Meyer, so nearly all that could be said about the "organic fraction" of soil was that it was colloidal, heterogeneous, dark-colored, and weakly acidic and that it contained, as a kind of graveyard of microorganisms, almost any naturally occurring organic compound one wished to find (3).

In 1945 Waksman predicted that the soil microbiologist was in a position to make important contributions to our knowledge of soil processes and plant growth and to the utilization of microorganisms in industry and public health. The development of antibiotics for arresting some human diseases and agricultural pathogens has of itself justified this optimism.

Expressing one point of view, Waks-

man emphasized that, for the microbiologist, soil is a unique medium in which organisms live, not in pure culture, but in highly complex populations comprising innumerable species which have a variety of associative and antagonistic effects. Control of these effects has, to a notable degree, allowed the agronomist to favor processes brought about by beneficial organisms and to suppress root and tuber pathogens (4). During the early period, enormous effort was expended in taking inventory of the denizens of the soil. There seem to be countless numbers of different "species" of bacteria, yeasts, protozoa, and so on in soil. In view of the phenomenon of transformation of one species into a closely related one with new, inheritable characteristics, by means of uptake of genetic nucleic acid markers from related bacteria, it is puzzling why there are so many rather than so few kinds of bacteria in soil. As a result of this phenomenon one might expect convergence rather than diversification of the microbial population of the soil. In assaying for unique biochemical activities, workers found that certain groups of these soil organisms were geared to elemental cycles such as ammonification, nitrification, and nitrogen fixation. Many laboratories and experiment stations devoted a large part of their research facilities to the analysis of steps in the decomposition of plant and animal residues in composts and

soil, with the hope of understanding these steps and, in addition, of explaining the formation of humus (3). It was recognized that acids produced during these decompositions facilitated the weathering of rocks, the liberation of phosphorus and potassium from clay minerals, and the mineralization of phosphorus and sulfur. From the many organisms with specialized activities, such as the nitrifiers, microbial biochemists prepared isolates for investigation in pure culture, and in doing so they ceased to be concerned with soil per se.

From a somewhat different point of view, Quastel (5) proposed another conceptual scheme—namely, that soil as a whole be considered an organ, comparable in some respects to a liver or a gland, in which enzymatic reactions can occur. To the soil are added various nutrients, including plant materials, rain, and air. Products of these reactions, such as chelating humic acids, are important as steps in elemental cycles, in the percolation (movement) of iron and aluminum humates, and in the formation of soil crumb structure, for example. The notion here is that the soil biochemist is more concerned with what the microbes are doing in soil than he is with their size, shape, or other properties on which taxonomic schemes are based.

With enrichment culture techniques these two approaches come together. If a sample of native soil is perfused repeatedly with glycine, together with the products of microbial action on glycine, those organisms capable of metabolizing glycine, ammonia, and nitrate will increase greatly in number, so that isolates of, for example, *Nitrosomonas* sp. will be more easily obtained. In this example certain cells of the soil-organ are encouraged to multiply and to "cooperate" in the conversion of glycine to nitrate, carbon dioxide, and water as principal products. Along with these reactions, however, and in the presence of carbohydrate, other soil organisms can synthesize ionic, high-polymeric substances,

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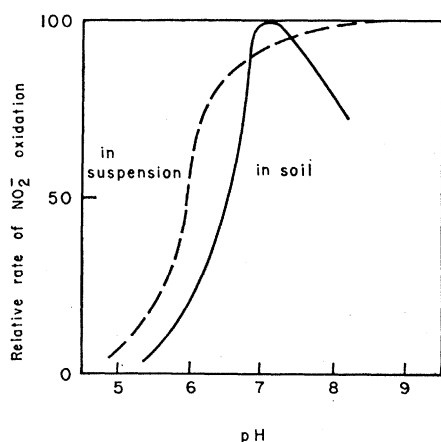


Fig. 1. Relative rates of nitrification of nitrite by *Nitrobacter agilis* in aqueous suspension and in soil as a function of the pH of the aqueous phases. [Unpublished data obtained with perfusion equipment by Skujins and McLaren]

some of which are absorbed on the clays (6). Only a kind of steady state exists at any one point in time, depending on the rates of addition and loss of metabolites to and from the soil. In this article I describe something of what we now know about these biochemical reactions in soil and relate them to some other aspects of soil science.

Ecological Conditions in Soil

First of all, let us consider the environment in which the soil microorganisms operate. It differs rather drastically from that of a culture flask or an agar slant. There is a point-to-point variation in concentration of all solutes in a matrix of clays, sand, and humus which characterizes a *microenvironment*. Furthermore, at the surfaces of these particles as well as at plant roots there is a variation in *molecular environment* characterized by gradations in pH and reduction-oxidation potential (7).

Morphologists have dissected soil into three principal horizons: A, the upper or eluvial layer, which receives litter and from which material is being, or has been, leached; B, the underlying illuvial layer, which is being, or has been, enriched; and C, the mineral, parent material underneath the A and B horizons. These horizons may be well delineated in profile, or the B horizon may arbitrarily grade into the other two. Pore space, varying in water and air content, occupies 30 to 45 percent of the soil. From horizons A to C the numbers of microbes per gram of soil

decline, and the microorganisms become less aerobic. Soils with higher content of humus or clay, or both, tend to hold more water, because of hydration and small particle size, than do soils with the larger silt particles. In "waterlogged" soils there are practically no air spaces; and in "air dry" soils the liquid water in the pores has almost disappeared, leaving only water films around the particles (8). Each horizon behaves somewhat differently from the others as an organ of biological activity (4).

Microorganisms cannot develop in the absence of water and below a relative humidity of about 85 percent. Their activities may depend on the thickness of the water film; for example, Rahn (9) found the optimum thickness of the film to be 20 to 40 microns for *Bacillus mycoides*, an aerobe. Winogradsky (10) determined the depths to which an aerobe, *Azotobacter*, and an anaerobe, *Clostridium*, could grow in wet soils: when the moisture content of the soil was 23 percent, the former occurred only at the extreme surface and the latter were found throughout the soil. In a recent study of widely different soils it was found that "change-over" from aerobic to anaerobic metabolism of organic materials takes place in widely different soils at an oxygen concentration of less than about $3 \times 10^{-6}M$, a very low concentration indeed (11). Decomposition of organic compounds in soil below this oxygen concentration leads to the accumulation of fatty acids. One implication is that water-saturated crumbs of more than about 3 millimeters in radius have no oxygen at their centers. Since crumbs of this size are present in most soils, this means that pockets in which there is no free oxygen are ubiquitous, and this provides an explanation of the universality of strict anaerobes. Oxidative processes, however, abound in relatively dry soils; for example, in certain soils of Colombia, the content of organic matter increases with increasing rainfall (12).

Waterlogged soils, high in organic matter, tend to become acidic through fermentation, whereas well-aerated garden soils are nearly neutral (that is, the pH is of the order of 6 to 7.5, as measured with wet pastes and a glass-electrode system). At higher pH, as in calcareous soils, growing plants may show iron or manganese deficiencies because of the insolubility of the corresponding phosphates and carbonates. The addition of sulfur results in the

lowering of pH and the liberation of these cations, as I discuss later.

Upon studying the effective pH at the surface of soil colloids, however, a new concept emerges. Wherever there are charged surfaces in contact with water, the effective pH of the surface (pH_s) will be lower than the pH of the bulk solution (pH_b). This difference has been expressed by Hartley and Roe as $pH_s = pH_b + \zeta/60$ at 25°C, where ζ is the electrokinetic potential of the particle (13). In fact, chymotrypsin acting on a protein adsorbed on kaolin has a different optimum pH for enzyme action than is found in solutions with the same substrate for this reason. The optimum pH for succinate oxidation with cells of *Escherichia coli* adsorbed on an anion-exchange resin differs from that for oxidation with free cells suspended in solution. Furthermore, the initial velocities of oxidation of this substrate with free and adsorbed cells at $pH_b = 7$ were quite different (14). The optimum pH for nitrification by *Nitrobacter agilis* in soils also differs from that in liquid media (Fig. 1). These concepts should be kept in mind in assessing the significance of pH optima for soil enzymes in sterile soil. Evidently the heterogeneity of soil as an environment for microorganisms extends from the gross-particle to the molecular level.

Some Metabolic Processes in Soil

The oxidation of sulfur in soil is a complicated process and is excellent for illustrating a useful biochemical technique with broad applications. Lees and Quastel designed an apparatus in which a column containing about 30 grams of soil in a water-saturated, aerated condition could be continuously perfused with a nutrient solution (15). The technique is splendid for characterizing nitrification (16) and sulfur metabolism (17) in soil and led these workers to conclude that soil could be enriched with the microorganisms involved until a "saturation" of numbers existed for a given metabolite. By way of illustration, we can examine the influence of the microbial oxidation of sulfur on the pH of an alkali soil. The use of sulfur for improving alkali soils was suggested as early as 1916 by Lipman (18), and the technique was developed by Kelley (19).

Sulfur (1 g per 30 g of soil) was added to a sample of Fresno fine sandy loam [a black alkali soil initially of pH

10.2 (20)]. The mixture was perfused with 200 milliliters of water, and the perfusate was analyzed for the presence of hydrogen ion and sulfate ions as a function of time (Fig. 2). After a few days of perfusion, sulfate was found in the perfusate, and the pH fell as a result of the overall reaction $1\frac{1}{2} \text{O}_2 + \text{S} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4$. The addition of *Thiobacillus thiooxidans* to the soil did not greatly modify the rate of the reaction—a finding which showed that related organisms existed in the field soil initially. After 20 days of perfusion the soil was washed free of sulfate and reperfused; oxidation took place without the lag shown in Fig. 2, and at the maximum rate. Clearly, at this point the soil is enriched with respect to sulfur oxidizers.

Microbial transformations of sulfur have counterparts in microbial transformations of nitrogen (21, 22). Sulfide and ammonia are reduction products of the decomposition of some common organic compounds; both may be oxidized by certain soil microbes, and a few autotrophs can use these oxidations as their sole sources of energy. A single sulfur compound, however, may yield different sulfur products on dissimilation by different microbes. From cystine, *Achromobacter cystinovorum* produces only elemental sulfur. In a soil perfused under aerobic conditions and containing an indigenous population, practically all sulfur in cystine becomes sulfate and the corresponding nitrogen becomes nitrate. The acidity of the soil increases, and the metabolism of other organisms may be inhibited.

On the basis of results with individual microbes isolated from soil (including bacteria and fungi) one might expect to find in soil the following intermediates: cysteine, sulfide, thiosulfate, trithionates, tetrathionates, pentathionates, sulfite, and dithionate, the relative amounts of these depending on the degree of aeration (17). Freney conducted an extensive study of the aerobic transformation of cysteine to sulfate in a soil-perfusion unit and found that both cystine and cystine disulfoxide appeared in the perfusate, but he did not detect taurine. (Taurine is an intermediate of animal metabolism.) When an enriched soil (that is, a soil enriched with cysteine), was perfused with cystine and cystine disulfoxide, the initial lag in oxidation that was observed the first time perfusion was carried out (see Fig. 2) was not observed. These facts, according to established thinking,

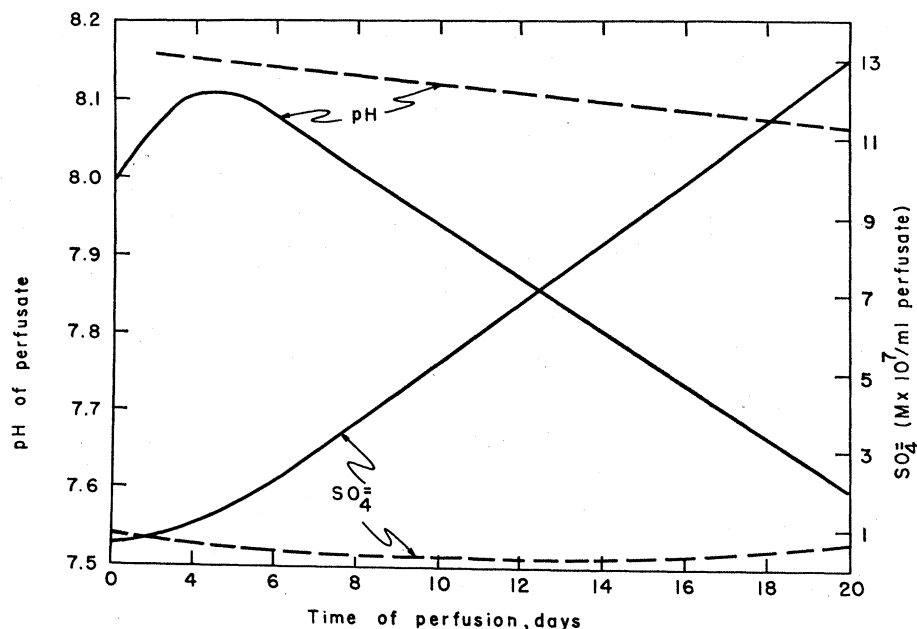


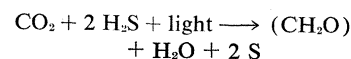
Fig. 2. Variation of pH and sulfate concentration as a function of time of perfusion of (solid lines) a black alkali soil containing elemental sulfur, and (dotted lines) a black alkali soil without added sulfur.

indicate that both compounds are intermediates in the transformation of cysteine to sulfate (22). On further investigation (23), cysteic acid was also identified in the perfusate, along with cysteine sulfinic acid. Other compounds found in this series of experiments were pyruvic acid, β -mercaptopyruvic acid, β -hydroxypyruvic acid, α -ketoglutarate, and a little sulfite. Since so many organisms are involved in these reactions, the pathway indicated below may be considered a logical overall reaction process for the soil viewed as an organ (it does not pertain to any particular organism): cysteine \rightarrow cystine \rightarrow cystine disulfoxide \rightarrow cysteine sulfinic acid \rightarrow cysteic acid \rightarrow sulfate.

In considering all such experiments it must be remembered that the amount of free cysteine normally in soil is minute (24). One of the great problems is that of deciding whether the reactions observed are typical of the microbial population under field conditions or only when the system is artificially enriched with substrate. An abundance of cysteine changes the oxidation-reduction potential of soil and leads to an increase in numbers of a group of organisms perhaps normally present as a relatively small fraction of the soil population. Nevertheless, in some areas of the world—for example, New England, Australia—most of the soil sulfur is locked up in some form of organic matter, and pasture lands in this region are, at present, mainly dependent for nutrition on a re-

lease of this sulfur by mineralization. Solubility studies have indicated that the organic sulfur is present in the form of fulvic acids, humus substances of high molecular weight (23). Fulvic and humic acids are evidently synthesized by microbes acting on plant materials, but the enzymatic nature of the transformation is obscure (3).

From the anaerobic decompositions of organic sulfur compounds, H_2S , methyl mercaptan, and dimethyl sulfide have been reported. To be useful to plants, all these compounds must be oxidized to an oxidation state of +6, and among the microorganisms capable of these oxidations are photosynthetic bacteria. Sulfide undergoes oxidation to sulfate with, frequently, temporary accumulation of elemental sulfur as globules within cells. The following reaction is representative:



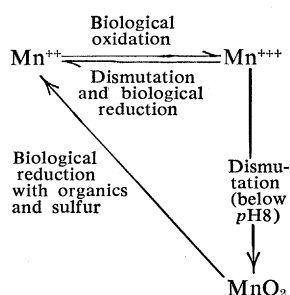
where (CH_2O) represents an energy-rich organic compound within the cell. Many species can oxidize sulfur and sulfur acids of intermediate oxidation states to sulfate in the dark. Such reactions can also be studied in soil by Warburg manometric procedures. For example, the oxidation of thiosulfate by an enriched soil requires the expected number of four atoms of oxygen (25).

Many microbes, like higher plants, satisfy their sulfur requirements with sulfate, but these reactions are less well

understood; the product accumulates on the inside of cells instead of on the outside. *Aspergillus nidulans* supposedly does this by reducing sulfate to thiosulfate and by then coupling thiosulfate with serine to give, first, serine thiosulfate and then cysteine.

Perhaps it is unnecessary to say that in the "organ" approach to soil we have no way of deciding which reactions may be attributed to a given species. If one studies isolates of single species, one misses the possible interactions among species—the mutual inhibitions and stimulations. This conceptual difficulty is not peculiar to consideration of soil as a tissue, however. A good illustration involves manganese metabolism. Bromfield and Skerman (26) found that manganese sulfate could be oxidized, with formation of a brown deposit of manganese dioxide on agar plates, if a certain two species of organisms were growing in colonies near each other. Two such pairs had *Corynebacterium* in common; the other organism could be either a *Flavobacterium* or a *Chromobacterium*. Obviously the existence of associative action between microbes makes it virtually impossible to count the number of Mn^{++} -oxidizing organisms in soil. An ascomycete, *Cladosporium*, can oxidize this ion without association.

If a neutral or slightly alkaline soil is perfused with manganese sulfate, it is found that after a few days all the Mn^{++} has disappeared from the perfusate. Above a concentration of 0.02M the rate of formation of manganese dioxide falls rapidly, indicating manganese toxicity. Also, metabolic poisons, including azide and iodoacetate, markedly inhibit oxidation. The detailed observations have been summarized as follows (27):

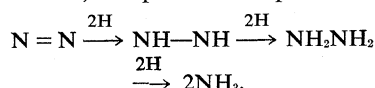


Reduction of manganese oxides in soils can be brought about by adding glucose, thiols, and polyphenols. These laboratory observations provide a useful explanation of the fact that application of manganese sulfate to field soils deficient in manganese is often ineffective, since manganese in the form of

manganese dioxide is not readily taken up by the soil. An increase in the concentration of manganese in a form that is readily taken up can be brought about by adding reducing forms of organic matter, thereby stimulating the growth of manganese-reducing organisms; by reducing the pH so as to shift the "cycle" toward Mn^{++} ; and by inhibiting oxidation through adding specific poisons. Here the sulfur and manganese cycles can be "overlapped" to advantage: the addition of sulfur favors formation of the reduced form of manganese through the formation of sulfuric acid, with a reduction in pH, and through slow production of thiosulfate. The addition of sulfur to a "manganese-deficient" soil stimulated the growth of beets and increased the manganese content of the beet. Similar results were obtained with peas (27).

Nitrification of ammonia has already been mentioned. The formation of nitrate in soil leads to enormous annual losses of nitrate to the ocean; nitrate ions are not adsorbed to the soil colloids and hence are easily leached out and carried away by rain. Since some agricultural crops can use ammonia very well, use of a cheap chemical which would inhibit growth of the nitrifiers without inhibiting that of the ammonifiers (which liberate ammonia from, for example, proteins) might greatly benefit agriculture. Ammonia is resistant to leaching, since it, as a cation, is held to soil particles. The insecticide chlordane is just such a reagent, although as much as 0.5 percent in soil is required (28); use of 2-chloro-6-(trichloro-methyl) pyridine has also been suggested (29).

Part of the losses of soil nitrogen by leaching are counterbalanced by the fixation of atmospheric nitrogen by soil microbes. However, more nitrogen is fixed in this manner than can be accounted for by our present inventory of known nitrogen fixers and their specific fixation abilities (4). Nicholas (30) has recently shown that gaseous nitrogen and gaseous oxygen used by respiring *Azotobacter vinelandii* compete with one another for electrons supplied by other metabolic activities of these cells. The intermediates must be very small in amount; the probable steps are



Iron, molybdenum, and cobalt are all required. When the oxygen content of a soil is low and there are large amounts of organic material, denitrification, with

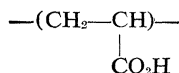
some loss of soil nitrogen back to the air, is also observed (4). The denitrifying bacteria are all "aerobic," but they can utilize nitrate as an oxidizing agent for growth. Both N_2 and N_2O are liberated, but such a loss can be kept to a minimum by maintaining a vegetative cover of the soil and suitable drainage.

The killing of weeds with herbicides may create problems. The main problem is that of deciding which herbicide to use, on the basis of considerations such as whether one wants it to persist long enough to inhibit growth of a second crop of weeds or not to persist long enough to spoil a subsequent commercial planting. Factors that influence the persistence of a herbicide are volatility, drainage, coverage of the land with an impenetrable foil, and nutrition of soil microorganisms. Organic herbicides break down most rapidly in soils rich in organic matter and under conditions of high moisture and high temperature. In general, where persistence of herbicides in soil is desired, there is a delicate balance between success or failure which is dictated by rainfall, period of fallowness, and crop rotation. The addition of borates to soil along with 2,4-dichlorophenoxyacetic acid (2,4D) reduces microbial attack on the latter; another approach is that of reducing the volatility of some of the less persistent agents (31).

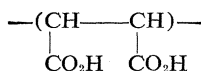
The effect of herbicides on soil microorganisms has been summarized, and the microbiological breakdown of these substances has been reviewed (31). At the concentrations at which it is usually applied in the field, the growth of nitrifying organisms and proteolytic activity are not suppressed by 2,4D. Indeed, when garden loam is perfused with 2,4D, the 2,4D disappears rapidly after a lag period of about 2 weeks. During the first 2 weeks the soil flora undergoes the familiar "enrichment" with respect to those organisms which can adapt to a new substrate. Once they have adapted, many organisms persist and retain a complement of enzymes that are active relative to this substrate long after the substrate has been removed.

Organic matter in soil plays an essential part in securing the structure that is required for high fertility. From microbial syntheses at the expense of carbohydrates of plant origin, macromolecules appear among the clay particles. These have the capacity to bind to the latter, presumably through, for example, $R-CO_2-Ca-clay$ bonds, ac-

cording to the scheme OO , where the arcs represent linear macromolecules and the circles depict negatively charged clay particles. These macromolecules include bacterial gums, alginic acid, pectic acid, and a large class of synthetic compounds having, as part of their copolymeric structures, these configurations, among others:



and



The net effects achieved by adding such substances to soils high in clay and low in organic matter are greater aeration, greater tilth, increase in rate of water percolation, and sometimes increase in crop yield, all resulting from the formation of a more stable crumb structure (Fig. 3) and an increase in stability against compaction (6, 32, 33).

Recently a refreshing viewpoint on the origin of other macromolecules in soil has appeared from the Russian and German schools (3). Aromatic polyphenols, formed by way of oxidation of quinones, can be condensed with amino acids. Enzymes of microbial origin—for example from myxobacteria—have been shown to promote these condensations under laboratory conditions, with production of humic-like substances. The polyphenols may be derived from lignin and cellulose, and certainly lignin is no longer regarded as a direct source of humic substances (3).

Enzyme Action in Soil

Thus far I have discussed some implications of biochemical activities in the fields of soil chemistry, physics, and fertility. In these matters the microbe is an active contributor. A number of investigators, however, have asked how much of the enzyme action in soil results from microbial activity at any given point in time and how much is attributable to an accumulated “background” of enzyme activity in soil per se—that is, activity that occurs without active participation of the microbe. One way to assay extracellular enzyme action is to add toluene to soil to suppress microbial activity. Although toluene and even gasoline are substrates for some organisms, in short-term experiments in which they are added to soil, life

activities may be considered to be suppressed. In Fig. 4 the optimum pH for glycerolphosphatase activity is depicted for some meadow and forest soils (34). Similar results are obtained if soil is first sterilized by means of an electron beam (35), and in the case of a Dublin clay loam, over half of the activity remained after complete sterilization through irradiation with a dose of 5 millirep. Any attempts to study the utilization of glycerolphosphate, per se, in sterile soil by a sterile plant are obviously frustrated by the phosphatase action of soil, and incidentally by the phosphatase activity of sterile roots (36). On the other hand, Hofman and Seegerer (37) suggest that an estimate of soil enzyme activity provides a very quickly obtained and good measurement of soil fertility.

Haig (38) has fractionated soils and found that most of the esterase activity is in the clay fraction. Intriguing questions, therefore, are, How is the enzyme held by the clay? How is it accumulated? How can it be isolated for study? Thus far all efforts at eluting soil enzymes from the soil colloids have failed (39).

An interesting and important problem of soil enzyme action requires a knowledge of the kinetics of enzyme action at solid-liquid interfaces. The phenomenon just described involves the action of an adsorbed enzyme on a soluble substrate. Conversely, microbes secrete soluble enzymes which act on insoluble substrates, such as cellulosic debris, soil organic matter, and chitin. The kinetics of these reactions is not represented solely by equations of the Michaelis-Menten type for classical reactions in solution. Instead, the limiting rates of reaction are governed by diffusion of substrates to surfaces, adsorption of enzymes on particles, and the local pH, discussed earlier (40).

In a recent lecture, Allison stated that a review of the contributions of soil microbiologists underlines the role that microbes play in almost all phases of soil science, and, in addition, that most present and future problems in soil-plant relationships will not be completely solved until careful consideration is given to the role of the microflora (41). A few interesting examples will suffice to show the close relationships among soil constituents, the plant root, and the microbe.

In Panama, the effective banana-producing lifetime of soils is determined by the rate of spread of *Fusarium* wilt. In an effort to correlate soil lifetimes

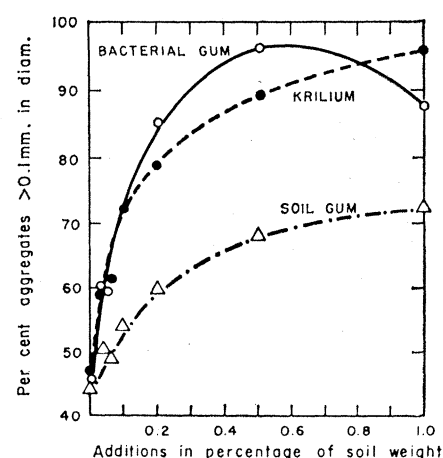


Fig. 3. Effects of bacterial gum, Kriliun (a synthetic polyanion), and extracted soil gum on aggregation of a silt loam soil. [D. A. Rennie *et al.* (33)]

with soil characteristics, Stotzky *et al.* (42) investigated soil texture, pH, soluble salts, cation-exchange capacity, available phosphorus, organic matter, drainage, and clay mineral content. Only one direct relationship was found to exist: montmorillonite-type clay minerals were present in all 20 of the “good” soils and were completely absent in all but two of the 14 poor soils. At present, it is not known whether the important mechanism involves a clay-host relationship or a clay-pathogen interaction. Montmorillonite is an especially good absorbent for some amino acids and for antibiotics, enzymes, and cations in general. It could function as an absorbent for either *Fusarium* toxins

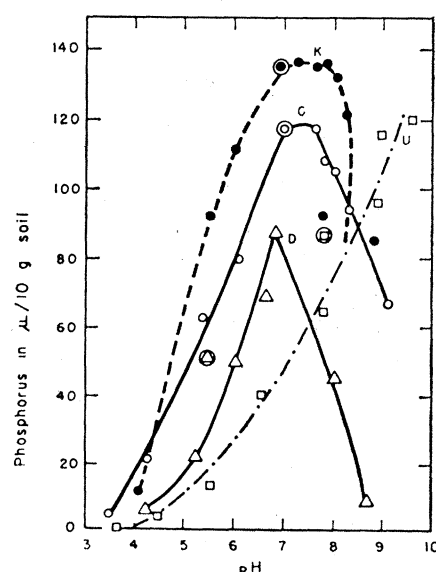


Fig. 4. Influence of pH on phosphatase activity (34). (C) Meadow soil, pH 5.6; (D) beach-oak forest soil, pH 4.8; (K) steppe soil, pH 6.8; (U) field soil, pH 7.9.

or *Fusarium*-stimulating compounds secreted by the plant.

Fusarium wilt of kidney beans can be suppressed by incorporating chitin in soil. The evidence at present suggests that the suppression is achieved through an enrichment of microbes which preferentially metabolize chitin and are thereby able to destroy chitin-containing fungi (43).

Plant-root systems normally secrete a wide variety of substances that are absorbed by the surrounding soil; these include amino acids and vitamins in amounts sufficient to modify the normal (that is, "indigenous") population of the soil (this is known as the rhizosphere effect) (44). Treatment of soil with pea-root-exudate solution alone resulted in an increase in the numbers of Gram-negative bacteria. These modifications can be important; for example, varieties of oat likely to develop a manganese deficiency support greater numbers of microorganisms capable of oxidizing manganese into forms that the plant cannot utilize than other varieties do (45), and, conversely, the rhizosphere population assists plants in the utilization of mineral phosphates (46).

The rhizosphere seems to contain a bacterial flora which is more active physiologically than nonrhizosphere soil in oxidizing glucose, alanine, acetate (47), and organic phosphates (48). Bacteria requiring amino acids for optimum growth are preferentially stimulated in this zone (49). Thus, any steady-state amounts of organic nitrogen and phosphorus in soil are greatly influenced by cropping. The competition between plant and microbe for a given element will be keen, but the dynamic equilibrium will be shifted as the plant pumps the nutrient out of the ground, so to speak (50).

On the other hand, products of rhizosphere microbes can affect root growth without apparent invasion of the root or damage or stunting of tops, as in tomato and subterranean clover (51). Familiar antibiotic substances may be involved (52). Rhizosphere organisms also seemingly stimulate barley to synthesize amino acids in proportions that differ from those under conditions of sterility (53).

Soil Development

Although there have been many rhizosphere studies made under glass-house conditions and in specially selected field tests, little attention has

been given to the rhizosphere flora of dominant species in naturally occurring plant communities. Sand-dune communities were chosen for study by Webley and his co-workers because the development of a soil microflora, accompanied by the formation of a soil, could be traced from the simple conditions of early colonization of bare sand through a series of communities of increasing botanical complexity (54).

Microbial counts were obtained from a transect across a dune at Newburgh (Aberdeenshire). As one progresses from open sand past early fixed dunes to dune pastures and heath, the number of bacteria rises from a few thousand to millions per gram as soon as colonies of vegetation appear in the sand. There is a fall in the numbers of bacteria, but not of fungi, when heather, with an acid humus, enters as a dominant plant. When one compares rhizosphere soil with soil near the rhizosphere, one finds far more microbes on the root surfaces of *Agropyron* and *Ammophila* than in the open sand, with *Corynebacterium*, *Mycobacterium*, and *Nocardia* predominant among the "bacteria" throughout. Among the fungi, however, *Penicillium* sp. predominated in open sand and *Cephalosporium* predominated near roots. Each plant species developed a unique rhizosphere flora which overlapped other species only in part. There can be little doubt that the activity of the microbes contributes to the development of the soil and the maturation of the habitat in a way which depends on the higher plants growing in a sand-and-salt milieu.

The first probes to land on Mars will be equipped with devices for investigating any biochemical and microbial activities extant. Meanwhile harsh terrestrial soil environments are being studied with this in view (55).

"The soil lies on the twilight of life, a connecting link between the living and the non-living, between material animated by vital forces and material subjected to physical forces" (56). Both here and elsewhere?

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Phosphohistidine

Its isolation from mitochondrial protein sheds new light on the process of oxidative phosphorylation.

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Principal deterrents to experimental progress on the mechanism of oxidative phosphorylation have been the extreme lability of the phosphorylation reactions in contrast to the oxidation reactions, and the lack of definitive chemical information about any possible intermediates in the process. The recent discovery of a protein-bound phosphohistidine by my laboratory group at Minnesota gives promise of increasing considerably our insight into how oxidative phosphorylation occurs. This article reviews current information about bound phosphohistidine and its metabolic role, and advances the hypothesis that all phosphorylations of oxidative phosphorylation funnel through the formation of bound phosphohistidine.

The formation of ATP from ADP and P_i (1) serves as the principal means of utilization of energy from oxidation of foodstuffs. In aerobic organisms, some 90 percent or more of the ATP formation occurs through oxidative phosphorylation, in which reduced cofactors are oxidized by molecular oxygen. A minor portion of the ATP arises from oxidation of substrates by cofactors. The mechanisms of these substrate phosphorylations, in contrast to oxidative phosphorylation, are relatively well understood. Most evidence about the mechanism of oxidative phosphorylation has been indi-

rect, so that formulation of reaction schemes based on relevant chemical information has been unfortunately limited.

Heretofore the phosphorylation reactions of oxidative phosphorylation have been observed only in intact mitochondria from cells, or in highly organized and insoluble subunits therefrom. Bound phosphohistidine appears to be an intermediate in oxidative phosphorylation by intact mitochondria as shown by its rapid labeling from either P_i^{32} or ATP^{32} and other factors governing its formation and disappearance. Our excitement was heightened when a remarkable soluble enzyme system was obtained which would catalyze the formation of bound phosphohistidine. Study of the phosphorylations unencumbered by the diffusion barriers and complexity of the mitochondria thus seemed possible, including delineation of discrete steps and reaction components. Explorations with this system are still in their early stages, but already the results are considerable. Interpretations and projections are made at this time because of present interest in the field, even though they must remain tenuous until our studies are more fully developed.

Our interest in possible intermediates formed from P_i in oxidative phosphorylation was an outgrowth of experiments demonstrating that P_i and not ADP lost an oxygen to water in the formation of ATP (2). Several years ago, we instituted a search for intermediates by incubating mitochondria for short pe-

riods with P_i^{32} and attempting to find any substances other than P_i or ATP which contained radioactivity. A readily soluble substance with expected properties of an intermediate was detected. This only resulted, however, in a rediscovery of carbamyl phosphate without shedding any light on oxidative phosphorylation (3, 4). We then directed attention to possible intermediates not readily extracted from the mitochondrial protein or lipid. This led to the detection of a trace of a phosphorylated component, which appeared to be an intermediate in oxidative phosphorylation, as reported at scientific meetings (4, 5) and in additional detail by Suelter *et al.* (6) in 1961. An isolation and fractionation program culminated in identification of the phosphorylated component as a protein-bound phosphohistidine, in which the phosphoryl moiety is attached to an imidazole nitrogen (7). Other concomitant studies showed formation of bound phosphohistidine directly from ATP^{32} by mitochondria, which added to the evidence that this phosphorylated imidazole structure was an intermediate in oxidative phosphorylation (8).

More recent experiments have led to the demonstration (9) of phosphorylation reactions that give rise to bound phosphohistidine from both P_i^{32} and ATP^{32} by soluble enzyme preparations from mitochondria. Relationships between precursor and product are difficult to establish unequivocally in structures as complex as the mitochondrion in which compartmentation of intramitochondrial P_i and ATP is possible. Thus, the convincing demonstration that bound phosphohistidine is formed directly from P_i or directly from ATP by a soluble enzyme system (9) strengthens the deduction that bound phosphohistidine is likewise formed directly from P_i or directly from ATP by intact mitochondria.

The soluble enzyme preparations retain the capacity for a dynamic equilibrium interchange between P_i and the phosphorylated imidazole group, as well as for net formation of the phosphorylated imidazole group from P_i upon an increase in pH or an increase in the

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