Review Article

THE PRODUCTION AND ROLE OF ANTIBIOTICS IN SOIL

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(Received for publication June 14, 1976)

Intensive research on antibiotics has been underway for approximately thirty-five to forty years. For most part, these recent studies have dealt with the search for new antibiotics—their therapeutic properties, structure, mechanism of action and biosynthesis. During a relatively short period in the late 1940's and early fifties there was a flush of activity on the question of the role of antibiotics in nature. This interest stemmed in the main from investigators who were seeking to understand the mechanism of microbial antagonisms in soil and its relationship to soil-borne plant diseases. At present there is a renewed interest in the role that antibiotics play in their ecology of soil and in the life of the microbes that produce them.

Most soils, whether cultivated, grazed or forested contain large numbers of microbes[70]. BRIAN[6] has estimated that one gram of surface soil contains the following populations:

Bacteria	— 1 to 100 million	Fungi	— 50 thousand to a million
Actinomycetes	— 1 to 10 million	Algae	— 10 thousand to 50 thousand
Protozoa	— 100 thousand to a million		

Their numbers vary a great deal depending on soil type, depth of the soil, season of the year, rainfall and temperature[87,70]. Under extreme conditions, such as in tundra, salt flats, and barren soil due to high or low temperatures, or moisture, the population of the soil is relatively homogeneous and their numbers greatly reduced.

Antagonisms and Disease Control

Antagonisms exist between members of the soil microflora[70]. Numerous experiments have shown the difficulty in introducing a new organism into a normal soil which has an established indigenous population and, in contrast, the relative ease with which they can be introduced into a sterilized soil. For example, four isolates of *Pythium* pathogenic to alfalfa when introduced into sterile soil were not virulent in a similar nonsterilized soil[69]. A similar result was obtained by COOPER and CHILTON[12] with *Pythium arrhenomanes* root rot of sugarcane. If soil was first sterilized and then allowed to recontaminate before infecting it with the pathogen the severity of the disease decreased with time of recontamination. HENRY[80] observed similar effects with *Helminthosporium sativum* (Table 1). Many of the antagonistic microorganisms have also been studied as useful agents for the control of plant diseases[78].

Table 1. Antagonism and disease prevention in soil when unsterilized soil was added to sterilized soil.[1]

Unsterile soil added (g)	Pathogen recovered (%)	Foot rot (%)
0	100	47.6
trace	30	7.8
1	0	7.0
5	0	3.1
50	0	5.5

[1] HENRY[80]

Root secretions of higher plants also influence the development of the soils microbial population as pointed out by SCHMIDT[60,61]. The complexity of the microbial antagonisms is shown in the studies of LOCHHEAD and LANDERKIN[42] in which a network of mutual antagonisms was found for eleven strains of actinomycetes which were all chosen for being antagonistic to *Streptomyces scabies*.

Of the many organisms living in soil a large proportion of them have antimicrobial properties. A compilation of results made for this review from six studies on actinomycetes isolated from soils shows that from 1 to 52% of the isolates in different experiments inhibited other microbes. The greater the number of test organisms, the greater was the opportunity for any one isolate to exhibit its activity. Some species are difficult to inhibit and in one experiment of the more than 7,369 isolates, only 1% inhibited *Fusarium oxysporum*. In another series of studies of about 7,642 isolates, only 4% inhibited *Escherchia coli*. Experiments by JOHNSON[35,36] on the types of microbes antagonistic to *Pythium arrhenomanes*, showed that 36% were fungi, 33% were actinomycetes and 0.9% were bacteria. In another experiment, the compiled results were different, but still high for fungi and actinomycetes and relatively low for bacteria. BRIAN[5] pointed out that antibiotic-producing organisms were more common among the soil inhabiting fungi than among those fungi that were parasitic on aerial plant parts. CONNEL[11] surveyed soil bacteria antagonistic to *P. arrhenomanes* and found that of 5,638 isolates only 3.5% were antagonistic to the fungus.

The concept of microbial antagonisms has also been used by ANWAR[2] to understand the reason nursery plots for flax wilt caused by *Fusarium lini* retained their high disease-producing properties year after year whereas nursery plots for *Helminthosporium sativum* root rots of wheat did not and had to be reinfested every year. There was an inverse correlation between the number of antagonists to the respective pathogens and their longevity in soil. The many studies with *Trichoderma lignorum* and *T. viride* are especially convincing in pointing to the possibility that antibiotics can be produced by antagonists in soil and inhibit the growth of other soil organisms[22,23,73~76,77,1,5]. The use of specific antagonists which had antibiotic effects has also been tried in the control of *Ophiobolus graminis* on wheat[8,59] and *Botrytis cinerea* on lettuce[49]. Some fungi and bacteria that had been isolated from soil suppressed the disease in the same soil from which they had been isolated[59]. A number of these isolates were grown in culture and produced same material in the filtrate which suppressed the virulence of *O. graminis* on wheat growing in similar soil. Adding living cultures to soil were more effective than were the active filtrates of these cultures and many soil inhabiting fungi and bacteria suppressed the pathogenicity of the pathogen.

Sometimes the ability of a microbe to control a disease in the field or in greenhouse pots is not similar to the activity of the antagonist in culture. Various isolates of a *Chaetomium* that controlled *Fusarium lini* in the field were not strikingly antagonistic in culture[68]. Antagonists of pink root of onion also were variable in their ability to protect shallots against *Pyrenochaeta terrestris*[17]. BROADFOOT[8] found no correlation between the ability of bacteria or fungi, originally isolated from soil, to control ophiobolus root rot and their ability to inhibit the pathogen in culture.

Antibiotic Activity in Cultures and Disease Prevention

A large number of correlations have been observed on the protection of plants from disease by antagonists, the activity of such antagonists in culture, and the production of antibiotically active culture filtrates. When TVEIT and WOOD[68] applied *Chaetomium globosum* to seeds with an oat straw culture to

protect them against pre and post emergence killing by *Fusarium nivale*, the following results were obtained in greenhouse experiments and similar ones in field studies:

Application of autoclaved oat straw culture	— 29% emergence
Application of unautoclaved oat straw culture	— 86% emergence
Soaking in a filtrate of the culture	— 85% emergence

In corn, with *Pythium arrhenomanes* as the root pathogen, there was a positive correlation between the inhibition of the pathogen in artificial media and the control of disease in soil which had initially been sterilized. None of the non-antagonists consistantly reduced the disease severity. The actinomycetes were most effective inhibitors[38]. As the soil became recontaminated, the amount of disease decreased. For sugarcane rot, the antibiotic potential of the soil was correlated with the highest yields of cane[12,44,11]. Pythium root rot of sugarcane was greatly decreased when antagonistic actinomycetes were added to soil but was not decreased by nonantagonistic isolates.

As indicated, a large number of experiments have been reported in which plants have been protected from disease by adding to soil a microbe which was antibiotic to the pathogen in culture. To obtain such results, the soil into which the antagonist was placed had first to be sterilized or amended with nutrients or both. *Trichoderma ligorum*, *Penicillium patulum*, and a streptomycete, A67, were effective in both sterile and nonsterile soil if 1% glucose had been added to the soil[22,23]. However such results can sometimes be attributed to the direct inhibitory effect of one organism on another *e.g. Bacillus mesentericus* on *Glomerella cingulata* and *Sclerotium rolfsii*[53].

In an attempt to explain the observation that of two adjacent fields, one badly infested with *Pyrenochaeta terrestris*, pink root of shallots, and the other almost free of the disease, the populations of antagonistic actinomycetes in both fields were determined. FREEMAN and TIMS[17] found that of the 532 antagonistic actinomycetes isolated, 355 were from the clean field and only 177 were from the infested field. Of 38 antagonists tested in steamed and artificially infested soil, the disease control ranged from zero to 100% with different isolates. Thus, even though there was a positive correlation between numbers of antagonists and disease control, no prediction could be made for the ability of any one antagonist to protect the host from the pathogen.

Soil Fertilization and Disease Control

The ability of antagonists to aid in disease control has lead to attempts to fertilize soils with organic plant and animal residues thereby encouraging the growth of the antagonists to the detriment of the pathogen[58]. In such experiments there was usually a sharp increase in the mixed soil population of microbes following the treatment. Many studies have been carried out on the control of root rot of

Table 2. Disintegration of sclerotia of *Phymatotrichum omnivorum*[1] in soil.

Days of incubation	Sclerotia recovered		
	Unamended soil	3% Manure	3% Sorghum fodder
15	190	76	77
75	152	55	45

[1] Two hundred sclerotia were added on day 0.
[2] MITCHELL *et al.*[48]

Table 3. Effect of adding organic material to soil.[10]

Treatment	After 7 weeks		
	Bacteria (millions/g)	NO_3N (ppm.)	Available phosphorus (ppm.)
Control	58	13.4	21.4
Chicken manure	636	114.7	129.3

CLARK[10] Data taken from Table 1.

cotton caused by *Phymatotrichum omnivorum* by applying animal manures or green plant manures to infested soil. For example, treating a field which had 71.1% disease in 1921 reduced the incidence to 2.2% in 1924[39]. The green alfalfa manured plots evolved 19~152% more carbon dioxide, and had more abundant numbers of bacteria, actinomycetes, and some fungi. There was less of the pathogen in fertilized soils with much less mycelium and fewer sclerotia[40]. When sclerotia of the pathogen were added to manured soil, many disintegrated[48] and peak periods of antagonistic activity coincided with most intense microbial activity (Table 2). Apparently the rapid growth of microorganism in the manured soil was harmful to *P. omnivorum*.

Adding organic matter to soil has also been studied to control *Ophiobolus graminis* root rot of wheat. The treatment greatly increased the bacterial population, from 58 to 636 million per gram. This increase was accompanied by an increase in nitrate nitrogen and available phosphorus. Such treatments reduced the incidence of disease but the pathogen was not eliminated unless the soil was kept under conditions favorably for microbial growth but devoid of susceptible roots[10]. Control with animal and green manures was also shown by FELLOWS[16] (Table 3).

Potato scab control has also been attempted with strains of bacteria that made CZAPEK's media unfavorable for the growth of *Streptomyces scabies*[56]. Scab was controlled by green rye in some soils but not in others and SANFORD[57] suggested that the control was due to the antibiotic properties of the predominant soil microbes. That antagonism other than by toxic materials, can be involved in the control was shown by MILLARD & TAYLOR[47], using the pathogen *Streptomyces scabies*, and the saprophyte *S. praecox*. In sterilized soil the addition of *S. praecox* in increasing amounts reduced the population of *S. scabies* in the same order as well as reducing the amount of scab on the potatoes and green manures also decreased the amount of scab. That an increase in soil microflora could be responsible for such control was demonstrated by ROUALT and ATKINSON[56], but in this study only soybean manuring decreased disease (Table 4).

Table 4. Effect of green manure on soil microflora and potato scab.

Treatment	Population/g oven-dried soil			Disease incidence (%)
	Actinomycetes (millions)	Bacteria (millions)	Fungi (thousands)	
Control	20	41.9	350	48.0
Rye	17.5	67.5	450	44.6
Red clover	29.8	240.0	790	41.4
Soybean	35.5	221.0	1200	10.3

ROUALT & ATKINSON[56]

Toxins

On the basis of the evidence available, the production of toxins in soil usually seemed a reasonable explanation for the antagonists relationships among microbes. The possibility that microbially produced toxins are present in soil was not without precedence. GRIEG-SMITH[24] had postulated their existance as early as 1910 but this concept did not find ready acceptance. Much later, WAKSMAN and WOODRUFF[72] demonstrated soil extracts with antibacterial properties and HESSAYON[31,33] held that such general toxins played a regulatory role in soil populations. The complex patterns of antagonisms observed by GARRARD and LOCHHEAD[18] among soil actinomycetes were thought to be brought about by

toxic substances. Similarly the reduction of "take all" by filtrates of an organism antagonistic to *Ophiobolus graminis* was ascribed to a toxin[59]. Soil extracts were deleterious to *Fusarium oxysporum* f. *cubense*–inhibiting germination, hyphal growth and sporulation[67].

Lysis of some fungi by bacteria have also been attributed to the secretion of enzymes. Lytic agents were present in soil and these could lyse viable or dead fungi, but if the soil was first sterilized by steam or propylene oxide lysis did not occur[9]. Diffusible fungi-toxic substances produced by streptomyces spp. were believed to also play a role in the lysis of fungi in soil[43].

In this early period, the strongest case for the formation and role of an antibiotic in soil was that of gliotoxin which was synthesized by *Trichoderma lignorum*. The infestation of soil with this fungus reduced the incidence of damping off of citrus seedlings caused by *Rhizoctonia solani*[73~76,77,1].

In nutrient culture, *T. lignorum* was antagonistic to the pathogen and caused lysis. The toxic principle was isolated and crystallized and the pure compound had properties similar to that of the filtrate. One difficulty in attributing the antagonism of *T. lignorum* solely to the antibiotic is the fact that the antagonist also directly parasitizes *R. solani*. This parasitism has been observed to occur, in the main, when the host mycelium was old and perhaps moribund due to the toxin[74,4].

Another impressive series of experiments in the toxin-soil area are those of the Wareham heath soils[5] on which conifers cannot grow well because of the absence of mycorrhizae. This in turn, is due to the scarcity of the appropriate hymenomycete mycelium. If soil is sterilized and an appropriate organism added, mycorrhizae form and pine seedlings develop. If a little unsterilized soil is added, the soil becomes "toxic" and growth of the plants is very poor. Normally, these soils contain a diffusible fungitoxin and the pine seedlings do not develop unless the soil is first detoxified. A number of fungi which produce gliotoxin, such as *Penicillium janczewskii*, and *P. terlikowskii* and one producing griseofulvin occur in and have been isolated from these heath soils. The toxins present in the soil appear to be associated with its microflora. A fungitoxin, produced in soil which was inhibitory to *Fusarium oxysporum* f. *cubense*, was related to the presence of the soil flora. All factors that reduced the numbers of these microorganism also reduced the toxicity of the soil or its extracts.

Though the data supporting the production and function of antibiotics in the soil is impressive, early warnings against the ready acceptance of an antibiotic hypothesis are in the literature. It had been pointed out that no strong evidence existed for antibiotic production in soil, and that penicillin or the *Penicillium* sp. producing it and the sensitive bacterium *Staphylococcus aureus* did not occur together in soil. WAKSMAN[71] held that there was no firm evidence for the production of specific metabolic products that gives the organisms producing them advantages in regard to available food or space. He worried

Table 5. Growth of *Bacillus subtilis* in the presence of a streptomycin-producing strain of *Streptomyces griseus*.

Incubation (days)	Viable cells in millions per gram soil	
	B. subtilis	*S. griseus*
0	20×10^{-5}	16
16	4.5	84
31	0	251
45	0	254

SIMINOFF & GOTTLIEB.[62]

Table 6. Growth of *Bacillus subtilis* in the presence of a nonproducing strain of *Streptomyces griseus*.

Incubation (days)	Viable cells in millions per gram soil	
	B. subtilis	*S. griseus* RM3380
0	37.5×10^{-5}	705
9	0	970
18	0	1,635
31	0	850

SIMINOFF & GOTTLIEB.[62]

whether soil contained enough nutrients for antibiotic production which usually occurred in highly artificial environments. However, PARK[50] pointed out that soil extracts do support the growth of fungi. But supporting growth is different from supporting antibiotic production. Nevertheless, the general concensus of opinion agrees with STALLING[65] that antibiotics are produced in soil and furnish protection in competative situations by eliminating other organisms in soil.

The data presented until now are without doubt strongly suggestive that antibiotics are produced in soil and play a role in its ecology. With the increasing discovery of new antibiotics that are produced by soil microbes has come an almost unquestioning belief in the hypothesis that they are made in soil and function there[41]. Other hypotheses for antagonism such as competition for food and for *lebensraum* are rarely considered. Now however, we are in the better position to re-examine the concept that antibiotics are formed in soil because many of the antibiotics have been chemically characterized and new sensitive methods are available for their detection.

The ideal evidence would be the identification of a known antibiotic in normal unaltered soil or at least in soil that had only been altered by usual agricultural practices. Very sensitive analytical methods would be needed for such studies and soil extracts from large quantities of soil could then be concentrated to a level that would be great enough to theoretically detect antibiotically active quantities. The next evidence in order of choice would be to infest an unaltered soil with an organism that is known to produce a specific, well characterized antibiotic in artificial culture and then seek for it in soil after an appropriate incubation period. I have seen one experiment in which this methodology was almost met. An actinomycete that was isolated from soil, produced an antibiotic in liquid shake culture. When the actinomycete was placed in nonsterile soil, and the inhibitory material extracted, this antibiotic had the same Rf as the original compound in the liquid culture, unfortunately the antibiotic was never identified.* The difficulty in such experiments would be for the antibiotic producer to establishing itself among the normal microbial components of the soil; because of this, most experiments are done on soil that has been sterilized.

Another criterion for the presence of a specific antibiotic in soil has been suggested by STEVENSON[66] for compounds which cause unique morphologic changes such as stunting, distortion, swollen areas, hyphal protruberances, or the curling of germ tubes. Unfortunately, most antibiotics are not that specific, though one such exception could be the curling of germ tubes caused by griseofulvin at low concentration. STEVENSON chose soil actinomycetes that were antagonistic to *Helminthosporium sativum*. Using the buried slide technique he found effects on hyphae in soil similar to those produced in culture even though the antibiotic could not be extracted from soil containing the antagonist. *Streptomyces antibioticus* was thus shown to produce actinomycin in soil. The explanation for the morphological affect and yet the inability to extract the antibiotic, was the accumulation of a relatively high concentration in the immediate vicinity of the streptomycete and the presence there of the susceptible microbe.

Population Growth

Changes in populations of soil microbes after sterile soil has been infested with them often lead to results that are difficult to interpret. SIMINOFF and GOTTLIEB[62] found that when a strain of *Bacillus subtilis* sensitive to streptomycin and a streptomycin-producing strain of *Streptomyces griseus* were

* These materials were seen in the laboratory of Dr. N. A. KRASSILNIKOV, Institute of Microbiology, U.S.S.R. Academy of Science.

introduced into sterile soil, *B. subtilis* did not multiply and died out whereas *S. griseus* increased (Table 5). One could then hypothesize that the decrease of the *B. subtilis* was brought about by the production of the antibiotic streptomycin in soil. But no such antibiotic could be detected in the soil nor could this *B. subtilis* which is very sensitive, be inhibited by as much as 500 μg antibiotic/g soil. Furthermore a streptomycin-dependent strain of *Escherichia coli*, requiring the antibiotic for growth, also did not grow in soil culture indicating the absence of free antibiotic. In addition, the *B. subtilis* did not grow and died out when soil was simultaneously infested with a mutant strain of *S. griseus* which did not produce the antibiotic (Table 6). Apparently the antagonism between the microbes was not due to the production of free streptomycin in soil.

Similar results were obtained with a *B. subtilis–Aspergillus clavatus* system. *A. clavatus* in culture, or in amended soil, produced the antibiotic clavacin (patulin) but produced no detectable antibiotic in unamended soil. Nevertheless the presence of the fungus prevented growth of the bacillus[21]. A system in soil of *B. subtilis* and *S. venezuelae* (a producer of chloramphenicol) allowed the growth of the bacillus as well as of the streptomycete. Small quantities of the antibiotic were produced by the mixed populations as well as by *S. venezuelae* alone but only after long periods of incubation[20]. It is possible that the antibiotic was produced too late to inhibit the multiplication of the bacillus in this system.

Soil has a protective affect even on those microbes that are sensitive to antibiotics *in vivo*, as we have seen with streptomycin. Similar protection occurs with terramycin and aureomycin[45] with chloramphenicol[70] with circulin, subtilin, neomycin, viomycin[46] and less with actidione and clavacin[21]. Even actinomycin which remains active in soil was somewhat protected in that milieu.

Production of Antibiotics in Unaltered Soil

Normal soil which has not been treated except for infestation by an antibiotic-producing microbe is rarely, if ever, a good substrate for antibiotic production. *Penicillium patulum* did not produce any antibiotic unless the soil was first sterilized and amended with a nutritive supplement[25~29]. *Fusarium vasinfestum* also could not produce the antibiotic fusaric acid unless these conditions were met[38]. *Streptomyces griseus* failed to produce cycloheximide and *Aspergillus clavatus* failed to produce patulin unless both conditions were present[21]. The few cases of production in unaltered soil are not definitive. The data on the formation in soil of trichothecin by *Trichothecium roseum* is based on some characteristic activity of the antibiotic action on *Fusarium oxysporum* not on the isolation of the compound[31~33]. The compound of KRASSILNIKOV previously mentioned as found in normal soil, has never been identified to my knowledge.

As far as I have been able to ascertain there is no unequivocal evidence that normal, nonsterilized and nonamended soil contains any of the known, identifiable antibiotics, although as previously indicated, unknown toxic materials do occur in soils. The most extensive studies in this area have been made with chloramphenicol[13,14]. Soils from 91 cultivated and grassland sites in nine states of the United States and from 13 other countries were infested with *Streptomyces venezuelae* and similar samples were not infested. No chloramphenicol was identified in extracts from either of these seeded and nonseeded soils even though the lower limit of detection was 0.3 mcg/g of soil. If the soils were sterilized, before seeding, chloramphenicol was found and identified in the infested soils. In other experiments, more sensitive analytical procedures allowed the recovery of 0.05 mcg/g of soil but again chloramphenicol was not found.

Experiments on large previously untreated soil samples that were infested with *S. venezuelae* revealed decreasing numbers of this streptomycete in normal soil and an increase in sterilized soil; correlated with these was the absence of the antibiotic in normal soil and its increase with time in sterilized soil. The isolates recovered from the normal soils were capable of producing the antibiotic in shake culture. Field experiments in which soil *in situ* that contained no chloramphenicol was infested with *S. venezuelae* and large soil samples, (1~2 kg of topsoil) were examined. The population did not increase with time nor were significant amounts of chloramphenicol detected.

A special study was made of soils from which *S. venezuelae* cultures capable synthesizing the antibiotic, could be isolated. However, no chloramphenicol was found in these soils. Furthermore, a study of 110 diverse soils from various sites, did not show the presence of chloramphenicol.

Nonsterilized and Amended Soil

A few antibiotics are produced even in nonsterilized soil if some nutrients are added to the soil. Under these conditions, the organism that has been added to the soil is in difficulty for it must compete with the indigenous microflora to establish itself in populations great enough to produce detectable quantities of the antibiotic. GREGORY[22,23] showed that the fungi *Trichoderma lignorum*, and *Penicillium patulum*, a streptomycete, A67, and a *Bacillus*, B6, produced antibiotic activity when 100 g of nonsterilized soil was amended with a mixture of soybean meal, 0.5 g; glucose, 0.5 g; calcium carbonate 0.2 g; cornsteep liquor, 0.15 ml.

There is here a difficulty of interpretation for these amendments themselves constitute a more or less typical medium for antibiotic production in laboratory media. In sterilized media, various other nutrients also supported the production soil, but only *P. patulum* produced the antibiotic in their absence. A strain of *Trichoderma viride* which produced gliotoxin *in vitro* was also capable of producing this antibiotic when clover was added to an acid soil that had not been sterilized[79].

When various seeds were inoculated with *T. viride* and planted in soil, gliotoxin was identified in extracts from the seed coats. Inoculation of pea seeds with the *T. viride*, *P. frequentans* and *P. gladioli* produced gliotoxin, frequentin, and gladiolic acid, respectively, in their seed coats. In other experiments, *T. viride* growing naturally in soil apparently infested the pea seeds and produced gliotoxin in the seed coat[81].

Production in Sterile Soil

Some antibiotics can be produced in soil that has been treated only by sterilization. Chloramphenicol, for example, was detected in soil that had been sterilized, then infested with *Streptomyces venezuelae* and incubated for a long period[20]. *P. patulum* produced traces of antibiotic activity under similar conditions[21,22]. *Trichoderma viride* synthesized gliotoxin in sterilized but non-amended soil[15] and *Trichothecium roseum* did the same for trichothecin[81].

Sterilized and Amended Soils

Sterilized and amended soil will support antibiotic production in some systems. In sterile soil, *Streptomyces griseus*, for example, though not producing cycloheximide in the absence of amendates, nor in the presence of oat or alfalfa straw, produced it after the addition of soybean meal to this soil. With increased amounts of the soybean, increasing quantities of cycloheximide were produced[21].

Aspergillus clavatus did not produce clavacin in sterilized unamended soil nor with anyone of a variety of amendates such as straws, alfalfa hay, glucose, or tryptone but did so when brown sugar was used. Other examples of production in sterilized amended soils are the researches of GROSSBARD[26] and of KALYANSUNDARUM[28] that have been previously mentioned.

Microbiological Degradation in Soil

Even if antibiotics were produced in normal soils, their role in antagonisms would still be moot. There is, for example, the question of concentration—which might be too low to affect the soil's microflora. A second disturbing feature would be the instability of the antibiotic, an observation that has been frequently made[3,25]. The relative inactivity of some antibiotics in soil also must be taken in account in claiming their role in regulating the microflora of the soil[45,46].

One factor in the decrease of antibiotic concentrations in soil is their degradation by its microflora, a phenomenon that has been shown for a number of antibiotics. Adding high concentrations of streptomycin to nonsterile soil, resulted in its gradual decrease; more than half of the antibiotic disappeared within two weeks[54,55]. In sterile soil, chloramphenicol concentration remained constant for 14 days but in nonsterile soil it decreased rapidly within 3 days and almost entirely disappeared within 14 days[20]. Similar results occurred in experiments with exogenous clavacin in soil[19]. In sterile soil, griseofulvin was stable for long periods but was so completely broken down in nonsterilized soil that all the chlorine of the molecule was released[33]. If either the streptomycin or chloromycetin soils were again and successively treated with more antibiotic each such addition was more rapidly degraded than the previous one. In both experiments microbes could be isolated that could carry out the degradation in culture. The organism responsible for vitiating griseofulvin depended on the pH of soil; at relatively high pH a bacterium, and at low pH a fungus seemed to be the organism carrying on the degradation.

With some antibiotics the results are not as clear. Practically all the added cycloheximide was lost in nonsterilized soil, but 70% also disappeared in the same time in sterilized soil. Apparently, only a portion of the breakdown was caused by the microflora[21] and most of the antibiotic reacted with nonliving elements of the soil. Another example is the decreased activity of streptomycin in muck soil[62], similar phenomena have been attributed to the clay content of even such high organic matter soils[51]. Data are available which indicate that biological degradation is most effective in soils of high organic content[34]. Tricothecin was also readily inactivated by soil, but at the same rate whether or not the soil was sterilized. The effect was attributed primarily to adsorption on soil particles and not to a chemical or microbiological breakdown[32].

Adsorption in Soil

Another well documented mechanism by which inactivation of antibiotics occurs in soil is their adsorption on its clay components[63,64]. Various clays make up different percentages of the soil and some of these are highly adsorptive. This is in part because of the negative charges on the clays to which positively charged compounds can be bound. Basic antibiotics, or amphoteric ones under acid conditions, are readily bound and thus inactivated[62,51,52]. The antibiotics spread the crystal lattices of the clays, neutralize their charges and flocculate them. Such basic antibiotics as streptomycin, kanamycin and neomycin are strongly adsorbed. Aureomycin, terramycin and bacitracin are amphoteric and their binding depends on the soil pH[45,51,52]. Small amounts of the adsorbed antibiotic can be replaced by

other basic molecules such as methylene blue and janus green. Adsorbed streptomycin is not biologically active[62] though under the proper conditions some inhibitory activity has been shown[54]. This probably is due to the removal of the antibiotic from the clay.

As would be expected, acidic antibiotics such as clavacin are not bound to clays[21] nor are neutral ones such as chloramphenicol[20] and cycloheximide[21,51,52]. The equal inactivation of trichothecin in sterilized and nonsterilized soil have also been attributed to adsorption[33]. Culture filtrates containing unidentified antibiotics have also been inactivated by the addition of clay[63,64].

The inactivation in soils follows mainly from the results that would be expected on the basis of the nature and condition of their clays[51,52]. Acid washed quarts adsorbed very little streptomycin, a sandy soil only moderate amounts, and an illite clay colloid, the most; two loam soils were intermediate[62]. Again, the reactions are not always consistant. A muck soil that contained only small amounts of clay, if any, inactivated or removed as much streptomycin as did loam soils with a high percentage of clay.

Other antibiotics such as subtilin, circulin and viomycin, which are peptides, were inactive in soil at concentrations at least as high as 500 μg per gm soil. In contrast, actinomycin, also a peptide, was active at very low concentrations[46]. Actidione is inactivated in sterile soil, though it is not adsorbed on clays. Apparently, the organic components of the soil play an important, though as yet unknown, part in the inactivation of antibiotics.

Discussion

Germane to the question whether of not antibiotics are normally produced and play a role in the ecology of soils is the definition of normal unaltered soil. That sterilized soil is not normal would be generally accepted and the production of an antibiotic in sterilized soils would not indicate that the same thing happens in nature. Similarly, we would not have much difficulty in recognizing that soils to which have been added organic plant and animal materials in the proportions used in laboratory media for antibiotic synthesis are no longer natural. On the other hand, if antibiotics could be found in uncultivated forest or grassland soils, one would readily accept the concept that antibiotics are produced in nature. In between these extremes there is more difficultly in trying to determine what is a natural state of soil.

A few examples would be pertinent to the problem. Would the incorporation of wheat straw into soil, a common tillage process, change that soil so that it would not longer be normal? *Trichoderma viride* produces gliotoxin on wheat straw in soil and, more important some antibiotic is found immediately adjacent to the straw[82]. Should one then accept this situation of straw in soil as a normal situation? If so, could we extend this concept of normality to alfalfa or clover amendates to soil for patulin synthesis in such soils[72]? Or even more, what shall we say about amendments such as soybean meal from the harvested beans[22,23]? Fruits and seeds of various plants naturally fall to earth, and become incorporated in soil; certainly this is a normal condition under any definition[79~83]. Could one then use the fact that *Penicillium expansum* produces patulin in apples in the laboratory[7] as evidence of its production in soil? Though this claim has not been made, its acceptance should follow if one accepts the concept that apples also fall to the ground and are decomposed in soil.

The antibiotic gliotoxin is produced in wheat, mustard, and pea seeds when they are inoculated with the fungus *Trichoderma viride* and sown in soil[81]. Furthermore, the prevalence of a *Pythium* rot of white mustard was reduced more in the presence a high gliotoxin-producing strain than in the presence of a nonproducer[80]. A number of other microbes also produced identifiable antibiotics in the seeds; *Penicillium frequentans*, and *P. gladioli* under similar conditions made frequentin and gladiolic acid, respectively. Another example of the production of antibiotics in nature is seen with a *T. viride* which was normally present in a soil where this fungus infected pea seeds sown in that soil. Under such conditions, gliotoxin was also identified in the seed coats of naturally soil infested seeds. In this case we

would certainly accept the thesis of antibiotic formation in soil under natural condition, once we accept the presence of seeds in soil as a natural phenomenon.

In my view of the evidence now available, the data still do not allow us to accept the thesis that antibiotics are naturally produced in soil and function there in antagonistic capacities. What shall one say about chloramphenicol on which most intensive studies have been made. Yet the antibiotic was never found in untreated soils in high enough concentrations to be detected. It is extremely doubtful that even if this antibiotic were, present in lower concentration, below levels of detection, it would antagonize some other members of the microflora in soil.

Wherever, an antibiotic has been demonstrated in soil there has always been some modifying factor to prevent an unequivocal demonstration of its presence in soil. Among these were 1) the evidence from disease control in which the proof is indirect, 2) the presence of a high population of antibiotic-producing organism in soil, is not in itself proof that the microbes produce such compounds in nature, 3) the decrease of sensitive microorganisms in the presence of a producing strain can be ascribed to other phenomena than the secretion of an antibiotic, 4) the use of sterilized soil is not natural, and 5) the use of nutritive amendments in high concentration is not normal to the microbiology of soil.

Even if antibiotics were produced in normal soil some of them might not play an ecological role because of inactivating mechanism in soil such as biological degradation, adsorption on soil clays, and reactions with the organic matter in soil.

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