

ANTAGONISTIC RELATIONS OF MICROÖRGANISMS¹

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Within recent years, there have appeared a number of reviews dealing with the antagonistic interrelations among various microorganisms; some of these have dealt with certain specific groups, and others with a great variety of organisms. It is sufficient to mention Holman (155), Buchanan and Fulmer (41), and Nakhimovskaia (231) on bacterial associations, Seitz (303) on mixed infections, Brown (40), Novogradsky (240), Weindling (355), Porter and Carter (261) and D'Aeth (64) on competition among fungi, Garrett (118) and Garrard and Lochhead (114) on the interrelations between soil-borne and disease-producing fungi, Nakhimovskaia (230) on antagonisms between actinomycetes and bacteria, and Waksman (345) on associations and antagonisms among microorganisms in different habitats. In this review, an attempt is made to present the broad antagonistic relations between microorganisms living in association, either in simple mixed cultures or in complex natural populations; the

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significance of these associations in natural processes; their relation to disease production in man as well as in domesticated plants and animals; the chemical nature of the active substances produced; and finally, the nature of the antagonistic action.

SURVIVAL OF HUMAN AND ANIMAL PATHOGENS IN SOIL AND IN WATER BASINS

Microbes capable of causing disease find their way into the soil and into water basins in very large numbers, either in the excreta of the infected host or in the dead and infected residues of the latter. If one considers the millions of years that animals and plants have existed on this planet, one can only surmise the great numbers of microbes causing the numerous diseases of all forms of life that must have thus been introduced into soils and surface waters. What has become of all the bacteria causing typhoid fever, dysentery, cholera, diphtheria, pneumonia, bubonic plague, tuberculosis and leprosy in man, mastitis and abortion in cattle, and of numerous diseases of other animals? This question was first raised by medical bacteriologists in the eighties of the last century. The soil was searched for the presence of bacterial agents causing infectious diseases and responsible for epidemics. The results obtained established beyond doubt that, with very few exceptions, organisms pathogenic to man and to animals do not remain alive in the soil for very long.

It is true that a few disease-producing microorganisms are able to survive in soils for considerable periods of time. One need only mention the organisms causing tetanus, gas gangrene, skin infections, actinomycosis and blackleg in cattle, coccidiosis of poultry, hookworm infections, trichinosis, enteric disorders in man. To these must be added certain bacteria, actinomycetes and fungi, which cause a variety of plant diseases such as potato scab, root rots, take-all of cereal crops, and the damping-off diseases of vegetables. However, the great majority of disease-producing microorganisms are able to remain in an active and reproducible state only for short periods outside of their respective hosts, and especially in soils and in natural waters. It is sufficient to cite the fact that typhoid and dysentery bacteria, which are known to contaminate watersheds and water supplies, sooner or

later disappear. No one now raises the question concerning the role of the soil as the carrier of these disease-producing agents or as the cause of severe or of even minor epidemics. This rapid disappearance of disease-producing bacteria may be due to several factors, such as (a) unfavorable environment, (b) lack of sufficient or proper food supply, (c) destruction by predacious agents, such as protozoa and other animals, and (d) destruction by various saprophytic bacteria and fungi considered as antagonists.

Frankland (108) was the first to establish that the typhoid bacterium may survive in sterilized polluted water or in pure deep well water for 20 to 51 days, but that it dies out rapidly in 9 to 13 days in unsterile surface water. Jordan and his associates (171) found that *Eberthella typhosa* survived in sterilized tap water for 15 to 25 days, as against 4 to 7 days in fresh water; it died off even more rapidly (in 1 to 4 days) in raw river or canal water. The degree of survival of this organism in water was found to be in inverse ratio to the degree of contamination of the water, the saprophytic bacteria being directly responsible for the destruction of the pathogen (292). Freshly isolated organisms survived a shorter time than laboratory cultures; and higher temperatures were more destructive than lower ones (163).

The presence of certain bacteria in water is often found to hinder the survival of *E. typhosa* (336). When *Pseudomonas aeruginosa*, on the other hand, is present in drinking water, it may not be accompanied by any other bacteria (280). Media inoculated with this organism and with *Escherichia coli* gave, after 13 days cultivation, only cultures of the former; however, the two organisms can coexist in sterilized water. *Vibrio cholerae* does not survive very long in fresh water, although long enough to cause occasional epidemics (139).

Typhoid and paratyphoid bacteria were found (368) to have only a very short life in sewage sludge, a reduction of 99% being reported after 6 hours' treatment of activated sludge (319, 153). There is a marked difference in the survival of different strains, Ruchhoft (290) having shown that whereas one strain died off very rapidly, two others died only in 8 to 10 days, and one survived for 13 days.

The addition of typhoid bacteria to a well-moistened and

cultivated soil brings about their rapid destruction (207). The same phenomenon occurs when a culture of these organisms is added to that of a soil microbe. An antagonistic relation is often found to exist in some soils but not in others, this being traced to the presence of specific bacteria. Frost (110) also reports a marked destruction of typhoid bacteria added to the soil, 98 per cent of the cells being killed in six days; it is suggested that in the course of a few more days all these cells would have disappeared from the soil. This is also true of sea water (167). On the other hand, under conditions less favorable to the antagonists, the typhoid organism survives not only for many days, but even for months.

E. coli is rapidly crowded out by other organisms in manure piles (227) and in soil (298, 308, 349). The dysentery and typhoid organisms disappear rapidly in sea water, namely in 12 and 16 hours; the paratyphoid organisms have been found to survive for 21 and 23 days (330). Sea water appears to contain an agent, other than its salts, which exerts a bactericidal effect (372).

Under conditions prevailing in southern England, *Mycobacterium tuberculosis* was found (364) to remain alive and virulent in cow's feces, exposed on pasture land, for at least five months during winter, two months during spring and four months during autumn; in summer, no living organisms were demonstrated even after two months; under protection from direct sunlight, the survival period was longer. Bovine tubercle bacteria have been detected in soil and manure, and on grass up to 178 days after infection, but not later (204). When *M. tuberculosis* was added to non-sterile soil, it was slowly destroyed (278, 313); the plate count was reduced to about one-sixth of the original in one month (33). *Brucella melitensis* survived in sterilized tap water for 42 days, as compared to 7 days in unsterilized water; it survived in sterilized soil 72 days, as compared to 20 days in unsterilized soil (161).

In spite of the gradual and even rapid destruction of some pathogenic microorganisms in the soil, the survival of others presents important problems to farmers raising hogs, cattle, poultry and other domestic animals. In order to overcome this

condition, the rotation of crops is usually practiced; several years are usually required to render infected pastures safe for use. A better understanding of the antagonists that are responsible for the rapid destruction of pathogenic organisms in the soil may throw light upon this problem and improve the methods of control.

SYMBIOSIS AND ANTIBIOSIS

In a natural milieu, such as soil and water, which is inhabited by a mixed microbiological population, numerous relations of association and antagonism occur. All organisms inhabiting such a medium are affected, directly or indirectly, by one or more of the other constituent members of this population. These relationships were at first visualized as due primarily to competition for nutrients, as was well expressed by Pfeffer (256), who said "the entire world and all the friendly and antagonistic relationships of different organisms are primarily regulated by the necessity of obtaining food." De Bary, in 1879, was the first to emphasize the significance of the antagonistic relations among microorganisms (66). When two organisms are grown on the same substrate, one overcomes the other sooner or later, and even kills it. The limited food supply in the culture medium was believed by many to be responsible for this (194), the fast-growing organism being favored as compared to the slow grower. Kruse (187) also suggested that this is a problem of food competition. When two organisms are capable of utilizing the same nutrients, but are differently affected by environmental conditions, such as reaction, air supply and temperature, the one that finds conditions more suitable for its development, will grow more rapidly and in time be able to suppress the other.

However, it soon became clear that antagonism among microorganisms embraces phenomena other than mere competition for or exhaustion of nutrients. E. F. Smith (309) pointed out, for example, that when two or more organisms live in close proximity, they may exert mutually antagonistic, indifferent or favorable effects. According to Porter (260), the effects produced upon each other by fungi in mixed cultures may be due to the formation

of substances which exert detrimental or beneficial effects. Lasseur (189) regarded antagonism as a very complex phenomenon: a result of numerous and often little known activities; it influences the morphology of the organism, the capacity for pigment production, and various physiological processes.

The terms "association" and "symbiosis" are used to designate mutually beneficial relations, as contrasted to "antagonism" and "antibiosis," which refer to a reduction in growth and in activities, as a result of the living of organisms in mixture (235). Bacteria may respond to such effects by exhibiting temporary or permanent modifications in their physiological characteristics (311). The morphology of diphtheria organisms may be influenced thereby, often accompanied by a reduction in virulence (151). Certain bacteria will form abnormal morphological cells under the influence of antagonistic actinomycetes (230); these changes are not hereditarily stable. Pyocyanase brings about morphological changes in *B. anthracis* (95). Penicillin, by inhibiting fission of bacteria, leads to abnormal growth of the cells, followed by autolysis (113). Different fungi will favor perithecia formation by other fungi and will influence the germination of ascospores (9, 276). Pigment formation by *P. aeruginosa* may be weakened in the presence of other organisms; *E. coli* may lose the property of fermenting sugar when grown together with paratyphoid bacteria (168). This type of antagonism has often been referred to as "functional antagonism" (231). The production of inactive lactic acid by *d*- and *l*-acid-producing bacteria, in the presence of certain anaerobes (323), as well as the formation of lactic acid by butyric acid bacteria, in the presence of other organisms (315), are other illustrations. "Synergism" is used to designate the living together of two organisms, resulting in a change which neither alone could bring about (155).

The injurious effects of one organism upon another range from antagonisms, of varying degrees of intensity, to the living or preying of one upon the other; the latter phenomenon may be classified with pathogenicity and disease production. Various types of antagonism have thus been recognized (231): 1. Antagonism *in vivo* vs. antagonism *in vitro*; the former being often desig-

nated as antibiosis (189). 2. Repressive, bactericidal and lytic forms of antagonism, as well as antagonism of function *vs.* antagonism of growth. 3. Direct, indirect and true antagonism. 4. One-sided and two-sided antagonism; antagonism between strains of the same species and among different species, or iso- and hetero-antagonism (88).

Duclaux (79) was among the first to demonstrate that the growth of a fungus renders the medium unfavorable to the subsequent growth of the same organism. Species of *Peziza* and *Aspergillus* have an antagonistic effect upon one another, which, according to Reinhardt (273), is a result of the production of acid, chiefly oxalic. Nikitinsky (238) suggested that the inhibiting effects are due to unfavorable changes in reaction. Culture solutions in which fungi have grown are not suitable for the germination of freshly inoculated spores and are improved by boiling (188); not only the same organisms, but also other species are checked in their growth (32).

Eijkman (88) demonstrated that bacteria produce in the medium thermolabile toxic substances; when such a medium is heated, it is again made suitable for bacterial development; the fact that growth is not as good as in fresh medium was explained as due either to exhaustion of some of the nutrients or to the formation of injurious substances. Bacterial spores are able to germinate again in the same medium, if this is boiled. Certain bacterial metabolic products, even when heated to 120°, have a strong influence upon the growth of various microorganisms (228).

According to A. J. Brown (37), staling of a culture is due not to the accumulation of metabolic products but to the exhaustion of substances which stimulate growth, as in the case of oxygen for yeast. Pratt (263) concluded that exhaustion of food is not a primary factor in staling; the latter is due largely to the formation of bicarbonate (39, 125). This effect is partially corrected by boiling and by adjustment of the reaction; treatment with ether removes the residual staleness; colloidal clay and charcoal also remove this effect. The phenomenon of staling was sometimes spoken of as "vaccination" of the medium (14, 275), and believed to be due to protein degradation products.

Fungi may produce (188, 202) not only growth-inhibiting but also growth-promoting substances; by means of certain procedures, it is possible to separate one from the other (244). The tendency of fungus hyphae to turn away from the region in which other hyphae of the same fungus are growing (negative chemotropic reaction) has been explained (111, 135) as a response to chemical substances produced by the growing fungus.

The repressive type of antagonism results in a delay in the growth of the antagonized organism (231, 109). The bactericidal type results in the destruction by the antagonist of another organism without producing any lytic effect, *Bacillus mesentericus* (*vulgatus*) being able not only to depress but also to kill diphtheria and pseudo-diphtheria bacteria (373). The production by an antagonist of metabolic products which possess lytic properties and which modify the growth of various bacteria has been designated as "direct antagonism." On the other hand, "indirect" or "passive antagonism" has been looked upon (231) as depending not upon the direct action of the antagonist but upon changed conditions of culture which become unfavorable for the particular organism (150); here belong changes of pH and rH values, and the impoverishment of nutrients in medium. Neufeld and Kuhn (236) limited direct antagonism to those phenomena where the action is due to the living cell itself, as in the repression of anthrax bacilli by intestinal bacteria (142).

Bail (11) suggested that there exists, for every bacterium, a typical constant number of living cells capable of living in a given space. When this concentration (M) is reached, multiplication comes to a standstill without the nutrients being exhausted or toxic substances produced. The same is believed to hold true when two bacteria live together (363). If the limiting concentrations of the two organisms are different, the one with a higher M value will repress the other; the weaker species may check the stronger one when planted in a sufficient excess (98). It has been suggested (144) that "biological activity" and "competitive capacity" must also be taken into consideration.

Antagonism may be either one-sided or two-sided, namely, when only one bacterium represses another, which is not antagon-

istic to it, or when each organism represses the other (115). One-sided antagonism may become two-sided under certain conditions of culture. *E. coli*, for example, is antagonistic to *E. typhosa*; however, if the latter is inoculated into a medium somewhat earlier than the former, the reverse is true (343, 122). Although antagonistic effects have usually been observed for one species of bacteria against another, very often one strain of the same species may exert antagonistic effects upon another strain (144, 221). Non-flagellated variants of typhoid bacteria are repressed by a flagellated form, smooth variants of paratyphoid bacteria by rough variants, etc. The fact that all bacterial cultures stop growing after a certain period of time has also been interpreted as a result of antagonism of some cells upon others. When the filtrates of such cultures are added to fresh nutrient media they may stop the growth of the same species as well as that of other species. Rahn (268) observed that the phenomenon of iso-antagonism is associated with the formation of a thermolabile substance, not passing through a filter. This substance is often destroyed by light (88).

Among the various types of antagonism, the most definite and the one which is best understood is that which results in the formation of antagonistic substances. The nature of these substances or toxins when produced by different bacteria and fungi is not always the same. Some are destroyed by boiling, by exposure to light or by filtration (202); others are resistant to heat and to ultraviolet rays; some are readily adsorbed by filters, from which they can be removed by special solvents.

The abundance of antagonistic substances produced by many fungi and bacteria is (97) greatly influenced by the energy and nitrogen sources in the medium. Schiller (300, 301) believed that antagonism could be induced by withholding certain nutrients: in a dilute glucose solution without nitrogen, yeasts were said to be "forced" to kill and digest bacteria, if a few loops of yeast suspension were added to a fully developed bacterial culture; the yeasts produced a bacteriolytic substance which was also active outside of their cells. On the other hand, various bacteria killed the yeasts when inoculated into cultures of the latter sus-

pended in distilled water. The destruction of the fungus *Ophiobolus*, causing the take-all disease of cereals, by soil organisms is believed to take place in a similar manner (116).

It has often been observed that certain organisms produce pigments in the presence of others, and that these pigments are in some way associated with the phenomenon of antagonism. *V. cholerae* produces, in the presence of *Sarcina lutea*, a dark violet pigment which is accompanied by an increase in agglutination and virulence (159, 278a). The destruction of *Dictyostelium mucoroides* by a red-pigment-producing bacterium is accompanied by intense pigmentation (258); the blue pigment of *Bacterium violaceum*, however, only delays the growth of the fungus. *Penicillium africanum* (72) produces a more intense pigment in contact with other fungi, such as *Aspergillus niger*; this pigment accumulates in the mycelium of the latter, which may thereby be killed. Nadson (229) demonstrated that the pigment of *Penicillium luteum* or *Spicaria purpurogenes* is used not only for purposes of protection, but also for attack upon other organisms, whereby the latter are killed and stained.

The various theories concerning the mechanism of antagonism may be summarized (231) as follows:

1. Exhaustion of nutrients in medium (249, 250, 109, 121).
2. Physico-chemical changes produced by the growing organism in the medium, including changes in osmotic pressure, surface tension, oxidation-reduction potential and reaction (8, 307, 109, 22, 28, 210, 334).
3. Production of specific enzymes, either by the antagonist itself or as a result of autolysis of the antagonized cells.
4. Production and liberation of specific substances, which have a selective bacteriostatic and bactericidal effect, or fungistatic and fungicidal action (36, 115, 109, 93, 77, 348, 104, 356).
5. Certain types of reactions, which may be designated as action at a distance (3, 253).
6. Space antagonism.

A great number of methods have been developed for measuring antagonistic action (109, 115, 314, 110, 265, 193, 213).

Porter (260) recognized that different organisms exhibit vary-

ing degrees of inhibition as well as different mechanisms of inhibition. Often one organism may completely check the growth of another; later, growth may be resumed, although not quite normally. The morphological effects produced by antagonists comprise changes in form, size and structure of hyphae, direction of growth, as well as complete cessation of growth and abbreviation of hyphal segments. Among bacteria, the spore-formers are strong inhibitors; and actinomycetes exhibit strong inhibitory action against most filamentous fungi. Phycomycetes and basidiomycetes are more or less inert. Ascomycetes and Fungi Imperfecti vary greatly in their inhibitory action. Some yeasts are strong inhibitors.

Very little is known of the defense mechanisms of microorganisms against the effect of toxic substances produced by antagonists. Green (137) has shown that extracts of *Brucella abortus* and other bacteria contain a factor, designated as "P", which specifically inhibits the bacteriostatic action of sulphanilamide. This substance stimulates the growth of many bacteria. The neutralization of a growth inhibitor of bacteria by a growth stimulant has been indicated for *p*-aminobenzoic acid against sulphanilamide (289). The ability of many bacteria to produce an enzyme which destroys the bactericidal agent of microorganisms has been demonstrated in the case of penicillin (2).

ANTAGONISTIC EFFECTS OF BACTERIA

Among the bacteria most frequently mentioned as possessing strong antagonism to pathogenic organisms, *Pseudomonas fluorescens*, *P. putida* and *P. aeruginosa* (*Bacterium pyocyaneum*) occupy, in the early literature, a prominent place. Garré (115) found that *P. putida* inhibits the growth of *Staphylococcus aureus*, *E. typhosa* and *Bacterium mucosus-capsulatus*, but not of *Bacillus anthracis* and others. Lewek (192) soon reported that *B. anthracis* is also killed by the above antagonist, whereas the growth of *S. aureus* and *Vibrio comma* is only retarded, and no effect at all is exerted upon *E. typhosa* and *E. coli*; *P. fluorescens* is antagonistic to *B. anthracis*, but not to other organisms. However, Olitski (245) demonstrated, in 1891, that *P. fluorescens*

inhibits the growth not only of *E. typhosa*, but also of *B. anthracis*, *V. comma*, *Serratia marcescens* and *S. aureus*. These and other apparently contradictory results were probably due to the specificity of the strains used by different investigators.

According to Laws and Andrews (190), the presence of *P. fluorescens* in sewage greatly reduces the period of survival of the typhoid organism. Horrocks (160) also found that the latter does not develop in gelatin upon which *P. fluorescens* was previously grown. The pathogen could not be detected in sterile sewage after the antagonist was present for seven days. An antagonistic effect against *E. typhosa* was also exerted by *E. coli*.

Frost (110) established, in 1901, that a number of different bacteria are able to exert a marked antagonism against *E. typhosa*. *P. fluorescens* exhibited the strongest effect; *Proteus vulgaris* acted more rapidly, but the active substance did not diffuse so great a distance into the medium. Filterable and thermostable antagonistic substances were produced; their action varied with temperature, being most pronounced at 37°; at ice-chest temperature, the action was so delayed that the pathogen had an opportunity to develop. This was believed by Frost to offer a possible explanation of the fact that when water supplies become contaminated in cold weather their power of producing infection is retained longer than when the contamination takes place in warm weather.

The activity of the influenza organism is largely dependent on the presence of accompanying bacteria (365); some of these, especially micrococci, are favorable to its growth, whereas others, such as *P. aeruginosa* and *B. subtilis*, are injurious. Lewis (193) observed that the luxurious growth of *P. fluorescens* in manured soil and in protein solution containing *B. cereus* is due to the antagonism of the former against the latter. *B. anthracis*, *B. megatherium*, *Vibrio comma*, *Chromobacterium violaceum* and *Rhodococcus* were also inhibited, *Salmonella* species were less sensitive, whereas *E. coli*, *Aerobacter aerogenes* and *Serratia marcescens* were highly resistant. *P. fluorescens* was found to produce a water-soluble, thermostable substance which was toxic to various bacteria, except the green fluorescent forms; it was also active against actinomycetes but not against fungi.

Spore-forming bacteria as antagonists

Among the spore-forming bacteria, *Bacillus subtilis*, *B. mycoides*, *B. mesentericus*, and, to a lesser extent, *B. brevis* and *B. simplex*, as well as some of the more heat-resistant types, the so-called *Tyrothrix*, occupy a prominent place as antagonists, as shown in table 1.

TABLE 1
Spore-forming bacteria as antagonists

ANTAGONIST	ORGANISMS AFFECTED	KNOWN PROPERTY	REFERENCE
<i>Bacillus anthracis</i>	Anthrax, typhoid and lactic acid bacteria		307, 293, 109
<i>B. brevis</i>	Gram-positive bacteria	Two substances crystallized	77
<i>B. mesentericus</i>	Typhoid, anthrax, Shiga, pneumococci Many bacteria Diphtheria bacteria <i>Helminthosporium sativum</i>	Bacteriolytic	237
<i>B. mesentericus vulgatus</i>	<i>C. diphtheriae</i>	Thermolabile, non-filterable	152
<i>B. mycoides</i>	7 to 20 species of bacteria <i>M. tuberculosis</i>	Lysis Thermostable, precipitated by tungstic acid	225, 226 176, 177
<i>B. mycoides</i> , var. <i>cytolyticus</i>	<i>Helminthosporium teres</i> Most pathogenic bacteria and many non-pathogens		260 107
<i>B. simplex</i>	<i>Rhizoctonia solani</i>	Thermostable	60
<i>B. subtilis</i>	Various bacteria <i>M. tuberculosis</i> , <i>E. typhosa</i> Rabies virus	Bacteriolytic	237 337, 18 206
<i>B. subtilis-mesentericus</i>	Mostly living gram-positive and dead gram-negative bacteria	Lysis	286, 287
Spore-forming bacteria	Fungi		270, 90, 12, 273, 260

The term "lysobacteria" was applied (286) to those capable of dissolving living and dead organisms. The following differences were recognized between the action of antagonists and that of phage: (a) the filtrate of the antagonist is active against other

bacteria; fresh filtrates of the antagonists are most active, the activity being destroyed at 70°; (b) both living and dead cultures of the antagonized bacteria are dissolved; (c) the action is not so specific as in the case of phage; (d) races of *E. coli* resistant to phage are dissolved by the filtrate of the antagonist. The filtrate acts upon intestinal bacteria not only *in vitro* but also *in vivo*.

Spore-formers are active antagonists against diphtheria and pseudodiphtheria organisms (10). Since these antagonists were not found in saliva, and the saliva bacteria were not active, the conclusion was reached that the action of saliva against bacteria was due to another factor rather than to its bacterial content. Dubos (77) isolated from a soil enriched with various living bacteria a gram-negative organism (*Bacillus brevis*) which has a marked lytic effect against gram-positive bacteria, including staphylococci, pneumococci, and others. An active substance was isolated which also acts upon these bacteria *in vitro* and *in vivo*. Hoogerheide (157) also isolated from the soil an aerobic, spore-forming bacillus which produces a very active substance: it prevents the formation of capsules by Friedländer's bacterium and is highly bactericidal. Strains of spore-forming bacteria producing antagonistic substances are widely distributed in the soil; they are non-diastatic, gram-negative and produce hydrogen sulfide (320).

Spore-forming bacteria are found to produce substances antagonistic not only to bacteria but also to fungi (table 1). Cordon and Haenseler (60) isolated a spore-forming bacterium (*Bacterium simplex*) which is antagonistic to *Rhizoctonia solani*, an important plant pathogen; the bacterium produces a thermostable substance, which inhibits the growth and even causes the death of the pathogen. *B. mesentericus* grown on artificial media produces an active substance, which suppresses the growth of *Helminthosporium sativum* (55). It increases sporulation, inhibits or retards spore germination, causes abnormal hyphal growth, and induces mutations in certain strains of the fungus. The substance is thermostable, diffusible, passes through a Berkefeld filter, is adsorbed by infusorial earth, withstands freezing and desiccation, and does not deteriorate readily. It is destroyed by alkalis

but not by acids, and is inactivated or destroyed by certain fungi and bacteria.

The antagonistic spore-forming bacteria produce substances which act primarily upon gram-positive bacteria, but also to some extent upon gram-negative organisms. It is of particular interest to note that living gram-positive bacteria are more susceptible to the action of these antagonists than living gram-negative bacteria, whereas the reverse is true in the case of dead organisms (287).

Non-spore-forming bacteria as antagonists

Since the early work of Bouchard (31), Emmerich and Löw (93) and others, numerous non-spore-forming bacteria have been shown (155) to be able to antagonize other bacteria (table 2); in many cases, the active substance was isolated and its chemical nature determined. Particular attention was paid to the pyocyaneus and fluorescens groups; and much consideration was also given to the members of the colon-typhoid group.

Wathelet (350) found that, in mixed culture, the colon organism gradually replaces the typhoid (60, 239, 148, 302, 180, 224). Chatterjee (51) noted that typhoid and paratyphoid bacteria fail to multiply when inoculated into media in which the colon bacterium has previously grown, a fact also reported by various other investigators (340, 209, 36, 343, 70, 254, 264, 370, 255, 324, 180). The antagonistic action of paratyphoid against typhoid bacteria has also been established (173). Nissle (239) introduced the term "antagonistic index" to express the relation between typhoid and colon organisms in a culture of the former inoculated with the latter. The term "minimum inhibitory ratio" was used to designate the ratio between two species at which one will overgrow the other (98, 236). Fulton (112) noted that when *E. coli* and *Salmonella schottmülleri* are grown in association, the second is at first inhibited, but, after *E. coli* passes its maximum development, it also makes a good growth. The occurrence of slow lactose-fermenting strains of *E. coli* in stools (168), as well as the inhibitory action found in certain stools seeded with *E. typhosa* was ascribed to the antagonistic action of the former (239). Different strains of *E. coli* appear to repress the typhoid organism

TABLE 2
Non-spore-forming bacteria as antagonists

ANTAGONIST	ORGANISMS AFFECTED	KNOWN PROPERTY	REFERENCE
<i>Achromobacter</i> sp.	Species of <i>Fusarium</i> , <i>Sclerotinia</i> , <i>Botrytis</i>		56
<i>Aerobacter aerogenes</i>	<i>B. anthracis</i> , <i>Pasteurella pestis</i>		142, 100
<i>Alcaligenes faecalis</i>	<i>Helminthosporium</i> sp.		260
Anaerobic bacteria	<i>M. tuberculosis</i> , <i>B. anthracis</i>		248, 155
Diplococci and pneumococci	Various bacteria	Thermolabile	199, 269, 247, 200, 100, 81, 165, 98, 144, 236
<i>Eberthella typhosa</i>	<i>E. typhosa</i> , <i>P. fluorescens</i> , <i>E. coli</i> , <i>B. anthracis</i>		115, 343, 131, 335, 305, 85
<i>Escherichia coli</i>	Typhoid, paratyphoid, diphtheria, staphylococci	Mostly growth inhibition	350, 239, 51, 18, 143, 180, 271
	Proteolytic and putrefactive bacteria		19, 329, 279
	<i>M. tuberculosis</i> , <i>B. anthracis</i> , and spore-forming bacteria		142, 126, 166, 306, 293, 335, 45, 172
<i>E. coli</i> strains	Other <i>E. coli</i> strains		239
<i>Klebsiella pneumoniae</i>	Anthrax, diphtheria, bubonic plague, etc.	Active filtrate	252, 305, 109, 19, 236, 18, 100
Micrococci	<i>V. comma</i> , <i>M. tuberculosis</i> , <i>E. typhosa</i> , <i>B. melitensis</i> , etc.		199, 81, 231
Myxobacteria	Plant-disease-producing bacteria	Thermostable, lytic	312
<i>Pasteurella avicida</i>	<i>B. anthracis</i> , <i>E. typhosa</i>		250, 109
<i>Proteus vulgaris</i>	<i>B. anthracis</i> , <i>P. pestis</i> <i>Clostridium sporogenes</i> and other anaerobes		335, 100 242, 13, 353, 156
	<i>Phytophthora</i> , <i>Basisporium</i> , <i>Sclerotium</i> , <i>Glomerula</i>		174, 260
<i>Pseudomonas aeruginosa</i>	<i>B. anthracis</i> , <i>E. typhosa</i> <i>V. comma</i> , etc.	Thermostable, filterable	31, 50, 23, 109, 93
	Gram-negative bacteria, <i>M. tuberculosis</i> , and yeasts	Depressed growth	280, 31, 369, 23, 138, 281

TABLE 2—*Concluded*

ANTAGONIST	ORGANISMS AFFECTED	KNOWN PROPERTY	REFERENCE
<i>P. fluorescens</i>	<i>E. coli</i> , <i>S. marcescens</i> , <i>C. diphtheriae</i> , <i>B. anthracis</i> , etc.	Thermostable, filterable	115, 150, 307, 192, 245, 160, 110, 193, 100, 288
<i>P. phaseoli</i>	Actinomycetes	Lytic action	211
<i>P. translucens</i>	<i>Fusarium</i> sp.		21
<i>Salmonella paratyphi</i>	<i>Ophiobolus graminis</i>		34
<i>Serratia marcescens</i>	<i>E. coli</i> , <i>B. anthracis</i> , <i>P. pestis</i>		168, 335, 100
	<i>C. chauwoei</i> , <i>B. anthracis</i> , staphylococci, micrococci, fungi	Non-pigmented, thermostable, lytic	80, 277, 282, 89, 208, 16
	Gram-positive but not gram-negative bacteria	Alcohol-soluble pigment	150
Staphylococci	Dead cells of various bacteria		134, 366, 367
	Gram-positive bacteria, anthrax, diphtheria, plague organism		101, 18, 82, 100, 61
Streptococci	Anthrax, diphtheria, etc.	Antagonism not associated with hemolytic properties or virulence	73, 91, 252, 109, 231, 302, 18, 57, 45
<i>Streptococcus cremoris</i>	<i>S. lactis</i>		362
<i>S. lactis</i>	<i>L. bulgaricus</i>	Thermostable, non-filterable	283
<i>S. muscosus</i>	<i>P. pestis</i>		100
<i>S. pyogenes</i>	<i>B. anthracis</i> , <i>Phytomonas tumefaciens</i>		25

to a different extent, freshly isolated strains being more active than stock cultures (310). However, young, actively growing cultures of *E. typhosa* inhibit the growth of *E. coli*, older cultures being non-antagonistic (343).

A bacteriophage was found (197) to develop as a result of the antagonistic action of *E. coli* against the Shiga bacillus; this was believed to occur in the intestines where antagonistic conditions are always present. A similar antagonistic stimulus was observed (99) for *Salmonella albus* to *E. coli*. The weakest antagonists

were said (148) to belong to the paracolon group, the strains of medium activity to the colon organism, and the strongest antagonists to the *E. coli-immobilis* type.

Gratia (129 to 131) demonstrated that one strain of *E. coli* may be inhibited by another (67); however, some cells of the former may remain immune against the action of the latter. An emulsion of dead cells may become clear, when living cells are added, an effect that was designated as *autophage*. The mechanism of this action was variously explained by a change in pH value of the medium, oxidation-reduction potential, or some direct effect of the bacteria. Thermolabile, filterable substances have frequently been demonstrated (59, 142, 205). These were considered either as auto-toxins or as proteolytic enzymes (255). Schilling and Califano (302) found that the filtrate of *E. coli* depressed only the dysentery organism of Shiga. The active substances produced by *E. coli* were believed (145) to be thermostable lipoids, which are capable of bringing about lysis of the colon and other bacteria.

An extensive literature has also accumulated on the antagonistic action of cocci. Holman (156) suggested that many chances of error are possible in the case of mixed cultures, particularly with closely similar forms; pneumococci, for example, were found to be able to live for long periods with non-hemolytic streptococci. Peculiar antagonistic relations between pneumococci and staphylococci were also observed (6). It was suggested (11) that adaptive alterations are to be expected in the growth of bacteria in mixed cultures; the antagonism of one or the other was believed to depend frequently upon their relative numerical abundance (98).

Lactobacillus bulgaricus was found able to modify the variation of *E. coli* from the *S* to the *R* phase, inhibiting its development and even bringing about its lysis; this took place only in the presence of the living antagonist; no active substance could be demonstrated, and lactic acid itself had only a limited effect (5). When a yellow sarcina was used as the "feeding organism," a stimulating effect was exerted on the growth of *Brucella* sp. on solid media; in liquid media, however, the life activity of the latter was repressed (182). A white staphylococcus exerted an antagonistic action on *Brucella* sp. both in liquid and on solid media.

Certain acid-producing aerobes were found to inhibit toxin production by *Clostridium botulinum* in glucose but not in non-carbohydrate media (146). Since acid itself was ineffective, Holman (156) suggested that the acid must be in a nascent state. A mixture of a *Clostridium sporogenes* with *C. botulinum* interfered with the development of the toxin by the latter; it was thought possible that this association might even cause the early disappearance of the botulinus toxin (170, 63, 106).

The antagonistic action of the non-spore-forming bacteria comprising a great variety of organisms is no doubt due to several agents and mechanisms. Very few of these are as yet sufficiently understood; although many attempts have been made to utilize these antagonisms for disease control.

Antagonistic effects of actinomycetes

The ability of actinomycetes to repress the development of other microorganisms appears to be widespread (table 3). In view of the difficulty of identifying these organisms as specific, well recognized types, most of the references are either to "white" or "pigment-producing" types, or just to plain "Actinomyces." Since there are hundreds of species now recognized and not all of them possess antagonistic properties (230), the identity of the antagonists may be considered, in most cases, to be unrecognized. This makes a comparison of the results of different investigators rather difficult.

In 1890, Gasperini (119) demonstrated that certain species of *Actinomyces* have a marked lytic effect upon bacteria and fungi. Greig-Smith (140, 141), in his studies on the presence of toxic substances in soil, found that the antagonistic action of actinomycetes was directed against many bacteria as well as against certain fungi; the fact that they grow only slowly in normal soils suggested the possibility that they comprise an important factor limiting bacterial development. In an attempt to find organisms that are effective against diphtheria of the pharynx, Rosenthal (285) succeeded in isolating from the air a species of *Actinomyces* which he designated as the true biological antagonist of Loeffler's organism. The surface of an agar plate was covered with an emulsion of diphtheria bacteria and inoculated in several spots with the antagonist.

At the end of two days, the plate was covered with the growth of the diphtheria organism, except that the colonies of the actinomycetes were surrounded by large transparent zones.

Gratia and Dath (133) suspended dead cells of staphylococci and other bacteria in agar and exposed the plates to the air. A white species of *Actinomyces* developed on the plates. When this organism was transferred to a suspension of dead staphy-

TABLE 3
Antagonistic action of actinomycetes

ANTAGONIST	ORGANISMS AFFECTED	KNOWN PROPERTY	REFERENCE
<i>Actinomyces anti-bioticus</i>	All bacteria and fungi, especially gram-positive types	Thermostable; largely bacteriostatic	348
<i>A. praecox</i>	<i>A. scabies</i>		219, 220
<i>Actinomyces</i> sp.	Bacteria and fungi	Lytic action	119
<i>Actinomyces</i> sp.	Diphtheria	Growth inhibition	285
<i>Actinomyces</i> sp.	Pneumococci, streptococci, staphylococci, <i>P. aeruginosa</i>	Lysis of dead cells. Substance thermostable	131, 133 359
<i>Actinomyces</i> sp.	<i>B. mycoides</i> , protoactinomycetes, mycobacteria	Bactericidal, with or without lysis	30, 185
Actinomycetes	Dead and living bacteria	Lysis	195
Actinomycetes	Spore-forming bacteria	Repression of growth	193
Actinomycetes	Gram-positive bacteria	Thermostable, produced on synthetic media, resembles lysozyme	230, 346, 186
Actinomycetes	<i>Pythium</i>	Thermostable	328

lococci in sterile saline, characteristic flaky growth was produced and the bacterial suspension became clarified in 36 hours. When the lysed emulsion was filtered, it could again dissolve a fresh suspension of the dead bacteria. This species of *Actinomyces* was found to be able to attack all staphylococci tested, as well as certain other bacteria such as *P. aeruginosa*; however, it was inactive towards *M. tuberculosis* and *E. coli*. This phenomenon was believed to resemble the "induced microbial antagonism" of Schiller (301), but it was distinct from bacterio-

phage. This type of antagonism was considered to be widely distributed in nature and directed against many bacteria, pathogenic and saprophytic. The fairly stable, active substance was present extensively in old cultures of this actinomycetes, and was regarded as a highly specific proteolytic enzyme. Some strains of the actinomycetes could also attack *E. coli*, but this property could be lost. The lysed material was designated as a "mycolysate"; it did not possess the toxicity of the non-lysed suspension, but retained the antigenic properties of the latter (132). Gratia later (131) asserted that the actinomycetes was also able to attack living cells of bacteria, except *E. coli* and *E. typhosa* which had to be first killed by heat.

Welsch (359) designated this bacteriolytic substance as *actinomycetin*. Some of the activity was lost on passage through bacterial filters. Welsch divided the bacteria in their relation to actinomycetes into three groups: (1) those organisms which are lysed by the aqueous extract of agar cultures, namely pneumococci and hemolytic streptococci; (2) bacteria which are not dissolved even by the most active soluble substance, but which are depressed by the mycelium of the actinomycetes, including various sarcinae and *B. megatherium*; (3) bacteria not acted upon either by the mycelium or by the active substance, comprising the colony-typhoid-paratyphoid and the pyocyaneus groups. When these bacteria are killed by heat or are placed under conditions unfavorable to multiplication, however, they are dissolved by the lytic substance. Cells of *E. coli* acted upon by radium emanation, which stops their multiplication, become susceptible to the lytic substance.

Various actinomycetes are reported (30) able to repress and lyse living cells of spore-forming bacteria. A thermostable toxic substance is produced, especially on agar media. The action of the toxin is weakened by an alkaline reaction and favored by an acid reaction. When *B. mycoides* and an antagonistic actinomycetes were inoculated together in peptone media, no toxic action was exerted, because the former alkalized the medium rapidly, thus making conditions unfavorable for the production of the toxin by the antagonist. The action of the toxin on *B. mycoides*

resulted in elongation of the vegetative cells, due to a delay in fission and suppression of sporulation. According to McCormack (211), aerobic conditions are necessary for the development of the antagonistic properties of actinomycetes; those requiring less oxidized conditions are themselves antagonized. *B. megatherium* was said to be antagonistic to certain species of *Actinomyces* but was itself antagonized by others.

Many species of actinomycetes (but not proactinomycetes) were found (185) to produce a substance which possesses a strong bactericidal action against various microorganisms, including proactinomycetes, mycobacteria and micrococci. The cells were either lysed, or killed without subsequent lysis. Spore-bearing bacteria were not killed but were stopped in their development; however, non-spore-forming bacteria, including nodule bacteria and *Azotobacter* sp., were not affected and actually grew in the filtrates of the antagonists.

Antagonistic actinomycetes are widely distributed in the soil (230). Out of 80 cultures isolated, 47 possessed antagonistic properties, but only 27 produced toxins. These acted upon the same gram-positive bacteria, but not upon gram-negative bacteria or fungi. No relation was observed between active antagonism and pigmentation of the colonies, formation of soluble pigments (346), manner of sporulation or shape of spores. Some strains were able (230) to excrete water-soluble toxic substances into the medium but others did not. The substances were thermostable: heating for 30 minutes at 1.5 atm. only reduced somewhat their activity. Since the capacity to produce antagonistic substances was possessed only by certain species, the utilization of this phenomenon for the systematization of actinomycetes as a whole was suggested. Based upon the action of the antagonistic substance, mycobacteria could be differentiated from non-spore-forming, especially nodule, bacteria. The production of the active substance was highest on synthetic media, and was rather weak or even totally absent in protein media.

Waksman and Woodruff (348) isolated from the soil an organism, described as *Actinomyces antibioticus*, which proved to be particularly active against a great variety of bacteria and fungi.

It produced a highly bacteriostatic substance designated as *actinomycin*. The organism was aerobic and produced dark-brown to black pigments on protein- and peptone-containing media; however, it was distinct in its physiology from the other chromogenic species.

Millard (219, 220) succeeded in controlling potato scab, caused by *Actinomyces scabies*, by the use of green manures and grass cuttings. The development of scab on potatoes grown in sterilized soil inoculated with *A. scabies* could be reduced by the simultaneous inoculation of the soil with *A. praecox*, an obligate saprophyte. By increasing the proportion of the latter to the pathogen, the degree of scabbing on the test potatoes was reduced from 100 per cent to nil. According to Goss (128), the general soil microflora has a controlling effect upon the development of scab; inoculation with *A. praecox* alone gave negative results. Sanford (294) was also unable to obtain control of potato scab by the inoculation of both steam-sterilized and natural soil containing different amounts of green plant materials with *A. scabies* and *A. praecox*. These organisms were perfectly compatible on potato-dextrose agar, as well as in a steam-sterilized soil medium. It was suggested that the control of scab obtained by Millard was possibly due not to the direct action of *A. praecox* but to certain other undetermined microorganisms favored by the presence of the green manure, or by other conditions (175).

ANTAGONISTIC EFFECTS OF FUNGI

A most extensive literature has accumulated on the antagonistic effects of fungi, especially from the point of view of causation of plant diseases. The various interrelationships studied involve the action of (a) fungi against bacteria, (b) of fungi against fungi, and (c) of bacteria against fungi (table 4).

In the study of staphylococcus variants, Fleming (104) observed that around a large colony of a contaminating fungus, which proved to be *Penicillium notatum*, the staphylococcus colonies became transparent and were obviously undergoing lysis. The pure culture of the fungus had marked inhibitory, bactericidal and bacteriolytic properties for many of the more common

pathogenic bacteria, including staphylococci, streptococci, diphtheria bacilli, gonococci and meningococci, but not for the organ-

TABLE 4
Antagonistic effects of fungi

ANTAGONIST	ORGANISMS AFFECTED	KNOWN PROPERTY	REFERENCE
<i>Cephalothecium roseum</i> Fungi	<i>Helminthosporium sativum</i> Fungi	Antagonism against the same or other species	136 89, 317, 274, 111, 380, 318, 280, 355, 64, 54, 240, 86, 304, 342
<i>Helminthosporium sativum</i>	<i>Ophiobolus graminis</i>	Thermostable	35
<i>Helminthosporium</i> sp.	Various fungi		260
Hyphomycetes	<i>Pythium</i>		75
<i>Penicillium luteum</i>	<i>Citromyces, Aspergillus niger</i>	Thermostable	351, 246
<i>P. notatum</i>	Staphylococci, streptococci, diphtheria, gonococci, meningococci, anaerobic bacteria, etc.	Lytic action	104, 58, 272, 48, 29
<i>Penicillium</i> sp.	Various fungi	Thermostable	35, 273
<i>Penicillium</i> sp.	<i>Helminthosporium sativum</i>	Suppression of growth	297
<i>Psalliota campestris</i>	<i>Mycogone</i>		53
<i>Torula suganii</i>	Various fungi		244
<i>Torulopsis</i> sp.	<i>Dematiaceae</i>	Inhibition of growth	215
<i>Trichoderma lignorum</i>	<i>Fusarium</i> , other fungi		124, 7
<i>Trichoderma, Gliocladium</i>	<i>Actinomyces scabies</i> <i>Rhizoctonia, Pythium, Phytophthora, Fusarium, Rhizopus, Sclerotium, Blastomycoides dermatitis</i> , other fungi	Lethal principle isolated	65 7, 260, 149, 354

isms of the colon-typhoid-dysentery group. The filtrate of the culture contained the active substance which was designated as *penicillin* (58, 272).

Chain *et al.* (48) succeeded in obtaining from the culture medium a water-soluble, stable, brown powder of penicillin which had great anti-bacterial activity. The impure substance inhibited, in dilutions of 1 part in several hundred thousand, the growth of many aerobic as well as anaerobic bacteria (*C. welchii*, *C. septicum*, and *C. oedematiens*). The material was also active *in vivo*, subcutaneous injections saving the lives of mice injected intraperitoneally with *Streptococcus pyogenes* or *Staphylococcus aureus*. Intramuscular infections of mice with *C. septicum* were also successfully treated by repeated subcutaneous injections of penicillin. Bornstein (29) found that twenty-seven strains of enterococci and six of *Streptococcus lactis* were resistant to penicillin; thirteen strains of *S. viridans* were susceptible. Other fungi also appear capable of producing bactericidal substances (361).

Harder (147), in a study of the behavior in mixed culture of fungi belonging to the Basidiomycetes and Ascomycetes, found that young colonies do not produce so much of the toxic substance as do older colonies, hence they can grow close to one another. *Coniophora cerebella* was held back by a species of *Penicillium*, its mycelium being considerably modified; in time, the former organism adapted itself to the latter and overgrew it at a rate eventually more rapid than that of a pure culture.

In some cases, as for example, the reciprocal influences of *Sclerotium rolfsii* and *Fusarium vasinfectum*, it was found (284) that, at pH values below 6.9, the former completely overgrew the latter, whereas in alkaline ranges the reverse process took place. Several species of *Penicillium* and other soil-inhabiting fungi were tested in steam-sterilized soil for their effects on the virulence of *Helminthosporium sativum* for wheat seedlings (297). Certain of the fungi exerted a marked degree of suppression, some had no effect, while others increased the virulence of the pathogen. There were marked variations in activity among the species of *Penicillium*.

Certain fungi commonly found in the soil (species of *Trichoderma*, later recognized as *Gliocladium*) have a decided inhibiting effect against various fungi, such as *Rhizoctonia* and other plant pathogens (354). This effect is due to the production of an active

substance in the filtrate of the antagonist. This "lethal principle," designated as *gliotoxin*, kills the antagonized fungus; it is also found to be highly toxic to *Blastomycoides dermatitis*, a causative agent of human skin disease.

The fungicidal effect of many bacteria has also been definitely established. Bamberg (12) isolated from corn plants certain bacteria which inhibited the development of *Ustilago zea* and destroyed the colonies of this fungus. It was believed that the widespread distribution of these bacteria might bring about a check on the multiplication of the pathogenic fungi in the soil. Four types of bacteria antibiotic to smuts and to certain other fungi were described by Johnson (169); some of the bacteria were found to produce enzymes that were able to dissolve the cell walls of the fungus sporidia. Carter (46) found that *Helminthosporium sativum* and a certain unnamed bacterium produce thermostable mutually inhibiting substances; the bacterium and its products inhibited the growth of this fungus as well as of other members of the same genus. The inhibitory agent was diffusible, acting upon *H. sativum* on potato-dextrose plates for a distance of 10 to 15 mm. The toxicity of *B. mesentericus* for this fungus has also been established (55).

Two bacteria belonging to the genera *Pseudomonas* and *Achromobacter* have been isolated (56) and found capable of bringing about the lysis of different species of *Fusarium* and other fungi. These bacteria were widely distributed in the soil, but were absent in certain flax-sick soils, in spite of the abundance of *Fusarium* sp. The fungus did not develop and the plant disease did not occur in the presence of the active bacteria. Nakhimovskaia (232) found that the presence of certain bacteria (*P. fluorescens*, *Serratia* sp.) in the medium inhibits the germination of rust spores; spore-forming bacteria and sarcinae did not exert any antagonistic action, but their presence affected the nature of the germination process, the spores giving rise to mycelium-like forms with great numbers of copulating filaments, whereas, in the control cultures, yeast-like forms prevailed and copulating cells were rarely encountered.

In the infection of wheat seedlings by *Ophiobolus graminis*, a

number of fungi and bacteria were found (296) to exert a marked antagonistic action; not only the living cultures, but the culture filtrates were also effective in many cases (96). The growth of *H. sativum* and *Fusarium graminearum* upon sterilized soil could be completely suppressed by the addition of small amounts of unsterilized soil or by the simultaneous inoculation with a number of other fungi and bacteria, so that no infection resulted when wheat seeds were inoculated with this soil (149). The fact that root-rot diseases of wheat are less severe when the crop is grown on summer-fallowed land than on land cropped to wheat for several years could be related to the growth of soil saprophytes, which in bare fallow have an advantage over the pathogens in competition for food. Infection of wheat seedlings by *O. graminis* in sterile soil fell off rapidly with the reestablishment of the original soil microflora (34). An inverse correlation was reported (223) between the degree of infection and the protective effect of the general soil microflora. An increase in soil temperature was found to increase the antagonistic action of the soil microflora to the parasitic fungus (149, 117).

The decay of fruits can also be suppressed or modified by inoculation with mixtures of known organisms (299). According to Potter (262), *Pseudomonas destructans*, the cause of rot of turnip, produces a potent, heat-resistant plant toxin, which is also destructive to the pathogen itself. By spraying turnips with this bacterial product, the disease could be checked. The same principle was found to hold true for oranges infected with *Penicillium italicum*. The injurious action of certain common soil bacteria on *Pseudomonas citri*, the cause of citrus canker, has also been reported (111). Wheat seedlings were protected from infection by *Helminthosporium* and flax seedlings from *Fusarium* by the use of antagonistic bacteria (260). A watermelon disease caused by *Phymatotrichum omnivorum* was reduced when certain fungi (*Trichoderma lignorum*) and bacteria were present in the soil together with the pathogen (38). The severity of the seedling blight of flax, caused by *F. lini*, was diminished when the pathogen was accompanied in the soil by certain other fungi (325). The pathogenicity of *H. sativum* on wheat seedlings was suppressed by

the antagonistic action of *Trichothecium roseum*, which is believed to produce a toxic substance (136).

The role of microbiological antagonism in the natural control of soil-borne fungus diseases of plants has been emphasized (295, 33, 296, 149, 34, 21). The principles of biological control have been outlined (118) as follows: The soil population is in a dynamic biological equilibrium. When a certain crop is grown continuously, the multiplication of various parasites capable of attacking the roots of that crop takes place (27). Organic manures are known to stimulate the development of various saprophytes in the soil. These multiply at the expense of the pathogens and are able to check their activity, either by preventing their growth (*fungistatic* action), or by attacking and destroying the mycelium of the parasites (*fungicidal* action). The biological control of plant diseases is said to be most effective against those organisms which have become highly specialized to a parasitic form of life.

Van Luijk (341) suggested that biological control of plant parasites may be obtained by inoculation of the soil with specific microorganisms selected for their antagonistic capacity, or by the addition of their growth products. However, Broadfoot (34) emphasized that the antagonism of a saprophyte to a plant pathogen, as measured by growth on artificial media, is not a reliable measure of the actual control that may be exerted upon the parasite in the soil. A lack of specific microorganisms is not considered to be a sufficient factor limiting biological control under natural conditions. No inoculation of soil with an antagonistic organism, such as *T. lignorum*, can have more than a temporary effect in changing the microbiological balance of the soil population. Similar results were obtained by Weindling and Fawcett (357, 101), in their attempts to control *Rhizoctonia solani* by the use of *T. lignorum*, and by Cordon and Haenseler (60), by the use of a strain of *Bacillus simplex*. Daines (65) found that *T. lignorum* produces a diffusible substance which is toxic to *A. scabies* in an artificial liquid medium. However, the toxic principle was rapidly destroyed by soil aeration. It was believed doubtful that this fungus could be of much assistance in combating potato scab.

Fellows (103) obtained field control of the take-all (*O. graminis*) disease of wheat in Kansas by the application of certain organic materials such as chicken and horse manure, alfalfa stems and leaves, boiled oats and barley, and potato flour. Garrett (117, 118) submitted evidence to prove that the factor chiefly controlling the subterranean spread of the pathogenic fungus along the roots of the wheat plant was the accumulation of carbon dioxide, with corresponding lowering of the oxygen tension, in the microclimate of the root zone. This could best be maintained by periodical additions of organic manures. Since organic matter low in nitrogen was more effective than high nitrogenous materials, it was postulated that the soil microflora uses the mycelium of *Ophiobolus* as a source of nitrogen. The addition of nitrogenous materials, either in an organic or in an inorganic form, was believed to protect the mycelium of the parasite by offering a more readily available source of nitrogen. Tyner (333) suggested that the differences in the microflora associated with the decomposition of different composts are largely responsible for differences in persistence and virulence of pathogens causing root-rots of cereals.

Considerable reduction in slime-disease of tomato plants was effected by the addition of green manures to soil before planting (338). Organic materials high in nitrogen, as well as supplementary nitrogen sufficient for complete decomposition of the organic material, were found to be most effective. Thom and Morrow (327) found that organic matter is most effective in depressing pathogenic fungi, during the period of its active decomposition. King and associates (178) utilized the antagonistic action of soil microorganisms for the inactivation of pathogenic fungi in the soil before the crop-growing season. Organic manures were added to the soil, in order to control *Phymatotrichum omnivorum*, the root-rot of irrigated cotton under continuous cultivation in Arizona. By the use of the Cholodny slide technique, it was possible to demonstrate (84) that microbiological antagonism represents the true mechanism of the control process. The development of saprophytic organisms was most profuse in the slides buried in the manured plots, whereas the mycelium of *P.*

omnivorum was most abundant on the slides in the unmanured plots. It was suggested that parasitism of the fungal strands by bacteria is one of the reasons for the decline of the pathogen in manured soils. Henry (149) believed that the biological control by the soil microflora could even be directed against internal seed infection, since appreciable infection of surface-sterilized flaxseed was found to occur in sterilized but not in unsterilized soil.

The addition of bacteria to unsterilized soil exhausted by growing flax was found to lower the percentage of plants diseased by *Fusarium lini*. Novogradsky (240) suggested the term "bacterization" for the process of treatment of seed with active bacteria in order to protect the plant against pathogenic fungi. It is concluded that the effect of bacteria on germinating seeds is due to the liberated bacterial products capable of depressing the development of parasitic fungi (15, 312, 184). Although, not in all cases conclusive, the results fully justify the hope that a better knowledge of the soil antagonists may lead, if not to complete control, at least to a certain amount of control over the numerous plant diseases caused by pathogenic fungi, especially those that persist for a certain length of time in the soil.

ANTAGONISTIC ACTION OF ANIMAL FORMS

Based upon the known fact that protozoa are able to feed upon bacteria, a theory was propounded, namely the "protozoan theory of soil fertility," that protozoa are responsible for the limited fertility of certain soils. According to this theory, the bacteria are viewed (291), as the sole agents responsible for the liberation of nutrients in the decomposition of soil organic matter and their transformation into forms available to higher plants. The protozoa, through their capacity of consuming bacteria, are considered as the agents controlling soil fertility. The increased fertility resulting from treatment of soil with heat and certain chemicals is regarded as a result of the destruction of the protozoa, the "natural enemies of the bacteria."

Subsequent investigations have not supported this theory (347). When protozoa were added to cultures of bacteria concerned in certain specific processes (53), they fed upon the bacteria and

brought about considerable reduction in bacterial numbers; this was not accompanied, however, by a detrimental effect upon the specific bacterial processes. Pure cultures of bacteria were found to multiply in a nutrient medium until a limiting population was reached (43). Protozoa grew in that medium without bacteria only when the concentration of the food supply was increased 100 to 1,000 times; in the presence of bacteria, they grew also in the dilute solution. The bacteria thus acted as collectors or concentrators of the food for the protozoa. Bacterial numbers were thereby reduced, but bacterial activities continued. The protozoa kept the bacteria below the saturation point, thus providing conditions for more continuous bacterial multiplication and for more complete oxidation of the organic matter.

The effect of protozoa upon bacteria may thus actually be beneficial to natural processes (62, 214, 233, 322), as for example in the fixation of atmospheric nitrogen, the liberation of ammonia from proteins, and the formation of carbon dioxide from carbohydrates. Cutler and Crump (62) suggest, therefore, that the presence of protozoa in the soil may keep the bacteria at a level of maximum efficiency. The favorable effect of partial sterilization of soil may be explained (193) by the destruction of the bacterial antagonists.

Various bacteria, especially *Pseudomonas aeruginosa*, have been found (52) to exert a toxic effect upon protozoa, limiting their development or bringing about their destruction (201, 257). The protozoa may develop a certain resistance against specific bacterial products (257). Protozoa are also known to feed on bacteria pathogenic to man (164, 102, 321, 267), animals, and plants (278, 154).

The importance of protozoa in the cycle of natural processes thus consists not in the mere destruction of bacteria, beneficial or injurious, but in establishing a variety of relationships with the bacteria, favoring the activities of some and impairing the activities of others.

A variety of bacteria, fungi and nematodes have been found capable of destroying the larvae of various beetles in the soil. Some of these organisms have thus been utilized for combating insect diseases of plants (216). Once these organisms have become

established in the soil, the beetles tend to disappear (123, 83). The ability of saprophytic nematodes to destroy root-knot nematodes has also been utilized for the destruction of the latter. Heavy applications of organic materials to the soil were found (196) to result in a greatly increased population of saprophytic nematodes. The decomposition of the organic residues supports large populations of plant and animal microbes destructive to the parasitic nematodes. Here belong the nema-capturing fungi (74, 68), the non-trapping fungal parasites, predacious mites, as well as a variety of bacteria.

CHEMICAL NATURE OF THE ANTAGONISTIC SUBSTANCES PRODUCED BY MICROÖRGANISMS

All bacteria were once said (26) to produce, at a certain stage of their development, antagonistic substances that are thermolabile and soluble in ether, alcohol and other solvents. It is now definitely recognized that only certain species and frequently only specific strains of microorganisms are able to produce bacteriostatic and bactericidal substances. These vary greatly in their chemical properties, in toxicity to animals, and in the mechanism of their action. The differences are often more of degree than of kind. For purposes of classification of these active substances the following criteria may be used: (a) solubility in various reagents, (b) specific chemical nature, (c) specific bacteriostatic and bactericidal properties, (d) toxicity to animals and action *in vivo*, (e) nature of the organism producing such substances.

Some are soluble in water, but not in organic solvents; some are soluble in alcohol or in chloroform, but not in ether and in acetone; some are soluble in these solvents, but not in water. Chemically, these substances may be classified as, (a) lipoids and lipid-like bodies, (b) pigments, (c) polypeptides, (d) sulfur-bearing compounds. On the basis of their biological activity, they vary considerably, some acting in low and others in high concentrations; some act best upon gram-positive bacteria and less well upon gram-negative organisms, whereas others act chiefly upon fungi; some are primarily bacteriostatic, some are bactericidal, and some are bacteriolytic. On the basis of their toxicity, they can be

classed as, (a) non-toxic or of low toxicity, (b) fairly toxic or (c) highly toxic. Some of the substances have been crystallized; information has been gained concerning the proximate chemical composition of others; the nature of most others is still imperfectly understood (table 5).

The active bactericidal agents produced by *Pseudomonas aeruginosa* have received the most attention (93). Recent evidence points to *pyocyanase* being a lipoid and containing unsaturated fatty acids (150, 20). *Pyocyanin* is a chloroform-soluble pigment which can be synthesized. The solution, left after the removal of the blue chloroform extract, when treated with ether gives a yellow pigment, a derivative of pyocyanin called (371) *hemipyocyanin*, which is also active (183). The *fluorescin* remaining in the culture, after the ether and chloroform extractions, is inactive. Pyocyanin diffuses more readily than pyocyanase (259).

Pyocyanase acts upon a variety of bacteria, including *E. coli* and *E. typhosa*, but not *Proteus vulgaris*. According to Gundel and Wagner (145), however, pyocyanase does not act upon the colon-typhoid group of bacteria. Different strains of *P. aeruginosa* may contain either pyocyanase or pyocyanin or both. Pyocyanase was at first looked upon as an enzyme belonging to the class of nucleases (92). It had, even in very low concentrations, a marked destructive effect upon diphtheria, cholera, typhoid and plague organisms, as well as on pyogenic streptococci and staphylococci; the cholera cells were rapidly dissolved. Bacterial toxins were rendered inactive in a few seconds. The action of the preparation was found to be proportional to time and concentration, and inversely proportional to the numbers of bacteria acted upon. It could be heated without much destruction, for two hours, in flowing steam, although some claimed (324) that the activity was thereby reduced. The enzyme nature of pyocyanase gained further support because (1, 218, 358) the activity of certain bacterial enzymes heated at 100°, for 15 to 30 minutes, is not always destroyed.

The enzymatic nature of pyocyanase was not universally accepted, however, because of its thermostability (179, 69). Dietrich (69) ascribed the action of pyocyanase to a change in osmotic

TABLE 5

Summary of certain selective bacteriostatic and bactericidal substances of microbial origin

SUBSTANCE	ORGANISM	PROPERTIES	REFERENCE
Actinomycetin	<i>Actinomyces albus</i>	Water-soluble, precipitated by alcohol; thermolabile; protein-like. Lysis of dead bacteria	134, 359, 360
Actinomycetin (Lysozyme)	<i>A. violaceus</i>	Water-soluble, ether- and alcohol-insoluble; thermostable; similar in action to lysozyme	185, 186
Actinomycin A	<i>A. antibioticus</i>	Soluble in ether, alcohol, benzol and chloroform, not in petrol ether; orange-colored pigment; toxic; highly selective bacteriostatic action	348
Actinomycin B	<i>A. antibioticus</i>	Soluble in ether, petrol ether, alcohol, but not in water; colorless; largely bactericidal	348
Gliotoxin (C ₁₄ H ₁₆ N ₂ S ₂ O ₄)	<i>Trichoderma</i> sp., <i>Gliocladium</i> sp.	Soluble in chloroform, benzol, alcohol; sparingly soluble in water; fungicidal and bactericidal	356
Gramicidin (C ₇₄ H ₁₀₄ N ₁₄ O ₁₄)	<i>Bacillus brevis</i>	Soluble in ether, acetone; lytic against gram-positive bacteria; toxic, active <i>in vivo</i> ; polypeptide	77, 162
Penicillin	<i>Penicillium notatum</i>	Alcohol-soluble, thermolabile, low toxicity	104, 272, 58
Pyocyanase	<i>Pseudomonas aeruginosa</i>	Lytic on many bacteria; thermostable; lipoidal; activity largely due to unsaturated fatty acids	92, 18, 94, 24, 100, 183, 259, 348
Pyocyanin	<i>P. aeruginosa</i>	Chloroform-soluble, blue pigment; thermostable; bactericidal action limited	150, 143, 183, 371
Tyrocidin (C ₄₄ H ₆₂ N ₆ O ₁₁)	<i>Bacillus brevis</i>	Insoluble in ether, soluble in absolute alcohol; lytic to gram-positive as well as gram-negative bacteria; toxic; polypeptide	77, 162

pressure. Raubitschek and Russ (269) emphasized that the solubility of the substance in ether, chloroform and benzol does not speak for its being an enzyme, nor the fact that temperatures of 0° to 37° do not influence its activity. It seemed to be merely a lipoid (145, 247, 344, 150, 75, 20). Hettche (150) reported that pyocyanase consists of a phosphatide, a neutral fat and a free fatty acid. The bactericidal properties were said to be due to the free fatty acids. A definite relation was found between the number of double bonds in pyocyanase and its bactericidal properties (150, 20). This information may be correlated with the finding (75) that most fatty acids exert bactericidal and bacteriolytic effects upon gram-positive bacteria, whereas gram-negative organisms are not lysed, which thus tends to explain the greater effectiveness of pyocyanase upon the former.

The pigment also was found (143) to possess lytic properties, a 1:1000 dilution of pyocyanin being able to lyse *E. coli* in 6 hours. Kramer (183) believed that pyocyanin acts only on gram-positive bacteria. Pyocyanin was said to be most effective in young cultures and pyocyanase in old cultures (150). The pigment appears to influence bacterial respiration (87).

An enzyme capable of decomposing the capsular substance of pneumococci has been isolated by Dubos and Avery (78, 76) from certain soil bacteria. This enzyme is highly specific, acting only upon one type of pneumococcus which is thereby rendered susceptible to destruction by phagocytosis. It is produced only in the presence of the capsular polysaccharide or of the aldobionic acid derived therefrom. The enzyme seems to be associated with a protein which passes through a collodion membrane with an average pore size of 10.6 μ , but is held back by pores of 8.2 μ (78, 127, 305).

The credit for having first isolated, from spore-forming bacteria, specific chemical compounds in crystalline form, aside from the work on the bacterial lipoids, is due to Dubos (77). By applying the principle of enrichment of soil with pathogenic bacteria, he isolated a spore-forming bacterium capable of bringing about lysis of various living gram-positive organisms. The active material, *tyrothricin*, is protein-free, non-volatile, non-dialyzable through

collodion, and heat-labile. It is alcohol-soluble, water-insoluble, stable at alkaline reactions, but is rapidly inactivated at reactions more acid than pH 5.5, even at room temperature. When redissolved in a neutral medium, the substance exhibits the lytic activity of the original solution from which it was extracted.

The bactericidal material can be further purified and separated into several crystalline preparations. *Gramicidin* is obtained by extracting the crude alcohol-soluble material with a mixture of acetone and ether, evaporating, dissolving in boiling acetone and cooling. The crystals are spear-shaped, colorless platelets, melting at 228-230°. The substance yields 62.7 per cent C, 7.5 per cent H and 13.9 per cent N, with a molecular weight of about 1400, suggesting the empirical formula $C_{74}H_{106}N_{14}O_{14}$. It has no free basic nor acidic groups, and contains 10 molecules of α -amino acids of which 2 or 3 are tryptophane residues.

A second fraction, designated *tyrocidin*, is insoluble in acetone or ether, and soluble in acidified boiling absolute alcohol, from which it precipitates on cooling. The product is recrystallized from acidified absolute methanol yielding clusters of microscopic needles melting at 237-239°, with decomposition. It is similarly built up of amino acids but contains one free carboxyl, one tryptophane and one tyrosine group per molecule. The molecular weight is given as about 900 and the compound represented by the formula $C_{44}H_{68}N_9O_{11}$. Gramicidin acts against gram-positive bacteria; while tyrocidin is also active against gram-negative bacteria (162). The two substances are highly toxic, especially gramicidin of which 0.3 mg. kills a mouse when injected intraperitoneally (203). These or similar compounds appear to be widely distributed among spore-forming soil bacteria (157, 320).

Actinomycetes are known to produce at least three types of active substances: (a) *actinomycetin*, (b) *lysozyme*, and (c) *actinomycin*. The first is water-soluble (134, 359, 360) and acts primarily against dead bacteria, although living bacteria were later also found (131) to be affected. It is thermolabile and destroyed by ultraviolet rays and by strong acids, but not by mild antiseptics. It is precipitated by acetone, alcohol and ammonium sulfate. Borodulina (30), however, found that the toxic agent of actino-

mycetes antagonistic to *Bacillus mycoides* is thermostable. These differences in heat resistance point to possible chemical differences in the nature of the preparations produced by different actinomycetes. Other Russian investigators (185, 186) also found a type of actinomycetin which is filterable and resistant toward radiant energy; it is said to resemble egg-white lysozyme, but not to be identical with it. It is soluble in water, but insoluble in ether, petroleum ether, benzol and chloroform.

Actinomycin obtained from *A. antibioticus* (348) is separable into two fractions: A, soluble in ether and in alcohol, but not in petroleum ether, and giving a clear solution in water; B, soluble in ether and in petroleum ether, soluble with difficulty in alcohol, and giving a turbid suspension in water. *Actinomycin A* is bright red in color, giving a yellow solution even in concentrations of 1 μ g per ml; it possesses extremely high bacteriostatic properties, but is rather slowly bactericidal. It is produced in liquid and in solid, inorganic and organic media, and is completely removed by charcoal; it is not affected by heat, and is only partly removed by passage through a Seitz filter. *Actinomycin B* has little bacteriostatic action but is actively bactericidal.

Penicillin, produced by *Penicillium notatum*, has a strong antibacterial action (104). Gram-negative bacteria are least sensitive and pyogenic cocci most susceptible. It is soluble in alcohol, but not in ether or chloroform; it is inactivated by oxidation and by evaporation at 40 to 45°, in acid and alkaline solutions, although it is fairly stable at pH 5 to 6. However, at pH 2.0 it is completely soluble in ether. The substance is extremely labile (58). It does not dialyze through collodion membranes and resists heating at 60 to 90° for short periods, and 100° for 5 minutes, but not for 10 minutes (272). Light (rich in ultraviolet rays), as well as oxygen, hydrogen and carbon dioxide bubbled through the medium prevent its formation or cause its destruction. The most active preparation completely inhibits the growth of staphylococci in dilutions of 1:800. If permanently adjusted to pH 6.8, penicillin retains its potency for 3 months.

Another type of compound produced by certain fungi, belonging to the genera *Trichoderma* and *Gliocladium*, was isolated by

Weindling and designated as *gliotoxin* (356). The period of greatest activity was produced in 2 days, soon after germination of the fungus spores. The nature of the medium and the final reaction are important for the production of the active substance. It is extracted from the culture with chloroform; the latter is then distilled off and the residue taken up in a small amount of hot benzene, or 95 per cent alcohol, from which, on cooling, silky white needles crystallize out. The substance, recrystallized from benzene or alcohol, has a molecular weight of 347 and the chemical formula $C_{14}H_{16}N_2S_2O_4$. It inhibits the growth of *Rhizoctonia* hyphae up to a dilution of one in three millions. The crystals, as well as the crude material, are also toxic to *Trichoderma*, but the minimum lethal dose is about 40 times greater than that for *Rhizoctonia*. The active substance is sparingly soluble in water.

DISEASE CONTROL BY UTILIZATION OF ANTAGONISTIC MICROÖRGANISMS

Numerous attempts have been made to utilize microorganisms for the control of various diseases in man, animals and plants. As early as 1877, Pasteur (251) noted that the development of anthrax in sensitive animals can be repressed by the simultaneous inoculation of *Bacillus anthracis* with various other bacteria. Pasteur may thus be looked upon as the first one to advance the idea of bacteriotherapy.

In 1885, Cantani (44) treated a patient suffering from tuberculosis with a culture of a saprophytic organism, designated as *Bact. termo*. The effects were highly favorable. He expressed the hope that other infectious diseases of a local nature or readily accessible might in time be treated with saprophytic bacteria that are antagonistic to the pathogens.

Emmerich (91) found that anthrax can be controlled by the simultaneous inoculation with other bacteria, such as species of *Streptococcus*, organisms once looked upon as agents for rendering the organism resistant to all bacterial infections. Pawlowsky (252) obtained resistance against anthrax infection by inoculation with Friedländer's bacillus. Bouchard (31) had good results from simultaneous inoculation with *Pseudomonas aeruginosa*; however,

this did not impart permanent resistance to the animals. There are numerous other instances on record of reduction in pathogenicity of one organism by the presence of others (23, 50, 94).

In order to overcome the destruction of pyocyanase in animal tissues, Emmerich and Löw prepared "immunoproteids," which consisted of a mixture of pyocyanase with blood or other animal tissues. Rabbits could thus be protected against anthrax. Woodhead and Wood (369) used a sterilized ten-day-old culture of *P. aeruginosa* and obtained healing action against anthrax infection, or at least a delay in the course of its development. Vaerst (339) succeeded in curing rabbits infected with anthrax by means of a pyocyanase preparation (11); and pyocyanase was soon (94) applied against various infections.

There has been considerable disagreement over the therapeutic action of pyocyanase. This was largely due to the variation in the nature of the preparations (200), and especially, the strain employed. Kramer (183) showed that the activity of pyocyanase depends on such factors as the nature of the strain, since not all strains are equally effective, the composition of the medium, glycerol-containing media being most favorable, and the method of extraction of the active substance.

Gaté and Papacostas (120) observed that mixed infections were usually mild (49); mixed cultures of the bacillus of Friedländer and *Corynebacterium diphtheriae* gradually gave a predominance of the former on repeated transfer. No toxin was produced when the filtrate of the culture of the antagonist was used for growing the diphtheria organism. The therapeutic use of filtrates was, therefore, suggested. The subcutaneous injection of beer yeast was found (332) to protect rabbits against fatal streptococcus and staphylococcus infections.

Gratia (131) prepared a vaccine for immunization purposes by allowing the specific organism to be acted upon by an antagonist. The culture was heated to 56°, dissolved by the use of an actinomyces, and the resulting solution was employed as the vaccine. Bumm (42) used a preparation designated as *neocolysin*, made up of living, proteolytic bacteria, which gave good results in chronic purulent conditions such as osteomyelitis. The bacteria were

supposed to continue growing as long as there was dead tissue available. The application of bacteriotherapy for treating chronic infections of the middle ear (266) and of actinomycosis (71) has also been suggested.

Besredka (17) used a filtrate from an anthrax culture for dressings or for intracutaneous injections; the results obtained were as good or even better than those obtained with the bacterial vaccine. Later, he utilized staphylococci and streptococci for similar purposes. He believed that a substance ("antivirus") secreted by the bacteria is dissolved in the filtrates, which substance checks further growth of the bacteria. Although in many studies of this phenomenon (47, 209, 243), suggestions were made that the favorable effect is due entirely to the medium (4), the therapeutic results of Besredka have largely been confirmed. The general opinion is that the filtrate does not act upon the infecting bacteria directly, but rather upon the tissue by way of local immunization. Although an occasional increase of resistance caused by the non-specific filtrates has been found, the protection produced by specific filtrates seems to be more intense and more dependable (243).

Morgan and Harvey (222) showed in 1909 that *E. typhosa* is inhibited by the free growth of antagonistic bacteria. As a result, there was believed to be particular danger when pasteurized milk becomes contaminated with this pathogen. Metchnikoff (217) suggested utilization of the antagonism between lactic acid and proteolytic bacteria for repressing the growth of the latter. Pure cultures of lactic acid bacteria are introduced into the food of man in order to repress in the intestinal canal the proteolytic bacteria which are supposed to bring about the intoxication of the system. In recent years, *Lactobacillus acidophilus*, an inhabitant of the human intestine and possessing antagonistic properties against undesirable intestinal bacteria, has come to the front.

Fleming (104, 105) suggested that penicillin could be used as a dressing for septic wounds. This preparation has little toxic effect and seems to be superior to dressings containing active chemicals. The difficulty in the use of penicillin, as in the case of pyocyanase, is due largely to the fact that the preparation does

not maintain its potency for more than a few weeks. Penicillin is not related to any chemotherapeutic substance now in use (48). As compared with sulphonamide drugs, it is (2) not inhibited by tissue constituents and pus, thus offering a definite advantage from a chemotherapeutic point of view.

Gramicidin injected intraperitoneally into white mice, was found (77) to exert a therapeutic action against experimental peritonitis caused by pneumococci and streptococci. However, it is almost completely ineffective when administered by the intravenous, intramuscular, or subcutaneous route. Particularly favorable results were obtained (198) with chronic mastitis. Sterile mineral oil was found to be a suitable, non-irritating medium for its administration. Of 31 quarters of cows naturally infected with *Streptococcus agalactiae* and treated by the gramicidin-oil mixture, 26 seemed to have responded by a complete disappearance of the streptococci. The infection in some of the cured cases was of a severe chronic nature. Gramicidin-like preparations have also been used (212) in the treatment of local infections, such as osteomyelitis.

Thus far, the utilization of specific microbial products for the control of plant diseases has made comparatively little progress (191, 60, 241). Various Russian investigators (15, 56, 240, 234) recommend the inoculation of plant seeds with bacteria ("bacterization" of seed) in order to combat infectious diseases. This phenomenon appears to involve, however, various complex soil-plant-microbe interrelationships (181, 316).

RETROSPECT

Ranging between the phenomena of true parasitism, where one organism lives in or upon the living body of another, and true saprophytism, where one organism merely destroys the waste products and dead cells of the other, there is a wide range of relationships between living systems which may be designated as associative and antagonistic: in the first, one organism assists the other, whereas in the second, one organism is injurious to the other. The antagonistic effects vary from those of space relations between the antagonist and its neighbor, where the prox-

imity of one organism is injurious to the growth of the other, to the production by one organism of definite chemical substances which injure or interfere with the growth of the other. As is generally the case with parasitic relations, the antagonized organism frequently develops a protective mechanism against the antagonist and is often able not only to neutralize its effect but even to destroy it. Often a balanced condition is established between the two organisms, where both are able to survive the antagonistic effects.

Numerous instances are found in nature where the presence or introduction of one organism leads to the destruction of another; whenever the latter, in its turn, leads a parasitic existence upon higher forms of life, the antagonist becomes a beneficial agent to these in their efforts to overcome the effects of the parasite or to destroy it. This phenomenon is largely influenced by the host species, by the type of parasite, as well as by the nature and degree of infection.

Just as the pathogenic bacteria are commonly believed to have evolved from the harmless saprophytes, so the organisms possessing antagonistic properties must have evolved in some manner from those that do not possess such properties. Otherwise, how could one explain the capacity of certain strains of such common universal saprophytes as *Bacillus subtilis* and *B. mycoides* to produce powerful agents capable of bringing about the lysis of numerous pathogenic and saprophytic bacteria? The fact that many pathogenic bacteria produce substances antagonistic to their own kind or to other pathogens has actually been utilized in an attempt to combat these agents of infection.

The mechanism of the action of the antagonist varies greatly and appears to depend largely upon the specific nature of the active substances. Some of these appear to be produced by only one organism, others are produced by many organisms; some antagonists, on the other hand, produce more than one active substance. The action of the antagonist may be either primarily bacteriostatic or largely bactericidal; the latter may or may not be accompanied by the lysis of the antagonized cells. Whereas in many cases the living cells of the prey are lysed by the antagonist, in other cases, the dead cells are lysed more readily.

The substances produced by these antagonists also vary greatly in their effectiveness, when injected into the animal body. Some possess a low toxicity (pyocyanase, penicillin); others are highly toxic (gramicidin, and especially actinomycin). This as well as differences in the solubility of these preparations account for the differences in their utility. Some appear to act better when applied to combat local infections, whereas others may become useful in attacking more generalized infections.

There is increasing appreciation of the fact that nature harbors many unknown organisms that are capable of combating disease-producing bacteria, fungi, worms and insects. Our knowledge of the activities, potentialities, and importance of these microbes is still incomplete. Man, in his struggle for existence succeeded, before the development of microbiology, in domesticating and utilizing the activities of many microbes. Here belong the lactic acid bacteria of milk, the wine-fermenting, beer-fermenting and bread-fermenting yeasts, the silage-producing, sewage-digesting, compost-producing and soil-inhabiting microorganisms. However, these represent only a small fraction of the microbial world. It is possible that we are finally approaching a new field of domestication of microorganisms for combating the microbial enemies of man and of his domesticated plants and animals.

Many practices in surgery, as well as old-time remedies, are based upon the creation of conditions favorable to the development of antagonistic microorganisms. The method of cast surgery, introduced in the Spanish Civil War, and the application of urine to cracked skin and local wounds, as practiced by certain farmers in this country may serve as illustrations. The chance contaminants may possibly serve as the antagonistic agents; to what extent the application of pure cultures of antagonists may improve such practices still remains to be determined. Trueta (331) states that plaster-treated wounds which gave, without the use of antiseptics, such marvelous effects during the Spanish Civil War, were found to contain aerobic bacteria with no one group predominating, except for *Bacterium pyocyaneum* tending to become more numerous, when the healing process has been established.

The utilization of fungi and bacteria for combating plant

diseases has also been variously attempted. The difficulty here is to establish the antagonist in the soil. This can be done only when conditions are modified, as by addition of stable manure or other plant and animal residues, which favor the development of the antagonists. Among the various other possibilities for utilizing antagonistic microorganisms in combating disease-producing and other injurious organisms, the methods of control of insects and other lower animal forms occupy an important place. The Japanese and other Asiatic beetles, which are so highly destructive to plants, have thus been combated rather successfully.

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