THE MICROBE AS A BIOLOGICAL SYSTEM

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PROLOGUE

At a time of the greatest emergency that has ever faced the civilized world, when our very existence as free men is threatened, it may appear trivial to devote time to a discussion of such an apparently abstract subject as "The Microbe as a Biological System." Perhaps because of this emergency, it is a logical time for reexamining values and ideals upon which our life is built, for summarizing existing knowledge in an effort to come to some understanding of the fundamental problems that face us.

The social obligations of science in general and the functions of the scientist in modern society in particular have been the focus of much attention in recent years, not only in the daily press, but even in scientific literature. The scientific worker, the investigator, the searcher of the truths that underly natural phenomena, has been blamed for many of the ills that have befallen society in recent years. Responsibility for the misuse that is being made of scientific discoveries to destroy man rather than to improve his life has been laid on the doorstep of science.

We have come a long way from the time of Dean Swift, when the scientist was ridiculed as one occupying himself with useless and even with vain fancies, from the time of Balzac, who said that "the advantage of science is that if you cannot make great discoveries, you can at least make up new names." Great progress has since been made. In the words of T. B. Robertson, "the investigator is the pathfinder and the pioneer of new civilizations; he is more than that, he is the interpreter of the infinite." Science has now reached a stage when virtually all phases of life are thoroughly permeated with the benefits derived directly or indirectly from discoveries in the many fields of research. One has come to expect from the scientist the solution not only of physical, biological, and mental ills, but also an answer to the complicated social problems, many of which are as old as man himself.

The scientist has come down closer to earth. There was a time when he looked upon himself as the high priest of a new form of philosophy (the philosophy of nature), which could soar above the problems of everyday life, without contaminating itself by making practical contributions to it. Without considering the social obligations of the bacteriologist, we may ask ourselves the more direct and important question: what are the present day scientific problems which are facing us? We are, of course, concerned, first of all, with the problems

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based upon the nature and activities of the microbes, and, secondly, with the application of these activities to human welfare. More than 20 centuries ago, the Greek philosopher Xenophon said, "The Gods have not revealed everything to men from the beginning, but men by searching in time find out better." The tendency has been, among many scientific workers, to emphasize scientific principles and let the laymen find their applications. Has not Virchow emphasized that it is the business of the scientist to establish facts, but not to philosophize about them? Fortunately, we microbiologists have not been hampered in our work by such rigid regimentation of ideas. We have never felt particularly disturbed by the possibility of finding applications for the scientific facts that we have uncovered. In this respect, we have had a great example before us. It was Pasteur who said: "For one who has devoted himself to science, the pleasantest thing that may come is to increase the number of his discoveries, but the cup of his pleasure is filled to its brim when the results of his work begin to be applied to practice."

THE MICROBE AND ITS NEIGHBORS

One may well begin by an examination of the problems concerned with the life of microbes in natural habitats and the interrelationship among microbes. Many of us, who are dealing with microbes as disease-producing agents or with the utilization of microbes in the preparation of foodstuffs or in industry, are accustomed to concern ourselves with pure cultures. These have come to be recognized as the sine qua non of the microbiologist. The isolation of an organism in pure culture has been one of the fundamentals of Koch's postulates, the ten commandments of bacteriology. Virtually all textbooks have accepted these postulates without question. The impression that one gains from a perusal of the scientific literature in the field of microbiology is that microbes can hardly be studied except in pure cultures. The available information on the role of microbes in living processes has thus become classified if not codified entirely on the basis of pure culture studies. This may be justifiable and even essential in the consideration of microbes as causative agents of disease and of methods for combating them, or in the utilization of microbes for the manufacture of chemical compounds; however, it is hardly sufficient, except as mere supplementary information, in the study of the many natural processes carried out by microbes, as in the soil, in the residual wastes of plant and animal life, in water basins, in sewage disposal plants, and in many other substrates.

It need not, therefore, appear strange to emphasize the fact that the great majority of microbes are found in nature and carry out their normal activities not in pure cultures, but in mixed populations. One may recall in this connection the remark of Winogradsky that some bacteriologists deal with hothouse varieties of bacteria, far removed from their naturally occurring progenitors, and would hardly recognize them when found in their natural milieu. Criticisms of this kind are often justified when one considers the fact that strains obtained from culture collections are employed for the study of the physiology of bacteria, in the belief, and often in the blind hope, that one is actually duplicating and
interpreting phenomena occurring in nature. One thereby overlooks the possibility and even the probability that physiological reactions brought about by such cultures may vary markedly from those of strains freshly isolated from a natural substrate, and especially from those that are active in their natural habitats.

Since my own interests are closely bound up with the soil microbes, I may be forgiven for drawing my illustrations largely from those microorganisms that inhabit the earth and that make up what may be designated as the soil population. Among the various methods employed by the soil microbiologist for the isolation of specific organisms, the "enrichment culture" method has received particular attention. It consists in enriching the soil with specific substances or compounds or treating it in such a manner as to stimulate the development of specific microbes capable of bringing about particular reactions. Such a process greatly facilitates the isolation of organisms, which would otherwise prove to be a most difficult task. By means of this and other methods, it has been possible to establish definitely that the soil harbors an extensive microbiological population, which is made up of thousands of species of bacteria, hundreds of genera of fungi, actinomycetes and algae, numerous families of protozoa, nematodes, and other worms and insects.

Some of the organisms are known to be concerned in the soil with highly specific reactions, such as the fixation of atmospheric nitrogen, the production of nitrite from ammonia, of nitrate from nitrite, of sulfuric acid from sulfur, and many others. Some of the processes can be carried out not only by a single organism, but by a number of different organisms. The decomposition of cellulose in nature, for example, can be brought about by many kinds of bacteria, possessing distinctly different morphological and physiological characteristics, by many fungi, belonging to widely different genera, by many actinomycetes, by certain protozoa and other invertebrates. The same is true of the decomposition of proteins, of hemicelluloses, and of various other organic compounds, such as hydrocarbons and phenols. Some of these processes may be brought about in chain-like reactions, where one organism uses the products of another, where one reaction leads to another, where the activities of one organism depend entirely upon those of others. The decomposition of proteins or of cellulose by complex populations and the two-step oxidation of the ammonium ion to the nitrate ion are good illustrations of this type of transformation.

All of the above processes, when considered separately from one another, fail to elucidate fully the complexity of the soil microbiological population, with its numerous interrelationships. Considerable evidence has now accumulated which serves to emphasize the fact that not only do microbes assist one another in creating favorable conditions or in preparing the required nutrients, not only do microbes compete with one another for the available foodstuffs, but they exert a variety of other functions, whereby they influence the activities of other living systems; in regard to many of these activities there have been so far only conjectures or unsubstantiated hypotheses. It is sufficient to mention, by way of illustration, the production by various microbes of stimulating substances, the
nature of which is not yet known, of injurious substances, comprising both toxins and phages, and a variety of other agents which result in the destruction of some microbes by others.

Any attempt to elucidate the numerous interrelations making up a complex microbiological population must, therefore, take into consideration a number of factors, which are based upon 1. availability and utilization of nutrients or food-stuffs; 2. the competition for available space; 3. the effect of environmental conditions upon specific microorganisms. To these may be added, 4. relationships among individuals within one group or one species (homogeneous population) and individuals or masses of individuals belonging to different groups (heterogeneous population); 5. relationships between young, growing, and reproducing cells, and older and respiring cells; 6. relationships between immune or resistant varieties and less resistant or more sensitive forms against attack by agents causing their destruction; 7. relationships between microbes that are able to become adapted to a symbiotic form of life, as in the case of the root-nodule bacteria or mycorrhiza-producing fungi, and the great majority of microbes which are not adapted to that type of life; competition between different strains of legume bacteria, either within or outside the plant, may result in the suppression of one strain by another, the latter then becoming the dominant strain, which is responsible for the production of the nodules. Finally we have, 8. relationships of microorganisms possessing the capacity to attack other living organisms, which have thus become adapted to a parasitic form of existence, and saprophytes or organisms that obtain their nutrients from simple elements and compounds or from dead organic residues. One may leave out of consideration, for the present, the phenomena of competition between microbes and Homo sapiens, as well as of the animals and plants for food, for space, or for the very tissue of the higher forms of life.

The above relationships comprise, in the broadest possible sense, the struggle for existence in the world of microbes, or as Pearl (1928) so aptly put it, the "dynamics of populations." Time does not permit consideration of these relationships in detail, and certainly not of their mathematical treatment, as has been done by Ross (1911) for malaria-host relation, by Volterra (1931) and Lotka (1932) for host-parasite relation in a very broad sense, and, in a more limited sense, by Buchanan (1918) and Rahn (1932) for pure bacterial populations, by Richards (1934) and Gause (1934) for pure yeast populations, and by Woodruff (1914), Johnson (1933), Gause (1934) and many others for mixed protozoan populations. It suffices to dwell here upon two types of interrelations in the world of microbes, namely, those of association and antagonism.

ASSOCIATIVE RELATIONS AMONG MICROBES

The physiology of an organism in pure culture is so markedly different from that of the same organism in a mixed population that one is often astonished to discover that an old friend, known and recognized by its specific reactions in given media, behaves quite differently in the presence of other organisms. Some of its well defined characteristics may be either gained or lost; it may show cer-
tains preferences or dislikes for certain nutrients or bring about reactions different from those that are taken for granted in pure culture.

A certain fungus, capable of decomposing cellulose, was found to prefer protein as a source of energy, when allowed to attack a plant material containing both substances; in the presence, however, of another organism which could not decompose the cellulose but could utilize the protein, the first microbe proceeded to decompose the cellulose. Another illustration: certain cellulose-decomposing and nitrogen-fixing bacteria were found to bring about the processes of cellulose-decomposition and nitrogen-fixation far more vigorously in the presence of other bacteria, which themselves could not effect either of the processes. This may be due to the destruction by the latter of the metabolic waste products produced by the primary organisms, or to the production of certain catalysts (growth-promoting substances), or to some other mechanism. Many other illustrations are found in nature of co-operation between different organisms, resulting in processes which neither could carry out alone. Aside from nutritive associations, there are other associations among microbes which may be designated as environmental, where one organism makes conditions favorable for the growth of another, as in the case of aerobes living together with anaerobes.

The role of associations of microorganisms in the causation of diseases of animals and plants has attracted much attention. Although some such associations have reduced the intensity of a disease, as in the classical experiments of Pasteur on the repression of anthrax by accompanying bacteria, others have aggravated the disease condition, as in the case of many mixed infections.

Not all investigators concerned with the problem of metabolism agree upon its fundamental unity or the laws governing it. According to some (Haldane, 1937), all the millions of species of living organisms, perhaps even the thousands of strains within each species, possess their own peculiar types of metabolism. According to others (Kluyver, 1931), however, the metabolism of all organisms is governed by certain fundamental principles, which are only slightly modified under certain specific conditions, the mechanism of the biochemical reactions always remaining the same. Should biochemistry of microorganisms become a study only of the intermediate metabolism or the "transformations undergone by matter in passing through organisms," and should life itself comprise "the active maintenance of normal and specific structure?" Should metabolism comprise a study of the numerous enzyme, hormone, and vitamin reactions in the living system, or should these reactions be considered as manifestations of life quite apart from the individual organisms concerned? Should one attempt to differentiate between the physiology and the biochemistry of the bacteria, or should one accept the dictum of a British biochemist that "bacteria have no physiology, only a biochemistry" (Yudkin, 1938).

In connection with the relationships discussed above, one may be justified in asking: What is, after all, normal and what is abnormal metabolism? I presume that the first refers to a type of growth of a living organism under a certain set of conditions, and the second under conditions which are in some way modified from the prescribed norm. When microbes are transferred from
their natural substrate, be it soil, water, or even a living plant or animal body, to an artificial medium, their growth upon this medium is said to represent "normal metabolism." When the particular medium is modified as by the introduction of salts of heavy metals, by changing the concentration of the nutrients, by modifying the reactions, or by changing conditions of growth, especially temperature and air supply, the previous or "normal" type of metabolism is changed, often very markedly, and a new type of metabolism results which is often designated as "abnormal metabolism." The type of growth of the microbe, its intermediary products, and the final metabolic products may now be quite different.

It is sufficient to cite the following illustrations concerning the modification of the metabolism of an organism. 1. Certain anaerobic bacteria produce butyric acid in the presence of CaCO₃, but yield largely butyl alcohol in the absence of a neutralizing agent. 2. Certain yeasts form alcohol in the absence of oxygen, whereas in the presence of the latter the carbon is largely consumed by the cell to give microbial substance, and little alcohol is produced. 3. Some fungi belonging to the Rhizopus group yield fumaric acid in the absence of zinc and in the presence of iron, but none or very little of this acid is formed in the medium when the two ions are present in reverse order. In these illustrations, the metabolic reactions are definitely modified, but they are not fundamentally changed.

The nature of the enzyme complex produced by one organism, the chemical composition of the organism, and even its physical appearance may be influenced to a marked extent by the presence of other organisms. It has been said that the bacterial cell contains three types of enzymes; some are organized to serve cell growth and cell division, some are only loosely coordinated, and some are mere free lances. A consideration of the question of constitutive enzyme vs. adaptive enzyme mechanisms would take us too far afield, but the bearing upon cell nutrition of the presence of other organisms, is, in this connection, quite obvious.

The problem of vitamin requirements and vitamin synthesis by microorganisms also has an important bearing upon the subject under consideration. Some microbes are capable of synthesizing their own requirements for growth-promoting substances, whereas others require an additional supply of some of the vitamins for growth and reproduction.

Could one say that these phenomena are manifestations of purposefulness vs. purposelessness in living systems? Is the development of a particular organism dependent upon the hidden capacity for a reaction characteristic of the species or upon the selection of certain strains of this organism from a large population of cells having such a capacity? What are the relationships between the mechanisms inherent in the cell and those that are influenced by other cells living in close proximity? The fact that different species within a single genus and even individual strains within the same species may vary considerably in their metabolic activities, when grown under exactly the same nutritional and environmental conditions, should lead us to analyze this concept more critically.

The phenomena of association among microorganisms are complicated further
by antagonistic effects. These as well vary greatly. They comprise relationships of competition for the available food supply, interference with certain types of physiological reactions including enzyme mechanisms, and the destruction of certain organisms by others, even the feeding of some microbes upon others. In the sum total of the economy of nature some of the latter effects may prove to be beneficial to the system rather than injurious. It is sufficient to cite the effects of protozoa upon many specific reactions brought about by bacteria, such as nitrogen fixation, cellulose decomposition, and ammonia formation; by consuming some of the bacteria, the protozoa apparently favor further bacterial multiplication and thus the continuation of the particular reaction.

**ANTAGONISTIC RELATIONS AMONG MICROBES**

Generally, the phenomena of antagonism may be expressed as follows: when two or more microbes live together, one may become antagonistic to or exert an injurious effect upon the others. The composition of the medium and the conditions of growth influence the mode of action of the antagonist. The metabolism and the very structure of the antagonized organism may become modified, or the cell may even be destroyed. There is no general rule that determines which of the two organisms living together will exert an antagonistic effect upon the other. In urine, for example, staphylococci may become antagonistic to *Escherichia coli*, or *vice versa*, depending on the initial numbers of the two organisms, or on the formation of specific metabolic products, or on the exhaustion of nutrients.

The antagonistic relationships among microbes were at first visualized as resulting from the competition for food. It was soon recognized, however, that this explanation alone was not sufficient to cover all the complex interrelations. De Bary emphasized, in 1879, the significance of the antagonistic phenomena in natural processes. When two organisms were grown on the same substrate, one overcame the other sooner or later, and even killed it. This relationship was designated by Ward as "antibiosis."

Organisms living in associations frequently develop characteristics that they do not possess when living in pure cultures. Schiller (1927;1930), for example, found that when beer yeasts were placed together with tubercle bacteria in a sugar-containing but nitrogen-free medium, the yeasts developed antagonist properties toward the bacteria and used the latter as a source of nitrogen. The yeasts secreted a bacteriolytic substance which was also active outside their cells.

A varied terminology has been developed for designating the phenomena of antagonism. "Direct antagonism" was differentiated from "passive antagonism," which was looked upon as depending not upon the direct action of the antagonist but upon changed conditions of culture under the influence of its growth; the latter may comprise a change in pH and rH of medium or an impoverishment of some of the nutrient constituents. "Direct antagonism" was often distinguished from "indirect antagonism," the first being limited to those phenomena in which the antagonistic effect is connected with the action of the living cell itself (a process often designated as "true antagonism"), whereas in
the second the metabolic products produced by one organism are injurious to other organisms. Intestinal bacteria, for example, were found to repress the anthrax organism only when the former were in an active living state.

Antagonism may be either one-sided or two-sided. In the first case, only one organism represses another, which is not antagonistic to it; in the second case, both organisms repress each other. A one-sided antagonism may become two-sided under certain conditions of culture. Escherichia coli, for example, is antagonistic to Eberthella typhosa; however, if the latter is inoculated into a medium somewhat earlier than the former, the reverse becomes true.

It has also been suggested that there exists, for every bacterium, a typical constant number of cells capable of living in a given space; when this concentration is reached, multiplication comes to a standstill, without the nutrients being exhausted or toxic substances produced. The same effect was believed to hold true when two different bacteria live together. If the limiting concentrations of the two organisms are different, the one with a higher value will repress the other, but the weaker species may check the stronger one when planted in sufficient excess.

The type of antagonism that results in the production of active chemical agents or antibiotic substances came to be recognized as of great significance in the development of microbial populations. The nature of the substance produced by different bacteria and fungi was found to be specific and characteristic of the organism. Some of the substances are destroyed by boiling, by exposure to light, and by filtration, whereas others are resistant to heat and to ultra-violet rays; some are readily adsorbed by filters and various adsorbing agents, from which they can be removed by special solvents, such as ether, alcohol, chloroform, or acetone, or by alkali or acid solution. Some of the substances have been crystallized; information has been gained concerning the proximate chemical nature of others; and the nature of many others is still imperfectly understood.

Among the numerous instances of antagonistic relationships of microorganisms in nature, the most interesting pertain to the behavior of pathogenic organisms brought in contact with a native population, such as that of soils, sewage, or water basins. Following the early work of Pasteur and others before the turn of the century, on the repression of pathogens by simultaneous inoculation with various other bacteria, several important contributions appeared. It is only within the last few years, however, that the subject has gained much significance. Frost demonstrated, in 1901, that a number of organisms show a marked antagonism against Eberthella typhosa. Pseudomonas fluorescens exhibited the strongest antagonistic properties; Proteus vulgaris acted more rapidly, but the active substance did not diffuse to so great a distance in the medium. Various soil bacteria were found by Frost to produce active substances that passed through a collodion film. Mixed cultures showed greater antagonism than pure cultures. This was believed to be due to the fact that the latter had been grown for a longer time on artificial media; the greater antagonism in the mixed cultures may also have been due to the combined action of two or more species. Frost concluded that (a) a marked antagonism is exerted by mixed cultures of
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bacteria against pathogens; (b) this antagonism results not only in checking growth, but in actually killing the pathogen, the death rate depending on the period of preliminary cultivation of the antagonist; (c) there is no evidence to show that the antagonistic substance exists in an active state in soil or in water, but rather that the phenomenon of antagonism depends on the rapid development of the bacteria present together with the antagonized organism.

Since Frost's work, an extensive literature has accumulated (Waksman, 1941). It is hardly necessary to review here the many contributions on the antagonistic effects of microorganisms. It is sufficient to mention the work of Fleming, Raistrick, Chain, Florey et al on penicillin, of Dubos on tyrothricin, and of certain others. Sufficient to say that many organisms have now been found capable of producing antibiotic substances when grown on artificial media. These organisms belong to a variety of morphological groups. The substances vary greatly in their chemical nature and in the mechanism of their action: 1. some act primarily upon bacteria, and others upon fungi; 2. some influence cell division, others affect respiration, still others interfere with the utilization of certain metabolites; 3. they are selective in their action, some being capable of acting upon a great number of organisms, and others upon only very few; 4. some act primarily in vitro and others act also in vivo; 5. they vary in their toxic effect upon the animal body, and in the reactions upon the tissues; 6. some are hemolytic and others are not; 7. some are water-soluble and others are only alcohol-soluble. Because of these properties, some can be used for general treatment, and others have only a local application.

These antibiotic substances offer great possibilities for many practical applications, including (a) the domestication of microorganisms for disease control; (b) the isolation of new chemotherapeutic agents for combating animal diseases; (c) the utilization of the activities of microorganisms for combating certain plant diseases. A new field of research has been opened to us; the possibility of elucidating many complex natural processes heretofore not sufficiently understood and of discovering new agents that may help man in controlling diseases caused by pathogens to himself and to his domesticated plants and animals is certainly inviting.

EPILOGUE

The microbe has come of age. The World of Microbes represents as complicated a biological system, with as complex interrelationships, and with as many varied applications, as the world of higher animals or plants. There is no field of human endeavor, whether it be in industry or in agriculture, whether it be in the preparation of foodstuffs or in connection with problems of shelter and clothing, whether it be in the conservation of human and animal health and the combating of disease, where the microbe does not play an important and often a dominant part. Knowledge gained by the microbiologist has been applied to practice; advantage has been taken of the activities of the useful organisms in furthering human progress and the harmful forms have been combated. May I be permitted to quote, in connection with the last phase of my address, from a
recent issue of the British Medical Journal: "Whether gramicidin or any other product of microbic origin will eventually be found to fulfill certain purposes better than either sulphonamides or any other class of antiseptic remains to be seen. That several classes of reagents should be competing for supremacy in different aspects of a task which not long ago was considered impossible of any real fulfilment is a truly remarkable position." This can apply with equal if not greater justification to penicillin and, I venture to say, to several other preparations that are already looming on the horizon.

One may well conclude with the words of Pasteur: "Messieurs, c'est les microbes qui auront le dernier mot!"

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