The Microbe's Contribution to Biology

A. J. Kluyver
C. B. van Niel

The Microbe's Contribution to Biology

A. J. KLUYVER

and

C. B. VAN NIEL

Harvard University Press
Cambridge, Massachusetts
1956

DISTRIBUTED IN GREAT BRITAIN BY
GEOFFREY CUMBERLEGE, OXFORD UNIVERSITY PRESS, LONDON

LIBRARY OF CONGRESS CATALOG CARD NUMBER 56-5343
PRINTED IN THE UNITED STATES OF AMERICA

The Microbe's Contribution to Biology

THE JOHN M. PRATHER LECTURES, 1954

Preface

This printed version of the "John M. Prather Lectures," delivered by the two authors at Harvard University in April 1954, is an unpretentious book. It is not meant for microbiologists, who must inevitably realize that it does not contain a harmonious summary of present-day microbiological knowledge. The lecturers were given to understand that their audience would be composed of staff members and students specializing in many divergent phases of biological science. Hence it seemed appropriate to devote the lectures to a brief survey of some aspects of microbiological research which, in the opinion of the authors, have contributed significant principles to an interpretation of the behavior of living organisms. We hope that this approach may appeal sufficiently to biologists in general to justify the present publication.

It should be evident that the time element imposed drastic limitations on the amount of material that could be treated. This has resulted in the neglect of some important fields of microbiological research, such as virology. Furthermore, the nature of the lectures implied that the authors had to draw freely on the work of many microbiologists; it is to be hoped that we have done justice to their notable contributions.

Although the printed version adheres closely to the text of the lectures as delivered, it was considered desirable to expand the documentation for some of the conclusions and to delete certain passages of a more digressive nature.

The references should not be considered as an adequate bibliography of the subject matter. They are mainly intended to guide the nonmicrobiologist to some of the classical papers and general reviews pertaining to the major topics. It seemed, however, desirable to include in addition a number of publications on which the salient points of the discussions are based. This accounts for the decidedly heterogeneous character of the lists.

On several occasions the authors have pondered the question of why the task of delivering these lectures was assigned to them. We have concluded that this should be attributed to the fact that for more than three decades we have followed developments in microbiology with keen interest. During the first six years of this period this was possible under conditions of close and almost daily personal contact; subsequently a spatial separation of some 6,000 miles made necessary individual effort, aided by correspondence and rare encounters. For us one of the great attractions of the Prather Lectures will always remain the opportunity thus provided to resume our personal contact after a lapse of nearly eighteen years.

We wish to acknowledge our profound indebtedness to the Committee on the Prather Lectures of Harvard University which issued the invitation, in particular to Dr. Kenneth V. Thimann, who has spared no effort to effectuate the project for which he took the initiative as early as 1951. The warm reception he prepared for us, and the many pleasant and stimulating discussions with him, will be gratefully remembered.

The hospitality proffered by the President and Fellows of Harvard University and by the staff of the Biological Laboratories is also deeply appreciated.

In addition, the authors wish to thank all those who have contributed to making this publication posssible, especially the scientists, editors, and publishers who have kindly permitted the reproduction of figures; the sources of these are acknowledged in the legends.

A. J. K.
C. B. v. N.

Delft, Netherlands
Pacific Grove, California
November 1954–February 1955.

The Microbe's Contribution to Biology

Contents

1

Microbial metabolism and the energetic basis of life

A. J. KLUYVER

Less than three full centuries have passed since microbes were discovered. Until that time mankind lived in blissful ignorance of the essential role which microbes play in the subsistence of the human race on earth. And it even took another 150 years after the discovery of the first microbe before this insight began to dawn, thanks to the penetrating studies of men like Ehrenberg and Pasteur. Nowadays most scientists are vaguely aware that something would go wrong if somebody were to succeed in exterminating the microbe world. Few people, however, realize how quickly terrestrial surroundings would deteriorate, transforming our greenest pastures and our tropical forests into barren areas. Further disconsolate horrors of life on a microbeless earth are excellently described by Rahn in his *Microbes of merit.*[1]

Without entering into details, we may, therefore, take as a starting point the recognition that all of us are personally indebted to the microbe without which only a few human individuals, by taking very special measures, would be able to lead a highly artificial and charmless life.

The purpose of these lectures is to consider the question in what respects the biologist's outlook would be different if he had continued to ignore—consciously or unconsciously—all forms of life invisible to the naked eye. I believe that such an attitude would inevitably have led to a serious underevaluation of life's potencies, and this volume is an attempt to document this statement.

Let us first of all try to imagine what would happen to a biologist who has restricted his attention exclusively to the higher forms of life, and is then suddenly confronted with microbes.

Now some will probably object that it is senseless to discuss such a hypothetical case, and observe that microbiology has developed very smoothly in the hundred years of its existence, so that its results have quite gradually become available to the biologist. To this I reply that since Pasteur's startling discoveries of the important role played by microbes in human affairs, microbiology as a science has always suffered from its eminent practical implications. By far the majority of the microbiological studies were undertaken to answer questions either directly or indirectly connected with the well-being of mankind. In the first half of the 19th century only a few scientists with a wider interest had made more or less incidental excursions into the microbe world, and the trophies brought home from these expeditions had only very incompletely been incorporated into the general biological picture.

Toward the beginning of the present century the situation began to change. Under the influence of the magnificent examples set by a few pioneers like Cohn, Winogradsky, and Beijerinck, the number of disinterested workers in the microbiological field increased steadily. Gradually so many data regarding the occurrence and behavior of microorganisms had accumulated that biologists began to realize that they could no longer continue to ignore microbial manifestations.

To a certain extent it is therefore justified to say that the biologist of the 20th century has been more or less suddenly confronted with the microbe world. And I think I may add that the digestion of all these findings has proved to be a slow process which even today is still far from finished. On the other hand, it is clear that the increased interest in the microbe also led to a gradual penetration of general biological principles into microbiology.

Now it seems to me that the student of only the higher forms of life, on becoming acquainted with the microbe world, would experience a first shock when he learns that vast parts of the earth's crust—which he always had considered to be devoid, or almost devoid, of life—are in reality teeming with invisible living organisms. This holds equally well for seemingly barren patches of surface soil and for deeper soil layers, surface and subsurface waters, and even the atmosphere. Our "macrobiologist"—as we may perhaps call him for the sake of simplicity—would probably be inclined to find some consolation in the idea that these tiny unicellular organisms can be considered as mere curiosities of minor importance for life's terrestrial activities. But this illusion would not last long either, for the microbiologist would draw his attention to the following facts.

Reliable estimates have shown that the amount of carbon dioxide consumed annually in the photosynthetic activity of the green plants is such that the quantity of this gas present in the atmosphere would be exhausted within some 30 years, if it were not replenished. Even taking into account the important carbon dioxide reservoir present in the oceans as a buffer system, such an exhaustion should have occurred within historic times. It is, therefore, clear that the green plants can continue to grow only because the assimilated carbon is in some way reconverted into carbon dioxide. At first sight this will not present any difficulties to our "macrobiologist"; he will refer to the slow combustion of the vegetable remains by man and animals. However, several independent estimates tend to show that the annual carbon dioxide production by this means amounts to only about 5 percent of the annual carbon dioxide consumption by the green plants. The conclusion seems inevitable that the remaining 95 percent is produced by the mineralizing action of the microbe. As far as is known, Louis Pasteur [2] and Ferdinand Cohn [3] were the first to realize the indispensability of decay for the maintenance of life on earth, and to state explicitly that microbes are the driving force in this process. The corollary of this conclusion is that the total weight of microbial protoplasm on earth exceeds that of animal protoplasm by many times. Ignoring the microbe would obviously mean that a very considerable part—perhaps almost one-half—of the living protoplasm on earth is left out of consideration.

We shall see, however, that this quantitative aspect is certainly not the main reason why the biologist cannot afford to live on in ignorance of the microbe.

It seems likely that a "macrobiologist" who entered the microbiological scene around 1910 would have been most impressed by the great diversity in properties of the microbial species to which he was introduced by the microbiologist. On inquiring into the natural habitats of all these different types of microbes he would get rather definite answers only for a certain number of specialized types, such as animal and plant parasites, or organisms living in environments where very specific conditions more or less constantly prevail, as in hot springs or salt lakes. Microscopic examination of such habitats often reveals the presence of readily identifiable microbes. But if he asks from what special materials the microbiologist had isolated the majority of his cultures, he would probably be referred to some arbitrary sample of soil, mud, or water, or even to the atmosphere. This answer would be all the more baffling to our "macrobiologist" because direct microscopic examination of such materials would fail to show anything like the large variety of microbes that can be isolated from them.

Then the microbiologist would come to his aid, and give him the following explanation. Experience has shown that a very large number of microbes may be considered to be almost ubiquitous. This does not mean that they are everywhere in considerable numbers, but that a few individuals of the species succeed in maintaining themselves at very divergent spots on earth, either in a dormant state or by temporary and localized outbursts followed by a slow decline of the micropopulation formed. Moreover, many of these germs are of the airborne type, and localities temporarily devoid of a certain microbe may soon be repopulated from places where the germ in question has just flourished. Taking into consideration on the one hand the dynamic state of conditions in most soils and waters, implying an almost continuous change in environmental conditions, and on the other hand the marked diversity in the nutritional requirements of various microbial species, it is clear that it is not easy to prophesy which germs will be abundant, which will maintain themselves at a low numerical level, and which will die off in a special locale at a certain moment.

That nevertheless the microbiologist so often succeeds in isolating specific microbes from a given sample of soil or water is due to a methodological principle first applied by Winogradsky,[4] and still more consciously developed with quite amazing success by my great predecessor in the Delft chair of microbiology, Beijerinck.[5] This principle has been dubbed by Beijerinck the ecological approach; its application depends on a well-considered selection of the conditions in a primary culture medium, thus causing preferential growth of a certain type of germ, ultimately leading to a predominance of the conditionally fittest. As soon as this stage is attained, isolation of the prevalent organism with the aid of methods commonly used by microbiologists is, of course, an easy affair.

Armed with this mighty tool, Winogradsky, Beijerinck, and those who followed them have made a thorough exploration of the microbe world. Besides the fact that these investigations have proved the practically ubiquitous occurrence of many microorganisms on earth, they have thrown a clear light on the surprisingly large diversity in nutritional requirements of the various microbial types. The remarkable result was obtained that nearly every soil sample seemed to contain germs willing to develop even in media with an extremely one-sided composition, for example, those containing one simple organic compound as sole source of carbon. In consequence, no sooner was one specialized microbe discovered than another with still greater achievements came to light.

The classical examples of the application of the microecological principle, usually designated as the principle of elective or enrichment culture, are, of course, well known among microbiologists. Yet it seems to me that not many are fully aware of its tremendous import. Is it not more or less a miracle that with its aid it is quite feasible to prove within a short time the wide occurrence of a microbe willing to feed on some new synthetic herbicide? [6] Or is it not quite amazing when a successful enrichment experiment shows that in an arbitrarily chosen soil sample microbes are present which are able to use carbon monoxide as sole source of carbon, especially when we take into consideration the fact that this gas so rarely occurs in nature? [7]

I think that we may expect that our "macrobiologist," on being confronted with a nearly endless diversity of such physiological monstrosities would find the microbiological scenery bewildering. On the other hand, the demonstration of the almost limitless applicability of the elective culture will make him ponder on its significance for microbial ecology. As a result he will be unable to escape the conclusion that in nature, with its continuously changing environmental conditions, successions of microbial forms will also occur. It is clear that this opens a new vision on the almost infinite capacity of life—this word used in its collective sense—for adapting itself to the immense variety of external conditions realized on earth. Is not the conclusion warranted that our "macrobiologist" has thus been forced to acknowledge the existence of potencies of life that he would never have discovered if he had stuck to his plants and animals?

However, our "macrobiologist" would certainly be most shocked by the fact that his clear notions regarding the energetic basis of life, derived from his studies of green plants and animals, had been thoroughly uprooted as a result of an acquaintance with the microbes.

Starting with the green plants he would have arrived at the conclusion that a living organism is an entity in which radiant energy is converted into chemical energy, thus enabling the organism to grow at the expense of carbon dioxide and some other simple mineral compounds. In addition he would have noticed that the utilization of the radiant energy was dependent on the presence of chlorophyll-containing cells in the plants. Next directing his attention to animals he would at first be at a loss on finding that any apparatus for an energetically important conversion of radiant energy apparently was lacking. On continuing his studies he would discover some characteristics of the animal forms of life, in particular, their need for a constant supply of free oxygen and of the dead remains of plants or of other animals.

Our "macrobiologist" would, therefore, be conversant with the idea that life had found two fully independent solutions for the problem of meeting its energetic and nutritional needs: a purely mineral nutrition provided that radiant energy can be utilized, and a nutrition depending on the availability of a complex organic food, part of which is burnt with the aid of free oxygen.

In view of all this we can understand the horror with which the "macrobiologist" would learn that there are microbes which are able to proliferate in completely inorganic media, but which, in contrast to the green plants, are not in need of radiant energy. Next he would meet with microbes that, like his animals, could use the system: organic matter plus oxygen in order to satisfy their nutritional demands. But again he would be startled when a deeper penetration into the behavior of these organisms taught him that, in contrast to what he had found for his animals, a large number of species would be perfectly happy with one single organic compound in their food. And his surprise would mount to amazement on finding that the different species showed an extreme diversity in the nature of the compound suitable for the maintenance of their life, and that among these compounds many are very remote from the normal substrates of animal nutrition. What could he think of forms of life for which, in addition to mineral salts, a substance chemically so inert as a paraffine hydrocarbon is the only requirement for proliferation? Or to make it still more paradoxical, how could our "macrobiologist" ever digest the idea that some microbes are able to accept certain phenols, so rightly renowned for their germicidal properties, as sole organic component of their food?

It seems possible that the "macrobiologist" would still find some consolation in the consideration that all these forms of life had at least one point in common with his animals, namely, the need for free oxygen which, here too, apparently was used to bring about a slow combustion of some component of the food. But scarcely having reached this conclusion, he would stumble upon various types of life that as Pasteur proved can flourish in the absence of free oxygen, and whose activities may even be inhibited by this gas.

It is easily understood that the microbiologist, so far largely occupied with an exploration of the microbe world, also began to feel the need for an inventory and subsequent evaluation of these findings, at first sight so chaotic. The first task in this respect was, of course, to draw up a survey of the various metabolic types encountered in microorganisms. A brief outline of the results obtained in these efforts to bring about a preliminary ordering follows.

From studies of the metabolism of the living cell the general experience has been gained that part of the components of the food that enter the cell are excreted again into the surrounding medium after having undergone a chemical conversion. It is usual to designate this part of metabolism as "catabolism" or "dissimilation," in contrast to those chemical conversions of food components that lead to the building up of cell constituents, and are summed up in the terms "anabolism" or "assimilation."

For reasons that will become clear later on, it is appropriate to consider the catabolic aspect of metabolism first. The foregoing definition of a dissimilatory process obviously implies that such a process can be important to the cell only from the standpoint of energy supply. And to this I may add at once that experience teaches that continuous transformation of chemical energy present in the food is an indispensable condition for maintaining the cell in an active state.

Now it has been well known since the time of Lavoisier that in animals the main dissimilatory process is a slow combustion of carbohydrates, fats, or amino acids with the aid of oxygen derived from the atmosphere, thus yielding carbon dioxide, water, and ammonia. This so-called respiration process is also encountered in the green plants, but here it is preceded by the primary energy-yielding process, the photochemical conversion of carbon dioxide and water to carbohydrate, which then acts as the substrate of respiration.

In view of the universal occurrence of the respiration process in the higher forms of life, one might expect that the microbial cell would also depend on this fundamental conversion for satisfying its energy requirements. And such has indeed been found in many different microorganisms.

However, a further investigation of the dissimilatory activities of microbes endowed with the faculty of bringing about this standard respiration process has led to some remarkable results.

In the first place it was found that in many microbial species, at least under certain, often ill-defined conditions, the oxidation of carbohydrate does not lead to the final oxidation products, carbon dioxide and water. Depending on both the specific organism and external conditions, different products of incomplete oxidation may be excreted by the cell. Table 1 lists some conversions leading to different products, all derived from the incomplete oxidation of glucose.

The second point that has emerged from the investigation of the microbial respiratory process is that for one and the same species a surprisingly large variety of chemical compounds can often serve as respiratory substrate. To cite an example: den Dooren de Jong long ago demonstrated that a strain of *Pseudomonas putida* can proliferate on a mineral medium to which any one of some 80 compounds was added as sole organic component.[8] It should be noted that these compounds include representatives of structurally quite unrelated groups.

In view of these facts it is clear that the "macrobiologist's" notion of respiration as being a more or less standardized process has to be definitely amended.

Another point of view resulting from the studies of microbial