BACTERIOLOGY

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A TEXT BOOK ON FUNDAMENTALS

BY

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TO

ROBERT WILLIAM HALL, PH. D.

PREFACE TO THE SECOND EDITION

Although a number of minor changes have been made in each printing of the first edition of this book, the rapid advances in the science and our changing viewpoint regarding some of its fundamental phases have made a thorough revision necessary. This edition, therefore, has been entirely rewritten. Modifications, additions, and omissions have been required in every chapter, and in most cases an entirely new method of presentation has been employed.

The success of the first edition has indicated that the type of subject matter chosen has been satisfactory. No change, therefore, has been made in the general plan of the book, although additional material on the morphology and physiology of bacteria has necessitated the inclusion of an additional chapter.

As in the past, the author has taken full advantage of the kind assistance of his colleagues and again extends his sincere thanks.

STANLEY THOMAS.

BETHLEHEM, PA.
March, 1930.

PREFACE TO THE FIRST EDITION

The great strides made in the science of Bacteriology during the past quarter century have been recorded in a mass of literature in scientific journals in many languages. To the lay mind, however, this science is still considered solely as an instrument in the hands of the medical profession. While medical bacteriology has been of invaluable service to mankind, and while there is no doubt that the impetus given by Pasteur, Koch and Lister has put this branch of the science ahead of other branches in pure achievement, the fact must not be lost sight of that medical bacteriology is simply one branch of a science which is, like its parent sciences chemistry and biology, of educational and practical value to mankind in many and varied fields of endeavor.

To the modern agriculturist a knowledge of that branch of bacteriology which teaches of the changes which take place in the soil due to the action of microörganisms, and the diseases of the higher plants, is essential. The engineer is interested in public water supplies and sewage disposal, and he cannot intelligently plan for proper sanitary construction without a knowledge of what "contaminations" are, and of the biologic factors in sewage treatment. The chemist is concerned with enzyme action, both from a theoretical point of view in his study of catalysis, and in the "practical" point of view in the manufacture of such chemicals as ethyl alcohol, butyl alcohol, lactic and acetic acids, acetone, etc.

The great canning industries, dairies, packing houses, tanneries, sugar refineries, and candy manufacturers, all employ expert bacteriologists both for routine analyses and for research investigations.

In teaching elementary Bacteriology to mixed classes of undergraduate engineers, chemists, premedical students, and general arts and science students, the author has come to a keen realization for the need of a text which treats of the fundamentals of this great science—fundamentals upon which the chemist, who later is employed in a fermentation plant, the medical student, and the sanitary engineer can build a superstructure of his own specialized knowledge. In an attempt to satisfy this need, this volume was written.

Inasmuch as some of our students take the course in elementary Bacteriology without any previous training in biology, an introductory chapter concerning the general conception of life and life's processes was considered essential.

The chapter on the Classification of Bacteria follows that recently adopted by the Society of American Bacteriologists and published by a Committee of the Society as Bergey's "Determinative Bacteriology" (Williams and Wilkins, Baltimore). While a name like *Escherichia coli* may seem like a queer appellation for our "old friend" B. coli, it is considered best that the proper generic classification be followed by the student, who can all too easily familiarize himself with nicknames.

In reading the various chapters on the bacteriology of air, water, sewage, food, etc., the experienced teacher will realize that only the barest outline is presented. In teaching certain groups of students, this will be sufficient; for others, this outline will serve as a basis for study which, as in the author's own case, can readily be elaborated upon by lectures.

The author expresses his thanks to his many friends who have aided in the production of the book, especially to Dr. Frank M. Huntoon, Medical Director, H. K. Mulford Co. and formerly of the Cornell Medical School, for his helpful criticisms of the chapters on Pathogenic Bacteria, and Immunity; to Prof. Frank O. Dufour, Head of the Department of Civil Engineering at Lafayette College; Prof. Ralph J. Fogg, Head of the Department of Civil Engineering at Lehigh University, and Prof. H. G. Payrow of the same department, for their aid in criticizing the chapters on the bacteriology of Water and of Sewage; to Dr. R. C. Bull, Director of the Student Health Service, Lehigh University, in the preparation of the chapter on Hygiene and Sanitation, to Dr. F. A. March, Professor of English at Lafayette College, for his aid in casting the manuscript; to Professors Babasinian and Long of the Department of Chemistry, Lehigh University, for their aid in the chapters on Bacterial Enzymes and Bacteria in Industry; to Dr. Sydney G. Paine of the Imperial College of Science and Technology, London, for his criticism of the chapter on Bacterial Enzymes; and, finally, to Dr. Robert W. Hall, Professor of Biology, Lehigh University, who has inspired the work and has read with critical eyes repeated revisions.

Numerous references are given to articles on special subjects, but no attempt can be made in a work like this to give due credit to statements which are of common knowledge to bacteriologists and which have become an integral part of the literature.

STANLEY THOMAS.

BETHLEHEM, PA.
June, 1925.

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CHAPTER I

INTRODUCTION

Bacteriology is that branch of science which treats of the life, activities, and functions of minute living creatures called "bacteria." Bacteria, or germs, or microbes, form an ill-defined group of microörganisms. Sometimes the term "bacteriology" is limited to the study of the members of a particular order of the class Schizomycetes or fission fungi. At other times it is used broadly as including the study of all of this class of fungi. Again, especially in medical bacteriology, the term includes not only consideration of the fission fungi but also of pathogenic yeasts and molds, and even extends into the animal kingdom and treats of a number of species of the Protozoa.

Regardless of which of these meanings is applied to the term, we must realize that, in the study of bacteria, we are concerned with living things. It is essential, therefore, that we understand some of the basic facts about living matter.

Life.—The term "life" is hard to define. The dictionary says life is the "state of being alive," and explains "alive" as "in a living state," which is what the logicians call "begging the question." From our store of observations, however, we can point out differences between a fossil rock or a petrified tree and a living worm, or tree, or dog. Moreover, we can distinguish between a dog which has been killed by a passing car, and his still running, barking, breathing playmate. These apparent differences are by no means the sole distinguishing features between living and lifeless matter—they are not in fact even the essential differences; but they serve to help us in determining what must be considered a mental concept—*life.*

The *origin of life* has been the subject of study and speculation from antiquity. Every system of religion and theosophy has offered its explanation. Science has attempted to create living things, and philosophers have always speculated on the "ultimate origin," or life's beginnings. One of the theories held by scientists for centuries, though now discarded, has had the greatest influence on scientific development—the theory of "spontaneous generation." According to that theory, animals developed spontaneously from decaying vegetable matter, from water, or from within other animals. Among the writings of the ancients are found exact directions for making mice from wheat, flies from decaying matter, and eels, frogs, and mice from mud and filth. Experiments similar to the modern boy's attempt to make snakes from horsehair were described seriously by writers two thousand years ago. The theory of spontaneous generation is one of those theories in which the burden of proof naturally rests on its opponents. It is known that life exists; that life must have started sometime; therefore, it may be reasoned, what is to prevent its being generated now? When the fact that the earth was at one time a molten mass of inorganic matter gained scientific acceptance, the believers in the theory of spontaneous generation thought that they now had absolute proof supporting their claim. And so they did—to this extent—that one or more living things must have arisen from non-living matter at some time in the remote past.

Most evolutionists incline to the belief that sometime in the past—but millions of years ago—there was one case, or, at most, a few cases, of life arising *de novo.* Those who accept the Biblical account of a fiat of creation, believe, of course, that life appeared upon earth in many different forms and all at the same time. On the other hand, those scientists who hold to the materialistic view of a fortuitous accident are apt to think of only one such appearance, because of the unlikelihood of a repetition of such necessarily complicated conditions. That unlikelihood and the lack of evidence are their only reasons for disbelief in present-day spontaneous generation.

As far as it is known at present, all living matter proceeds from preexisting living matter (biogenesis), a portion detaching itself and bringing with it the same power of reproducing. In non-living matter, nothing which is in any way similar to this property of living matter is known to exist.

It is now an accepted fact that, no matter through what cause life first appeared on the earth, a living creature cannot be made today, except through the agency of some already existing form of life.

For the purpose of this book, this statement may be made to include another idea. It is not only true that life cannot arise spontaneously, but also that life must arise from preexisting life *of its own kind.* In other words, the offspring, while differing in minor ways, must be similar to the parent in all biological essentials. The common observation that only dogs can breed dogs, and oak trees, oak trees, are simply examples of the general law that includes every species of living matter, from the typhoid germ to man.

At the same time, it must be understood that while the above is true of successive generations, one's viewpoint must be modified when considering the history of a species over a long period of time. Just as it is realized that life must have had a beginning at some time in the remote past, so also if a primitive form of life is assumed, one must admit that this "first life" has evolved into a myriad of forms which are recognized today as biological species. Thus, it is believed that in the remote past there existed on the earth an animal form whose descendants today are known as wolves, foxes, and dogs. Yet one cannot breed dogs and obtain fox offspring. The process of biological change through which new species originate is known as "organic evolution." This is explained by using such terms as adaptation, segregation, geographic isolation, etc. But one is forced to admit that while the fact of evolution is scientifically reasonable, the mechanism of the origin of species is still highly theoretical.

Composition of Living Matter.—One of the most interesting facts about living things is that no matter how widely separated they are in the scale of life, chemically they are made up of the same few elements. From the *chemical standpoint,* it is known that living matter always consists of complex compounds of the elements carbon, oxygen, hydrogen, and nitrogen, generally sulphur and phosphorus, together with various amounts of many other elements, in the form of salts or electrolytes. These elements, which, taken in as food, become a part of the living organism and are then living matter themselves, are obtained in various ways. Hydrogen and oxygen may be utilized by both plants and animals in the form of water, though animals obtain their nutritive supply of these elements from organic matter. Carbon can be utilized in the form of carbon dioxide by the green plants, through the aid of chlorophyll and sunlight. The non-green (non-chlorophyll-bearing) plants almost universally obtain this element from the same source that animals do, that is, from substances which are prepared for them by the green plants. One genus of bacteria has the power of oxidizing methane (CH_4). Plants obtain their nitrogen from simple nitrogenous compounds (as saltpeter) in the soil, and animals obtain it from plants (see nitrogen-fixing bacteria, p. 146). Phosphorus, sulphur, calcium, etc. are obtained by both plants and animals from simple organic or inorganic compounds.

What chemical change takes place when these elements become living matter is not known. They are combined into substances which are known as "proteins" (or proteids), which cannot be made outside the living organism. Although all proteins are not living matter, there cannot be living matter except in the form of proteins or combinations containing them.

The *proteins* form a large group of chemical compounds made up of nitrogen, carbon, oxygen, and hydrogen, quite often sulphur, sometimes phosphorus and rarely other elements—iron, copper, bromin, and iodin. These substances are susceptible to heat, to acids and alkalies, and to certain ferments called "proteolytic enzymes." When a change once takes place, it is impossible to get back the original protein molecule; the reaction is never reversible.

The protein molecule is a complex structure of derivatives of the amino acids. The amino acids are saturated fatty acids in which the hydrogen atoms of the radical CH_3 are replaced by the group NH_2. Thus: amino acetic acid (glycine, $CH_2(NH_2)COOH$) is acetic acid (CH_3COOH) in which an atom of the CH_3 group has been replaced by NH_2. In the same way, alpha amino proponic acid (alanine, CH_3CHNH_2COOH) is proponic acid (CH_3CH_2COOH) in which an atom of hydrogen from the alpha CH_3 group has been replaced by NII_2.

Amino acids are combined in the protein molecule by what is called the "peptide linkage," that is, through the carboxyl and the amino groups:

$$CH_3CH(NH_2)CO(OHH)HNCH_2COOH \quad \text{(alanylglycine)}$$

Many different organic groups enter into the protein molecule. The benzene group, various carbohydrate groups, the pyrrol group (NC_4), the immazol group (N_2C_3), and the indol group (C_8N) are only a few of the "rings" which are found in the different molecules.

As no loss of weight occurs when a substance ceases to be alive, no ponderable substance is lost, and it is possible, by chemical analysis, to find out the amount and the relative proportions of the various elements that make up a living organism. Yet, because chemical analysis kills living matter, it cannot be taken for granted that the substance analyzed is just the same as it was when alive. It may be that some change, such as a molecular rearrangement of the elements, takes place when living matter dies. Moreover, the difficulties of analysis are increased by the fact that only a relatively small part of a living organism is alive. Water, waste products of life, reserve food supplies, as in bone or cartilage, form a part of the living organism and yet are in themselves lifeless. Although it is not known in what form proteins exist in living matter, it is known that proteins form the chemical basis of living substance.

The mere knowledge of the chemical constituents of living matter is by no means sufficient to enable us to create life. In fact, up to the present time, the chemist has been unable to make true proteins.

The Physical Basis of *Life—Protoplasm.*—Looking at living substances from the physical rather than the chemical viewpoint, it is found that all living matter is built up in somewhat the same way. In the first place, water comprises more than half of the weight of all living matter. The rest consists of the chemist's proteins, together with other, though in themselves lifeless, ingredients—salts, carbohydrates, and fats. The various combinations of these substances, as found in living matter, are grouped under the collective term "protoplasm," or the essential form or "make up."

From the chemical point of view, protoplasm is a combination of proteins, and proteins are rightly called "the chemical basis of life." From the structural point of view, it is not, however complex in composition, a single chemical substance, nor does it always represent the same combination of chemical substances. Protoplasm is composed of a large number of different chemical substances united physically to form structures that are remarkably complex. Protoplasm has been defined by Huxley as the *physical basis* of life, and is generally recognized as a structure which forms living things. It is jelly-like in consistency, practically transparent, but not perfectly homogeneous. Consisting as it does largely of proteins which are insoluble in water, it may be considered as an emulsion or colloidal suspension of many and various substances of different densities in water.

The Cell.—Long before either the chemical or the physical nature of protoplasm was recognized, the fact had been observed by microscopists that living matter is always divided into very small units—which were called "cells."

Man or snake, oak tree or fern, when examined through the microscope is seen to be built of definite units analogous to the bricks in a building.

This unit structure of living things was first described by Robert Hooke, some 260 years ago (1665). Hooke described cork as being made up of "little boxes or cells distinct from one another," and showing a striking similarity in arrangement to the cells of a monastery. The name itself shows that Hooke regarded the cell as a cavity surrounded by solid walls. Unfortunately, Hooke saw and described the lifeless remains of what had been living matter. Cork, which is obtained from the bark of certain trees, is composed of what might be termed the "exoskeleton," or protective tissue of matter previously living, in which, however, the life substance has dried up and disappeared. (An analogous case would be an oyster, described after an examination of the oyster shell.) Even up to 1839, when Schleiden and Schwann demonstrated that all living things are made up in the same way, the solid walls were regarded as the essential part of the cell.

The essential part, however, of the cell is not the walls—the hard, solid substance which Hooke saw—nor the cavities; it is the matter which fills the cavities during the life of the cells: the protoplasm.

Unicellular and Multicellular Life.—Just as until quite recently the chemist looked upon the atom as the unit of matter, so, at the present stage of biology, a single cell is regarded as the unit of life. It is quite reasonable to think of the existence of a formless or structureless life, however, and, although we have given up the "ferment" or unorganized life idea, it may well be that such a life once existed and still exists. Some day we may understand the life force as we are beginning to understand the structure of the atom. Biologists today, however, think of all living things as composed of cells, and look upon the single cell as the unit of life.

All of the plants and animals about us are made up of innumerable cells, of many different kinds and shapes, and having different functions. In animals, nerve cells, muscle cells, epithelial cells, etc. all serve different purposes, and are made up of combinations of different proteins. Even in plants, it is readily seen that the cells in leaves fulfill a different purpose than those in the roots.

As different forms of animal life are studied, a great variation in the number and development of certain kinds of cells is noted, and, if these organisms are arranged in the order of complexity, it is found that there is a gradual scale, ranging from man down through the reptiles, the fishes, the worms, the jellyfish until a point is reached where there are practically no differences in the cells of the organism, and, finally, where all of the processes of life are carried out in a single cell. Such an animal as the amœba is spoken of as a unicellular animal. A similar scale can be drawn in the vegetable kingdom from the oak tree to the yeast plant.

Moreover, the life history of any multicellular individual can be traced to a single cell—the fertilized egg.

In the growth of a multicellular organism we start with one cell, which divides and ultimately produces innumerable cells, all of which are a part of the living body, and each of which plays a particular rôle in the body economy. These cells are incapable of independent existence; when the body dies they die. In the case of unicellular organisms, on the other hand, such growth does not take place; cell multiplication goes on just the same, but instead of building themselves up into a complex organism, each new cell maintains an independent existence. Thus, looking at life from the point of view of the single cell, growth in the multicellular forms corresponds to reproduction in the one-celled forms.

The Ideal Cell.—As was stated previously, multicellular organisms contain a great variety of cells which differ from each other in shape, size, and function. They represent different chemical entities, all, however, falling into the category of protoplasm. In the case of the one-celled plants and animals, these differences give rise to separate and distinct species.

If there is visualized a single cell, having no differentiation of characteristics, either in itself—*e.g.*, the power of the malarial parasite to produce disease, or the paramecium to swim—or in its relation to other cells, in a physiological division of labor in a higher organism,—*e.g.*, as in a nerve cell, a muscle cell, or an epithelial cell—a generalized cell which may be called an "ideal" or "typical" cell may be thought of. Such a cell cannot be imagined as existing today, but may be thought of as a cell which existed in the remote past and which had acquired no specialization but had merely the ability to live and propagate.

The ideal cell may be conceived of as spherical in shape, the protoplasm being confined by a limiting membrane or wall. Two forms of protoplasm are to be distinguished. The main mass of the protoplasm is called "cytoplasm," or cell substance. In the center of the cytoplasm lies a denser spherical mass of protoplasm called "karyoplasm," or nucleus. The latter always contains a readily stainable protein of acid character known as "chromatin." Near the nucleus of the cell and very close to each other lie two small bodies called central bodies, or "centrosomes." Other bodies, such as plastids and acid vacuoles, together with various differentiated granules, may be present. Each of these parts of the cell plays a functional rôle in the life of the cell.

The Evidences of Life.—Although one cannot lay his finger on anything and say "This is life," yet certain characteristics possessed by living things which are lacking in inanimate objects may be pointed out. In examining these evidences of life, it seems best to explain them in reference to the simplest living mechanism—the cell.

1. *Metabolism.*—Living matter is always in a state of physicochemical change called "metabolism." That is, it is constantly changing its material and transforming energy. In other words, living matter is continually undergoing waste and repair. In the performance of its life function, the cell (*every* living cell, in the case of multicellular organisms) is burning up its substance or undergoing the process of "katabolism." To make up for this waste, new material must be taken in and assimilated.

In this process, lifeless substance is being converted into living matter. This is called "anabolism." This exchange of old material for new takes place by osmosis through the cell wall. In the case of higher multicellular animals, the food material is brought to the cell by the blood stream, which also carries away the waste products of the cell life. In the case of unicellular creatures, they can only carry out this life process when in a moist or liquid medium. Anabolism and katabolism of course go on simultaneously.

In the life of the average cell, three periods are manifest—the growth period, in which anabolism goes on more rapidly than katabolism; the stationary or adult period, in which the two processes are approximately equal; and the disintegration or old-age period, in which katabolism takes place faster than anabolism, and at the end of which death ultimately ensues.

The living organism, then, is constantly taking in food and giving off waste; the particles of the body are constantly changing, but the form remains the same. Professor Huxley says:

> To put the matter in the most general shape, the body of the organism is a sort of focus to which certain material particles converge, in which they move for a time, and from which they are afterwards expelled in new combinations. The parallel between a whirlpool in a stream and a living being, which has often been drawn, is as just as it is striking. The whirlpool is permanent, but the particles of water which constitute it are incessantly changing. Those which enter it on the one side are whirled around and temporarily constitute a part of its individuality; and as they leave it on the other side, their places are made good by newcomers.
>
> Those who have seen the wonderful whirlpool, 3 miles below the Falls of Niagara, will not have forgotten the heaped-up wave which tumbles and tosses, a very embodiment of restless energy, where the swift stream hurrying from the falls is compelled to make a sudden turn towards Lake Ontario. However changeful in the contour of its crest, this wave has been visible, approximately in the same place and with the same general form, for centuries past. Seen from a mile off, it would appear to be a stationary hillock of water. Viewed closely, it is a typical expression of the conflicting impulses generated by a swift rush of material particles.
>
> Now with all our appliances, we cannot get within a good many miles, so to speak, of the living organism. If we could, we should see that it was nothing but the constant form of a similar turmoil of material molecules, which are constantly flowing into the organism on the one side and streaming out on the other.[1]

Still another analogy is pointed out by Dr. H. H. Newman,[2] with a clear distinction between the metabolism of living matter and its apparent counterpart in lifeless matter.

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[1] Huxley, "The Crayfish as an Introduction to the Study of Zoölogy," pp. 84–85.

[2] Newman, H. H., "The Nature and Origin of Life," in "The Nature of the World and of Man," p. 173, Chicago, 1926.

The question arises whether metabolism is a unique property of living matter or whether equivalent processes go on in lifeless materials. A common parallel to metabolism is the candle flame. If allowed to burn in a quiet atmosphere, the flame, with its central colorless core, its luminous zone and its outside zone of carbon dioxide and water vapor, remains constant in form and structure in spite of an ever-changing content. It is constantly taking in new materials, transforming them into flame, and giving off products of combustion and energy in the form of heat and light. Why is this not fully an example of metabolism? Certainly the analogy is very close, and if the ability to do this kind of thing were the sole criterion of life, the candle flame would be alive. But can the candle flame grow and reproduce other candle flames like itself? In the anabolism of an amœba or a nerve cell, a particle of carbohydrate is converted into living nerve cell or amœba. If the same particle of carbohydrate had been taken by a muscle cell or yeast cell, it would have become living muscle protoplasm or part of a living yeast cell.

2. *Growth.*—In the process of repair, the addition of the new material takes place by interposition between the existing molecules of protein, that is, the replacement goes on throughout the cell. This is known as "intussusception." When anabolism exceeds katabolism, growth, or, increase in mass, results.

A crystal can be made to grow if suspended in a saturated solution of matter similar to itself, but there are many striking differences in this growth from the growth of living matter. In the first place, a crystal can only be made to grow in, and at the expense of, material the same as itself, while a cell grows at the expense of materials different from itself. Again, crystals only grow by accretion at the surface, while the growth of living matter is always by intussusception. Crystals can exist indefinitely, but the life of the individual organism is limited. Finally, the size of the living cell is limited, the individual growing more or less rapidly until it reaches a certain size, after which growth entirely ceases. Crystals have no definite limits as to size.

3. *Irritability.*—Even the simplest forms of life possess that power of responding to external stimuli, known as "irritability." This response is due to the instability of living protoplasm, and the energy involved is greater than that of the stimulus. For example, Paramecia, which belong to the group of single-celled animals, are attracted by oxygen. Bacteria will be attracted toward dilute solutions of certain chemicals, and repelled by

other chemicals. Heat is another stimulus which affects the protoplasm of living things. In the higher complex animals, the characteristic of irritability is magnified in special cells—nerve cells. In general, the various manifestations of living things, such as metabolism, growth, motion, etc., are controlled to a large extent by this property of protoplasm of responding to the stimuli produced by internal or external conditions.

4. *Movement.*—The power of locomotion, while not exclusively characteristic of living matter, is commonly present in animals and quite frequently in plants, especially among the lower classes. Among the lower animals, locomotion is accomplished by a protoplasmic streaming, as in Amœba, or by flagella or cilia, as in Euglena and Paramecium. In the higher animals, the ability of movement is not possessed by the individual cells (except certain wandering cells within the organism, like white blood cells and by spermatozoa) but is a property of the complex organism as a whole. This is usually accomplished by the alternate contraction and relaxation of muscle fibers, either along the entire body, as in the snake, or of specialized organs, as in the wings of birds, the fins of fishes, and the legs of horses.

5. *Reproduction.*—Finally, in respect to propagation, living matter is quite unlike non-living matter. A whirlpool or the flame of a candle may be likened to the metabolism of living things; a crystal may grow; the response of an iron nail to the magnet may resemble irritability; and the movement of the steam locomotive may resemble that of an animal. But nothing in the inanimate world even approaches the ability of living things to create other living things like themselves. Even a catalyst, like amylase or pepsin, which can hasten chemical changes in matter far greater than its own bulk, cannot produce more of its material than was originally present. In fact, biologists are convinced that it is only living matter which has the power of reproduction.

Several different methods of reproduction are observed in living organisms. Usually, each species reproduces in a certain way, although some species have a number of different methods which lead to rather complex life cycles.

Asexual Reproduction.—Among the lower forms of life, asexual reproduction is prevalent. In the unicellular plants and animals, each individual is potentially a reproductive cell. In the lower multicellular forms, certain cells seem to be set apart for the purpose of reproduction, but there is no differentiation in sex. There are quite a few distinct types of asexual reproduction:

1. *Binary Fission.*—The simplest method of reproduction is by what is called "binary fission";[1] that is, the organism simply divides through the middle to form two similar and approximately equal halves, each of which is then a new organism. Each half grows to the size of the parent, and the process is repeated. In this method of reproduction, the parent loses its identity entirely in its two offspring. In fact, if the growth of the offspring is thought of in its proper anabolic light, it may be said that any cell existing today, of a species which reproduces by binary fission, is a part of the original cell of that species, and that there is on earth an example of eternal life.

Bacteria, like most organisms which reproduce by binary fission, divide in a plane perpendicular to the long axis. This process is called "transverse fission." In many cases, multicellular organisms are capable of reproduction by means of normal fission, or even by fission that is the result of an accident. For example, if a twig be cut from a tree and placed under favorable conditions of growth, it will become a new tree. This fact is of great value to horticulturists, who use the method of transplanting cuttings from particularly valuable trees or bushes. Again, if an earthworm (one of the segmented worms) be cut in halves, each part is capable of regeneration and continuity of life, with all the original characteristics of the whole. In both of these cases, plant and animal, the new individual has the remarkable property of replacing the lost part, and of developing specialized cells from the offspring of cells whose functions were entirely different.

2. *Mitosis.*—In the division of developing or embryonic cells of all multicellular organisms, and of a great many unicellular plants and animals, the process is somewhat more complicated than that of simple fission. This complex method is known as indirect cell division, karyokenisis, or mitosis. While there are many variations in the mitotic cycle, in general the plan is as follows:

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[1] It may be that when more about the nucleus of certain microscopic forms of life is known, the prevailing ideas regarding binary fission will have to be changed. The inheritance of unit characters is hard to explain by simple fission.

It will be remembered that the cell consists of an outer limiting membrane within which is a nucleus surrounded by cytoplasm. Among the formed bodies in the cytoplasm are two small bodies near the nucleus, known as "centrosomes." While the nucleus is the site of mitotic division, the directing force seems to be in the centrosomes. When a cell is about to divide, the first noticeable change takes place here. The two centrosomes draw apart and migrate to opposite sides of the nucleus forming what is called an "aster." Starlike lines radiate from these bodies in all directions. In the meantime, the chromatin granules in the nucleus, which are the basis of heredity, and which, under ordinary conditions, are reticular, or in the form of a network, gather in the form of a continuous string called the "spireme." Gradually, the spireme breaks up into rod-shaped bodies (chromosomes), which lie in a plane perpendicular to a line drawn between the two centrosomes. The next step is a longitudinal division of each chromosome so that every chromatic granule is split in two. Now, seemingly, by attraction of the asters of the centrosomes, the two halves of each chromosome separate and move in opposite directions toward the centrosomes. Ultimately, all of the chromosome halves on the one side gather in a definite spot, surround themselves with a membrane, break up into chromatin granules, and form the chromatin of two new nuclei. In the meantime, the cytoplasm divides along the line of the plane in which the chromosomes were, the cell membrane forms a dividing line, and two complete cells appear, each of which contains exactly one-half, quantitatively and qualitatively, of the nuclear material of the parent.

3. *Conjugation.*—Some plants and animals at times go through a process which is in many ways similar to mitosis and in others quite the opposite. This is called "conjugation." Two adult cells in which there is no evidence of sex distinction join together and exchange nuclear material. As in the case of mitosis, the chromatin granules form chromosomes which split into two equal parts, each containing one-half of each original chromatin granule. After a more or less complicated process of division, one-half of the chromatic material of one cell is replaced by one-half of this material from the other cell. After a time the two cells separate. Although it is not strictly a sexual process, conjugation resembles sexual reproduction in that the resultant cells are not of the same material as the parent, but each new cell represents both

5. *Budding.*—In some plants and animals, as the yeast and hydra, the method of reproduction is by what is called "budding" or "gemmation." This is not to be confused with the "buds" of rosebushes or fruit trees. In the case of the yeast, the parent organism sends out a small bud or shoot from its periphery, which after a time becomes detached from the parent and is an independent organism.

Sexual Reproduction.—Most of the multicellular plants and animals propagate by what is called sexual reproduction. The body of the adult animal is made up of innumerable cells, such as nerve cells, muscle cells, blood cells, etc., which are grouped under the term "somatic" or body cells. In a definite organ of the body, however, are located special cells which are solely concerned with reproduction. In this case there are two sexes, each carrying a distinct kind of germ or reproductive cell. It is through the union of these two different cells that the offspring results. While differing greatly in the manner in which these two kinds of germ cells are brought together, all forms of sexual reproduction are essentially the same.

The mechanism of sexual reproduction is, in a way, the reverse of the mitotic division of somatic cells. In mitotic division, a single cell divides into two daughter cells, each of which has quantitatively one-half of the chromatic substance of the original cell. In sexual reproduction, two cells, male and female, unite to form a single cell, which then proceeds to divide, furnishing the daughter cells with an equal proportion qualitatively of the chromatic material of the two uniting germ cells.

The preparation for the union of these two different germ cells continues for some time before actual union takes place. The original germ cells (primordial germ cells) which are present at birth in the male and female are not themselves the cells which take part in reproduction. A multiplication takes place, by mitotic division, producing many individual germ cells from each primordial cell. Now ensues the so-called synapsis or fusion stage in which each two chromosomes unite to form one, thus decreasing the number of chromosomes by half, but not reducing the amount of chromatic material in the cell. Following this come the maturation divisions. In this case, the process differs in the male and female in regard to the number of germ cells produced, but not in its fundamental purpose, which is to produce cells having one-half the number of chromosomes possessed by

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Now fertilization may take place. This is simply the union of the two germ cells, male and female, each with one-half the original number of chromosomes, thereby restoring that number. The fertilized egg now contains chromatic material from male and female parent in equal amount, both qualitatively and quantitatively. Now begins that series of cell divisions in the building of a new individual. As the first division, and all subsequent divisions (with exceptions which do not interest us here) is by typical mitosis, each of the two daughter cells receives, by chromosome splitting, equal representatives of paternal and maternal chromatic material—hence, of hereditary trends.

Species.—Accepting literally the Mosaic account of creation, early biologists defined species as fixed groups of plants and animals between which existed definite distinctions. Among the individuals in a particular species many races and variations could be recognized, but never could the biological changes giving rise to these variations be sufficient to permit the characteristics of the variants to cross the boundary line of the species, nor could new species be formed.

Charles Darwin, in his "Origin of Species," opposed the Biblical notion of the fixity of "kind," and suggested that new species have arisen from previously existing species. In accepting the doctrine of organic evolution, our definition of the term "species" naturally needed revision. With the impassable boundaries between species removed, it became difficult exactly to define the group. Various plans for determining the limits of the species group have been suggested. One of these is on the basis of interbreeding. The sterile offspring of the horse and the donkey seemed to indicate a species distinction between these two animals, and suggested a rule. Exceptions to the rule, however, in the case of other animals that have been interbred destroy its value as a scientific law.

Today the species is recognized as a group of closely related forms which *in the opinion of the observer* differ from the members of other groups. The term has become an expression of opinion, not of fact. Thousands upon thousands of species, between which at times only very fine distinctions can be drawn, are recognized by some biologists, while others look at species as large groups which are subdivided into subspecies, races, subraces, and varieties. The first group of naturalists have been called "splitters," and the latter, "lumpers."

Bacteriologists have recently recognized a new phase of this difficulty in the possibility of complex life cycles among bacteria. What have been formerly known as distinct species may be simply different stages in the cycle of a single kind of bacterium. It would be an analogous case if the various embryonic and larval stages in the life histories of animals were assumed to be separate species.

Variations in Species.—Organic evolution is an accepted truth of the modern biologist. A study of comparative anatomy, embryology, paleontology, geographic distribution of plants and animals, and finally the use of blood tests have convinced him of its reality. The mechanism of evolution or the basic reason for the development of new species is not so clear. It is certain, for example, that disease germs, which, phylogenetically, are much older than man, must have developed their ability to keep alive in the body of man only after modern man had appeared on the earth. Again, among the pathogenic germs we find one species producing syphilis, another typhoid fever, another diphtheria, etc. How did these different bacteria get their specific characters? It is likely that many factors are involved. One, seemingly, is, that the chromatic granules of a cell are not in a state of absolute chemical stability and that changes in the germ plasm are taking place constantly. When the variations are transmitted to the daughter cells, the new type of cells or the multicellular organism lives or dies according to its adaptability to the environment. If it finds the new conditions suitable, it lives and propagates more of the new kind; if not, it dies and it is not known that a change ever took place. In other words, the principal factor is chance, and chance determines the formation of new species and survival of the organisms best adapted to the environment.

The Relation of Living Things to Each Other.—It is apparent that there are two great kingdoms of living things: the animal and the vegetable. It is easy to distinguish between a man and a tree, a grasshopper and a lily, a worm and a fern. It is harder, however, to place in the animal or in the vegetable kingdom the lowest of one-celled organisms. To facilitate the study of living things, and, for the purpose of the evolutionist, to show phylogenetic relationships, all living things are divided into groups.

Starting with man, for example: except for minor differences, all men are alike and can be placed in one definite group. This group is called *Homo sapiens*, or thinking man. Paleontological researches have brought to light men who are quite different from modern man, yet who are closer to us than to any other modern animal. Now we make a larger group, Homo, which includes both modern and prehistoric man. Then certain of the higher apes—the anthropoids—more closely resemble man than do any other animals, so we make a still larger group, Homimides, which includes modern man, prehistoric man, and the apes. Then we find the lower monkeys quite closely resemble this group and we make a still larger group which includes them. This large group we call the "Primates." Again, in an important characteristic—the suckling of their young—other animals are similar to man, and there results a still larger group containing man, prehistoric man, the anthropoid ape, monkey, dog, cat, horse, cow, etc. This still larger group is called the "Mammalia." Once more it is found that, in the possession of a backbone, birds, fish, frogs, and snakes are similar to man and dissimilar to all lower animals; and another still larger group, the vertebrates, or "backboned" animals, is formed.

In this way biologists have divided all animals and plants into a few great divisions or "phyla," each phylum containing numerous subdivisions, as an army is divided into divisions, regiments, companies, and squads. For classification, names have been given divisions and subdivisions, and a system of nomenclature has been worked out which is generally accepted and adhered to in scientific writing.

According to this system of classification, living organisms are divided into two great kingdoms, the plant and the animal. Each of the kingdoms is divided into a few phyla, each phylum into several classes, each class into one or more orders, each order into families, each family into genera, and each genus into species. All similar individuals make up a species.

In the scientific classification of living organisms, individuals are not named. The generic name, *i.e.*, genus, is given (it is usually a Latin word, capitalized) followed by the name of the species (generally an adjective in Latin form, not capitalized), thus: *Homo sapiens*, modern man; *Canis domesticus*, the dog; *Escherichia coli*, the colon organism; *Eberthella typhi*, the typhoid germ; etc.

CHAPTER II

THE HISTORY OF BACTERIOLOGY

The history of a science, like the history of a nation, must be more than simply a list of dates. Happenings, or events, and men who took part in them, naturally form a part of history, yet in the final analysis great events are simply the concluding phases of a series of occurrences which led up to them. For example, we are apt to think of the signing of the Declaration of Independence as coincident with the birth of our nation, yet historically we must understand the Crown land grants in the Ohio Valley, the New England tax returns, the real purpose and result of the French and Indian War, and many other factors that cannot be listed as events, before we can properly understand the reasons for the first Fourth of July.

The birth of bacteriology as an independent science took place about the time of the Civil War. But just as the history of the Confederate states must include the history of slavery in America, the cotton industry in the southern states, and even the fundamental ancestral differences between the southern planters and the northern manufacturers, so must the history of bacteriology include many discoveries in other fields of knowledge which contributed to the formation of a new science. It would seem to be a far cry from the water-filled globes of Seneca to the modern bacteriological microscope. It is difficult to believe that Aristotle, in 384 B. C., could have had anything to do with scientific discoveries in 1860, or that the speculations of Hippocrates four hundred years before Christ could have had any influence on the labors of Pasteur, Lister, or Koch. Yet all of these are facts, and it is only through an understanding of the development of theories—not excluding theories proven false—that the student of bacteriology can get a true background for the appreciation of present-day knowledge.

The Microscope.—Because of the extremely small size of bacteria, it was impossible for man to realize the presence of these organisms until he was able to see them. The first line of development, therefore, which led to the establishment of bacteriology, was the perfection of the magnifying lens. The first definite record of the use and properties of lenses was given by Roger Bacon in 1266. There is no doubt but that by this time lenses were used as aids to the eye as glasses are used today. At the beginning of the seventeenth century, more than 350 years later, the Dutch had perfected telescopes and microscopes of a magnifying power of about 30 diameters. Of such were the instruments used by the Jesuit priest Athanasius Kircher, who saw blood corpuscles; and Robert Hookê, who first described the physical unit of living matter, the cell. In 1683 Antony van ~~Leeuwenhoek, a~~ Dutch linen draper who ground lenses and devoted his leisure to the study of microscopic specimens, succeeded in producing a simple lens of sufficient power to enable him to distinguish living organisms in saliva, water, and other fluids.

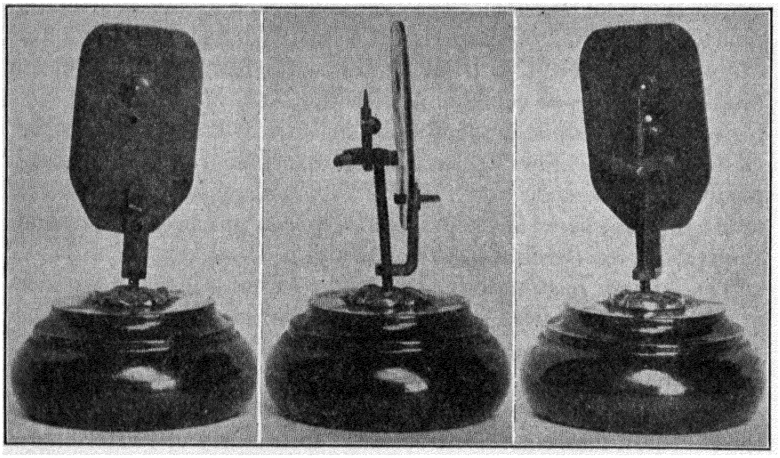

FIG. 3.—Three views of a van Leeuwenhoek microscope.

In a letter to the Royal Society of London, dated Sept. 14, 1683, he said

> I saw with wonder that my material contained many tiny animals which moved about in a most amusing manner; the largest of these showed liveliest and most active motion, moving through the water or saliva as a fish of prey darts through the sea; they were found everywhere, although not in large numbers (*A*). A second kind was like that marked (*B*); they were present in large numbers. These sometimes

spun around in a circle like a top, and sometimes described a path like that shown in (*D-C*). A third kind could not be distinguished so clearly—now they appeared oblong, now quite round They were so very small that they did not seem larger than the bodies marked (*E*), and, besides, they moved so rapidly that they were continually running into one another; they looked like a swarm of gnats or flies dancing about together. I had the impression that I was looking at several thousands in a given part of the water and saliva mixed with a particle of the material from the teeth no larger than a grain of sand, even when only one part of the material was added to nine parts of water or saliva. Further, the greater part of the material consisted of an extraordinary number of rods, of widely different lengths but of the same diameter. Some were curved, some straight, as shown in (*F*); they lay irregularly and were interlaced. Since I had previously seen living animalculæ of this same kind in water, I endeavored to observe whether there was life in them; but in none did I see the smallest movement that might be taken as a sign of life.

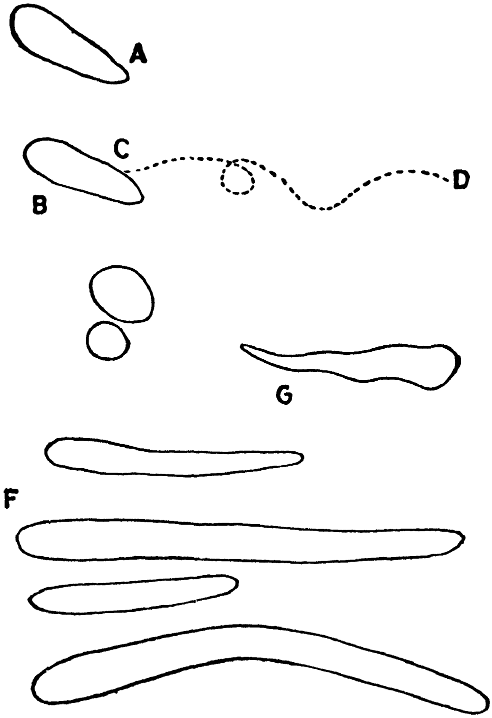

FIG. 4.—Leeuwenhoek's figures. The oldest known drawings of bacteria. (*After Fisher.*)

The drawings which accompany van Leeuwenhoek's description show that his "tiny animals" were really bacteria.

The relation of the development of the microscope to the science of bacteriology did not end with the discovery of bacteria. Even though bacteria had been seen, it is doubtful if any further advances could have been made with the lenses which van Leeuwenhoek had. But scientific interest in things unseen, stimulated by the writings of Kircher, Hook, Malpighi, van Leeuwenhoek, and others, demanded better and still more powerful lenses. During the next hundred years the compound microscope, first made by Johann Janssen in 1590 and discarded by van Leeuwenhoek, was undergoing constant improvement. Differences between kinds of bacteria were noted and attempts at classification were made.

In 1844, John Dolland developed the use of the oil-immersion lens. This was the last radical improvement needed to make the essential tool of the bacteriologist sufficiently powerful for the science to become exact.

During the life of Pasteur and the period of fundamental discoveries in bacteriology, another improvement was made by Ernst Abbé, a German physicist, in the invention of the substage lens for illumination of the microscopic object. Since that time, most advances have been in the nature of mechanical improvements. The modern bacteriologist has at his disposal a microscope which will enable him to see clearly organisms magnified 1,000 diameters, and to take pictures by the use of ultra-violet light which will bring out details magnified over 2,000 times. Physicists tell us that the limit has been reached, that lenses cannot be made small enough, and with sufficient curvature, to give much greater magnification; also, that the object examined cannot be of less size than one-half the length of the light rays used to illuminate them. Yet every year manufacturers offer new microscopes with unquestionable mechanical improvements.

Interest in Form.—For more than a century and a half following van Leeuwenhoek's description of his "animalcules," the interest in bacteria was that of the natural scientist. What were these creatures? Were they true entities of an hitherto invisible world, or were they simply self-perpetuating seeds or germs of higher plants and animals? Linnæus, the greatest systematist of the eighteenth century, and the one who laid the foundation of modern biological terminology, believed bacteria to be tiny animals (vermes) and invented a name for them—

"chaos"—which showed the scientific state of mind of the hundred years following van Leeuwenhoek. A Danish biologist, O. F. Mueller, near the close of the same century, while still believing bacteria to be animals, placed them among the lowest group, the Protozoa. During the fifty years beginning with 1800, there was a constant succession of attempts to give bacteria a place in nature and to divide them into groups among themselves.

It was not until the middle of the nineteenth century, however, that Joseph Leidy, Naegeli, and Cohn proved that bacteria were one-celled vegetable organisms. Even after the early researches of Pasteur, when the arguments as to the origin of bacteria and the overwhelming interest in the relation of bacteria to disease were at their height, the attempt at scientific classification was not entirely abandoned. Arguments over methods of fission, over pleomorphism, and over the relative significance of form and physiological activity were sufficient to interest a small group of men who were more concerned with what bacteria are than with what they do. Today, after fifty years of "practical" bacteriology, a returning interest in "pure science" is noticeable. The recent work of Löhnis, Hadley, Henrici, and Mellon on "life cycles," "dissociation," and race history of bacteria are receiving the attention of every bacteriologist.

Interest in Communicable Disease.—No history of medicine would be complete without mentioning the experiments of Pasteur, Koch, Tyndall, and others of the early bacteriologists. Lord Lister, the Father of Antiseptic Surgery, said, "Truly, there does not exist in the entire world any individual to whom the medical sciences owe more than they do to Pasteur." Today, bacteriology forms a large part of the study of medicine, and no physician would attempt to practice his profession without a thorough knowledge of the relations of bacteria and disease. On the other hand, the development of medicine as a science had a most intimate bearing on the growth of this younger science. The theories, both false and true, which were drawn from medical observations, acted as mental stimuli to those who were later to be known as bacteriologists.

The Demonic Theory.—It is a curious fact that all primitive peoples held one theory of disease, namely, that it was simply a form of evil, like drought, or flood, or crop failure, and was caused by evil spirits or demons. The modern jest that the priest endeavors to get man into heaven and the physician labors to keep him out, had no foundation in the minds of early men. Then the priest and the physician were one and the same, and the prevention and cure of disease were clearly duties of the tribal priest.

Egyptian medicine was in the hands of priests, each priest confining himself to a particular disorder. Here, more than three thousand years ago, was a forerunner to the modern idea of specific diseases, and to present-day specialists. The Babylonian physicians were also priests, and fought a myriad of demons by incantations. Among the early Jews, disease was regarded as a manifestation of God's wrath, as punishment for sin. The ancient Greeks, with their beautiful idealism, worked out a complete system of deities for the transmission, prevention, and cure of disease. Æsculapius, the god of medicine—usually portrayed with his daughters, Hygieia and Panacea, his caduceus or snake-entwined wand in his hand—was constantly endeavoring to ward off the evil effects of the arrows the great gods sent to punish mortals, or to hold in check the countless ills set free by the inquisitive Pandora.

A good example of this primitive union of medicine and religion may be found in the beliefs of the American Indians. Here the priest is the medicine man, who not only consults the gods as to favorable times for battle and the planting of corn, but by the use of fetishes or charms wards off the evil spirits that are supposed to be constantly trying to work evil on the warriors and squaws of the tribe. Even with this, the duties of the medicine man do not cease, for a good priest not only keeps the demons of disease from his own people, but encourages them to attack the enemies of his tribe.

"Medicine making" referred to crops, to battle, and to successful voyages, as well as to the cure of bodily ailments. The "good medicine" of the Indian can almost be translated into the "good luck" of the white man.

The demonic theory of diseases has not entirely disappeared with civilization. Christ cast out devils from the Gadarenes, according to the gospels. The burning at the stake of witches in order to kill the demon that had taken possession of a human body was common in the Middle Ages. Even today there may be found, among the less highly educated, a firm belief that a maniac is "possessed of a devil," and that it is possible to cause bodily ailments by "hexing."

The Humoral Theory.—The Greeks, with the rise of the philosophical schools, seem to have given up the idea of Pandora's box as the source of all disease. At the time of the Trojan war the physician, or at least the surgeon, had different duties than the priest. Following the numerological philosophy of Pythogoras, there was developed a concept of disease based on the physiological fluids in the body. These fluids or "humors," four in number, corresponding to air, water, fire, and earth, were blood, phlegm, yellow bile, and black bile. The four humors were supposed to be present in the body in definite proportions. These proportions were invariable for any particular person but differed with different individuals, as is indicated in our modern expressions of temperament: sanguine, phlegmatic, bilious, and choleric. If the adjustment of these humors became deranged, illness resulted. Cure consisted in restoring the balance.

The idea of blood letting to bring about a proper adjustment in certain disorders, while accepted in humoral pathology, did not have its inception in Europe. The early Chinese and Egyptians practiced venesection, probably on the principle of purification symbolized by the phenomenon of menstruation.

The development of the humoral theory is typically Greek, though it has an outward counterpart in the Chinese belief in the necessity of a balance between two essential vital principles: Yan (life) and Yin (death).

The greatest figure in ancient medicine was Hippocrates. A believer in the humoral theory, he first developed the scientific method in the diagnosis and cure of disease.

As in the case of the demonic theory we find traces of the humoral idea of disease persisting long after its fallacy was apparent. George Washington was bled on his deathbed and today one does not have to look far for people who welcome an occasional cut as "something that's good for them." The nickname "leech" for a physician has a historical basis in its reference to the use by physicians of the blood-sucking annelid.

Following Hippocrates, there was a gradual development in the science of therapeutic medicine for a period of nearly a thousand years. Theory followed theory, however, as to the cause of disease. Solidism, sthenicism, Broussais' theory of irritation, etc. each held a temporary sway, to be retained only as a name when succeeded by a new speculation. Finally, we come to the close of what was really an age of scientific advancement with the great theorist Galen. The pharmacology of antiquity contained many curatives used today, diagnosis had become in a way exact, and even the means of transmission of epidemic diseases was suspected in a way which accounts, for instance, for the building of the Roman aqueducts; but the ideas as to the true causes of disease remained purely speculative.

The Alchemic Theory.—In order to understand fully the medicine of the thousand years between 500 and 1500 A. D., one must be a close student of medieval history. One must get the atmosphere of the times in order to interpret them. The replacement by Galen's theorism of Hippocrates' scientific system of observation gradually led to a monastic scholasticism in which the study of things was subordinated to a study of words or formulæ. A remedy in itself was of less importance than the words to be spoken while administering it. It is easy to see how the original Hippocrates numerology could be seized upon and transformed into a means of stimulating the inward-looking, vague thoughts of acetics seeking a *Universal Spirit.* The four humors now became simply elements of a *Principle,* or Essence, which was not confined in the body but simply manifested itself there in its elements. If one could find a way of keeping the abstract Principle stabilized, disease could be prevented. The remedium was the physician's concept of the philosopher's stone.

Yet it is during this hodge-podge of faith healing, exorcism, and theurgy that an idea was born which was to have a remarkable influence on the founding of the science of bacteriology. This was based on the assumption that the unbalanced Principle could be passed from one person to another and that a sick person could transmit a disease to a healthy individual.

The Contagium Theory.—With the end of the Dark Ages came a revival of scientific method, and observation again took the place of conjecture. By the time of the discovery of America, the fact that great plagues or pestilences were passed by some means from one individual to another had become apparent.

The nature of this means was, of course, unknown, and a great many theories as to the qualities of *contagium* were brought forth. In general, there were supposed to be three main sources of contagium, none of which was wholly responsible for it but all of which were associated with a fourth factor. This factor, in turn, was dependent on the climatic conditions of the country in which the plague was rampant. The first of these sources lay in certain peculiar conditions in the air. Excessive heat, or immoderate rains or droughts, or obnoxious or foul odors were supposed to make the air pestilential. The second factor was diet. Of course, no physician would lay the blame for contagium on the diet of his own particular people, but he did single out the peculiar diet of the people of some other country as the origin of the contagium. Thus, the Chinese, who ate birds' nests, or the American Indians, who ate turtles and eels, and who smoked tobacco, were supposed to be peculiarly apt to cause the starting of a contagium. The third factor dealt with the national customs of foreigners, and when no other cause for an epidemic could be found, the foreigner's peculiar habits could always be made to account for a pestilence. Coitus with menstruating women on the island of Haiti was said to be responsible for the syphilitic contagium brought to Europe by the sailors of Columbus.

From the idea of contagium, an effluvium, a miasma, or a malaria, it was only a step to the thought that instead of an indefinable ethereal substance being responsible for disease, a material body carried in the air might be the agent.

Interest in Fermentation and Decay.—As early as the time when men first made a written record of their observations, the changes which take place in animal and vegetable matter and which today are called fermentation and decay were noted down.

Various methods for the prevention of decomposition following death were practiced in prehistoric times. The art of embalming was known to the Egyptians more than six thousand years ago, but even before any artificial means of preservation of the dead was used, burial under desert sand, with the evident realization that dryness hindered decay, was customary. The ancient Egyptians believed that the soul could exist independently of the body after death, and could at some future time reinstall itself in the body. If, however, the body was allowed to disintegrate after death, the soul also died. Various means were employed in Egypt for the preservation of the dead. The simplest and least permanent was to immerse the dead body in brine. Another method was to inject into the body cavity cedar resin or pitch. The best form, and the one practiced on the bodies of dead kings, was quite complicated, but essentially consisted of removing the brain and entrails and filling the cavities with balsams and spices.

Embalming was practiced by other ancient peoples, who probably learned the art from the Egyptians. The early Assyrians used honey as a preservative; the Persians wax; and the Jews, aloes and various spices.

That foods could be preserved from decay was also known in prehistoric times, and among primitive peoples. The American Indians dried and powdered fish to keep it for future use, and buffalo and venison pemmican were staple articles of diet. Squaws made the pemmican by cutting buffalo and deer meat into strips, drying it, and pounding into it fat and berries.

That the process of fermentation could be used to advantage in the preparation of foods was also well known to ancient man. Evidently, bread was made at a time as ancient as the stone age. Leaven, or sour dough, was being used by many peoples at the dawn of history and had probably been in use long before as a means of "raising" bread. The Jews used leaven before the migration from Egypt.

Beer, another product of fermentation, was made by nearly every ancient people. The ancient Egyptians, Greeks, Chinese, and Japanese all had methods of preparing this alcoholic beverage. The South African aborigines prepared a beer from millet seeds, and Caesar found the inhabitants of England making beer in about the same method that is used today. Wines are also of prehistoric origin, and it is safe to say that when ancient man ceased his nomadic existence and settled down to form civilizations, he started growing the grape and making wine.

Among Eastern peoples the art of fermenting milk is of prehistoric origin. The milk of the cow, horse, goat, buffalo, and camel was used to prepare staple articles of diet known among different peoples as kumiss, yohourt, kefir, matzoön, and leben. The Laplander prepared his *pina* from reindeer's milk.

So, at the dawn of history we find these processes of fermentation and decay well known, although little thought was probably given to the causes of the phenomena.

During the Middle Ages the alchemists conceived the idea that fermentation was a purifying process. They tried to "purify" everything, in order to get the essential *stone*—and believed that the bubbling of an acid, when in contact with a metal, and the bubbling of wine were the same thing.

It was not until alchemy gave way to the science of chemistry that there is found any hint at the true explanation of what goes on in the process of fermentation. Van Helmont, about 1600, first showed that the gases emitted in alcoholic fermentation, in the burning of wood, and in the effervescence from chalk treated with an acid were the same substance. He called this gas "gas carbonum." Fifty years or more later, Johann Becker prepared alcohol by fermenting sugar.

About the time of the American Revolution Lavoisier tried to explain the chemical change involved in fermentation. He thought that sugar was an oxide which was broken up by fermentation into two other oxides, carbon dioxide and alcohol. The *chemistry* of fermentation was beginning to be understood.

At the beginning of the nineteenth century, the *cause* of fermentation was first suggested. In 1803, L. J. Thenard stated that a microscopic organism, yeast, was the cause of fermentation. This observation in itself was correct, but it lead to a scientific debate which was far reaching in its effect.

It in fact reopened a question which had lain dormant for centuries—the question of spontaneous generation. In 1839, J. von Liebig, the greatest chemist of his day, denied that microörganisms caused fermentation, and proposed the opposite hypothesis: namely, that during the chemical process of fermentation, conditions were right for the spontaneous birth of microörganisms from inanimate substances.

The Theory of Spontaneous Generation.—The spontaneous-generation theory, whereby animals sprang full grown from lifeless matter, or like Athena from the forehead of Zeus, had been pretty well discarded by the beginning of the seventeenth century. The discovery of microörganisms, however, and the realization of the existence of an invisible world, reopened the question in an entirely new form. Speculation was replaced by observation and, while the conclusions drawn by some observers were not correct, these very fallacies had a stimulating effect on other workers.

Up to this point, the various lines of endeavor which have had a bearing on the science of bacteriology as independent forces have been described. Beginning with the eighteenth century, these lines gradually converged until the entire scientific world, whether interested mainly in natural history, in fermentation, or in disease, was engaged in a battle royal, with scientific data as ammunition. Out of the melée arose the new science, bacteriology.

Within a few years following the development of the microscope to the degree of efficiency of that used by van Leeuwenhoek, it was shown repeatedly that decaying animal and vegetable matter invariably contained microörganisms. Less than a hundred years later Spallanzani demonstrated that microörganisms were so closely related to the process of decay that the latter could not take place in the absence of the former.

In 1836, Theodor Schwann went a step farther and showed that decay ceases when all living microörganisms in the decaying material are destroyed. He also attempted to prove, what had been suggested by the chemist Robert Boyle about two hundred years earlier, that microörganisms were the cause of disease. A few years later, Jacob Henle carefully summarized the scientific findings of the day and made a clear statement of the germ theory of disease. He then enunciated what was later to be repeated by Robert Koch and what is today known as Koch's postulates. In 1849, Pollender, and in the following year, Davaine, showed the presence of a bacterium in the blood of animals suffering from anthrax.

Concomitant with the idea that fermentation and disease are both associated with the presence of microörganisms, these phenomena were soon likened to one another, and a new term was coined in medical language: zymotic disease.

At this point the proponents of the germ theory of disease divided into two groups, debating whether the germs caused the disease or whether the conditions in the body during disease caused the spontaneous development of microörganisms.

Louis Pasteur.—At the middle of the nineteenth century, the scientific stage was set. Discoveries of prime importance were being made in biology, chemistry, and medicine, and, as in the case of a nation in time of crisis, one man had to assume the stellar rôle. The part fell to Louis Pasteur.

Pasteur was born in Dôle, France, Dec. 27, 1822. His early studies were devoted to mathematics, and later to chemistry, under the influence of J. B. Dumas. His first interest was centered around tartaric acid and its isomeric forms. From 1844 to 1848 he worked on his "crystals," almost incessantly. Finally, he discovered that racemic acid is composed of two acids, which, being opposites, neutralize each other when polarized. From this discovery he formulated his doctrine of molecular dissymmetry, upon which the whole structure of stereochemistry is built. The same year he was appointed professor of chemistry at Strasbourg, where he stayed until 1854. His continued researches in stereochemistry during this period won for him the ribbon of the Legion of Honor.

In 1854, he was made dean of the Faculty of Sciences at Lille. Here his work on the tartrates led to studies of fermentations of their salts, and, as there were great alcoholic distilleries in Lille, he became interested in fermentation in all its branches. He was induced to investigate the cause of inferior fermentation and, by discovering that there were different organisms appearing in good and poor fermentations, proved for the first time that fermentation was due to the presence of specific living things. This view was directly opposite to the views of the great German chemist, Liebig, and to all the teachings of chemistry at that time. In addition to the opposition of the German chemists, Pasteur also stirred up, by this discovery, the proponents of the theory of spontaneous generation, who were led by Pouchet.

While this discussion was at its height, Pasteur was persuaded to study an epidemic that was destroying the silkworm industry in southern France. For three years he devoted a large part of his time to an endeavor to determine the cause of the disease. Finally, in 1868, he finished his experiments and proved that the disease was bacterial in origin and could be eradicated by sanitary measures.

Lord Lister obtained from this work, and from Pasteur's work on fermentation, the principle upon which he founded the practice of antiseptic surgery.

In 1877, Pasteur proved a distinction between the anthrax bacillus and the *vibrion septique* of malignant œdema, thereby opening the whole subject of specific bacterial disease and the true nature of contagium. From this time on he devoted his life to the study of bacteria in their relations to disease. Puerperal fever, chicken cholera, and many other diseases of man and animals claimed his attention, and, while he did not succeed in demonstrating the causes and cures for all, he did an immense amount of research which has been invaluable in putting later workers on the right track.

In 1885, Pasteur made the first rabies vaccine inoculation into a human being. Hydrophobia, a disease which up to that time was practically always fatal, was conquered.

Pasteur died near St. Cloud Sept. 28, 1895. His principal works, as outlined here, were sufficient to place him in the front rank as a chemist, bacteriologist, and scientist. It is impossible, however, to measure his value to humanity in terms of his own researches. He was a pioneer and as such opened up vast fields of research. In this respect his superiority is acknowledged. Opposed by the foremost thinkers in every line of his endeavor, he surmounted the greatest difficulties and established his name in history as one of the greatest men of all times.

The Period of Rapid Growth.—While Pasteur was preeminent in the new field of bacteriology during his lifetime, it must not be thought that he was solely responsible for the enormous strides taken by the new science. He happened to be the "man of the hour"—a man who was a natural leader in his field. Around his name, even during his life, there arose a glamor, composed of both fact and unfounded tradition. That he played an important part in starting the scientific world on the right track and by his keen scientific mind added a large individual share to our knowledge cannot be gainsaid. Yet it would be foolish to affirm that without Louis Pasteur there would still be no science of bacteriology. The time was ripe for scientific investigation, and other men, though less colorful, perhaps, in method and personality, were independently making discoveries which were bound to lead to present-day knowledge.

It was during the lifetime of Pasteur that the most remarkable and fundamental discoveries in this new and uncharted field were made. The work of John Tyndall is of interest because it shows the intimate connection between the rise of the science of bacteriology and the decline of the theory of spontaneous generation. While the idea that small animals such as mice, frogs, and eels obtained life spontaneously from decaying organic matter had many adherents up to the beginning of the eighteenth century, biologists generally had discarded it. The discovery of the minute microörganisms revived the question.

For more than a century, biologists interested in these microscopic forms were divided into two groups. The one maintained that microörganisms were formed and obtained life in the changes which are recognized as fermentation, decay, and disease; the other was equally convinced that, instead of being a result of these processes, microörganisms caused fermentation, putrefaction, and disease. Pasteur's work on fermentation stimulated Tyndall to prove definitely that microscopic life did not spring up as the result of such processes, but that microscopic life, coming from the air, was the cause of these processes.

Carl von Weigert, in 1871, first stained bacteria with carmine and five years later introduced aniline dyes as bacterial stains. When a student today compares stained and unstained specimens in the laboratory he readily understands the enormous importance of this laboratory procedure.

Robert Koch, in 1881, a decade after bacterial stains were first used, devised the use of gelatin as a liquefiable solid culture medium and invented a "plate" method of bacterial study. Up to this time the isolation of single species of bacteria involved much difficulty and almost always a certain measure of uncertainty. So long as investigators were not positive that they were dealing with a single species and not a mixture of several species of bacteria, no general conclusions could be free from doubt. This discovery did more for bacteriology than any other single discovery since the time of van Leeuwenhoek.

While Rayer, Pasteur, Davaine, Pollender, and others had seen Bacillus anthracis, and had established the proof that an animal could be made to contract the disease by inoculation with the blood of an animal suffering from anthrax, Koch was the first actually to study the germ in pure culture outside the animal body. He was able to observe the morphologic characters of the bacterium under the microscope. Finally, he succeeded in causing the disease in animals by injecting into them the culture he had grown on artificial media. So, for the first time, the demonstration of the causation of disease by bacteria was accomplished.

Koch outlined this method of proof and formulated the requirements to be fulfilled in order to prove the relation between the bacterial cause of any particular disease and the disease itself.[1] According to Koch, the bacterium must be present in the diseased animal; it must be isolated and grown on artificial culture media; it must be able to produce the disease from the artificial culture by inoculation into a healthy, susceptible animal; and it must again be isolated from the animal thus infected. These requirements are known as "Koch's postulates," and have been of invaluable service in pathological technique.

Koch also applied and developed the use of aniline dyes, first introduced into microscopic technique by Weigert. His study of the organism causing tuberculosis is a classic in respect to the thorough manner in which he carried out his research. In fact,

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[1] "Koch's postulates" were first proposed by Henle.

it may be said that, if Pasteur's researches gave rise to modern bacteriology, those of Koch established it as an exact science.

During this period the causative agents of most of the bacterial diseases were discovered: that of puerperal fever by Pasteur, of typhoid by Eberth, of diphtheria by Klebs, of cholera and tuberculosis by Koch, of gonorrhea by Neisser, of meningitis by Weichselbaum, and of bubonic plague by Kitasato and Yersin.

The work of these pioneers has been carried on to greater completion within the lifetime of the present generation. The outposts had been to a large measure already captured, but the coordination and the calm summarization of the early data have been carried on in recent years.

It is indeed difficult to realize the results of the labors of these men. The additions to knowledge that have been given by Priestley in chemistry, Darwin in biology, Kant in philosophy, and Calvin in theology have been admired and praised. How much more should the efforts of these men and their gifts to humanity in the prevention and cure of disease be appreciated. As a reminder of some of the results of their labors, Dr. Sedgwick (1855–1921) in his address as president of the American Public Health Association (1915) said:

> Bacteriology laboratories for the quick detection of dangerous infections have been installed almost everywhere. We have begun the medical and sanitary supervision of schools and school buildings. We have invented, and put within the reach of all but the very poor, the most complete, convenient, and salubrious heating and ventilating appliances for houses, theaters, halls, hotels, and workshops.[1]

There is, however, plenty of work still to be done. The causes of measles, cancer, infantile paralysis, and other diseases· are as yet unknown. In the United States alone it is estimated that 160,000 persons die each year of tuberculosis. That is, about 9 per cent of the deaths in this country are due to tuberculosis, and of the 100,000,000 people now living in the United States, 9,000,000 are doomed to die of this disease. Pneumonia claims about an equal number of lives yearly. Great epidemics like the comparatively recent scourges of infantile paralysis and "Spanish influenza" still threaten humanity. These, moreover, are all

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[1] SEDGWICK, W. T., "American Achievements and American Failures in Public Health Service," *American Journal of Public Health*, vol. 5, no. 11, p. 1103, Sept. 7, 1915.

communicable diseases and could, under perfect sanitary conditions, be eliminated entirely.

It is the distinct duty of the educated man to his community to understand and to help to further public-health measures. As preventive medicine has been effective, it is easy for opponents to make it appear unnecessary. This is exemplified in the oft-repeated question "Why should I be vaccinated against smallpox when there is no smallpox around?" In Manila, with a population of a quarter of a million, not one death occurred from smallpox during a period of 7 years. When, in 1918, preventive measures were not effectively carried out, more than 700 deaths were caused by this disease. Any number of such examples are of scientific record.

Nor does the importance of a thorough understanding of bacteriology end here. The term "bacteria" is not synonymous with disease, as so many seem to think. Only a very small proportion of bacteria are in the least way harmful. For example, in the great dairying industries, in butter and cheese making, bacteria have an essential rôle. In the disposal of sewage, in the manufacture of wines and important organic acids, and of food such as sauerkaut, pickles, etc., in the preparation of hemp, flax, and tobacco, and in the tanning of leather bacteria play an extremely important part. Through the discovery that certain bacteria have the ability to "fix" free nitrogen and so make it available for plants, a distinct branch of the science, agricultural bacteriology, has sprung up. Were it not for the bacteria which decompose dead organic matter, the earth long ago would have been uninhabitable because of the accumulated refuse. In fact, the "good" bacteria are just as important to human life as the "bad" bacteria.

Bacteria are found almost everywhere in nature. From the air, the water, and the soil, and from all living creatures, bacteria may be isolated. They are on everyone's clothes, on desks, chairs—on the pages of this book. Even in the normal, healthy body, bacteria are present on the skin, under the nails, in the mouth, in the intestinal tract, in the upper respiratory tract, and, in fact, in any part of the body which is exposed to, or receives substances directly from, sources outside the body. This applies to the lower animals and the plants, as well as to men. The only part of a living organism where bacteria are not found, in fact, is within the healthy tissues, whether of plants, or animals, or men.

CHAPTER III

CLASSIFICATION OF BACTERIA

One of the fundamental desires of the human mind is to separate facts and things into logical groups. In the world of living things it is immediately apparent to the thinking mind that there are certain outward resemblances among large groups of animals and plants. The birds, the beasts, the fishes, the worms, the trees, the bushes, and the grasses, all form more or less definite aggregations which have some characteristic in common among themselves, and it is these characteristics which set them apart from other kinds of animals and plants. Again, within any particular group, smaller groups, made up in the same way, with closer and lesser similarities, are seen. The fruit trees are distinguished from the evergreens. The rabbit and the squirrel more closely resemble each other than they do the hippopotamus or the hog. And even among these smaller groups differences and similarities are seen, and the tiger is called a "great cat," and the dog and fox are spoken of as belonging to the wolf "family."

Homology and Analogy.—When the reasons for placing an animal in a certain group are analyzed, it is found that the classification is made on the basis of certain outstanding characteristics. It is the *type* of these characteristics used which makes a grouping scientific or unscientific. The whale, the water snake, and the trout all swim. The bat, the moth, and the eagle all fly. Man and the ostrich run on two legs. Yet man and whale and bat are really more closely related to one another than each is to the ostrich, the trout and the moth.

Biologists recognize two types of characteristics. One is that based on function or purpose or use. The wings of the bird and of the butterfly are both used for flying. Such characteristics are called *analogous* characteristics. On the other hand, the flipper of the whale, when examined anatomically, very closely resembles the hand of a man and the paw of a dog or a cat. Characteristics of this second type, which are based on anatomical structures, are called ***homologous*** characteristics. Modern scientific groupings or classifications are based solely on homologous characteristics. Professor Osborne says:

> In structure Aristotle observed the law of Analogy, as, for example, in his comparison of the functions of the fore and hind limbs. But the principle of Homology, or fundamental likeness of type structure between the fore and hind limbs, was first pointed out by Vicq d'Azyr in 1805. Now analogy is the Will-o'-the-wisp of Evolution; it is always leading us astray, for functionally similar forms, and forms with an external resemblance are produced over and over again in Nature, and do not always point to phyletic affinity, while Homology is one of our safest guides.[1]

The Reasons for Classification.—The purpose of scientific biological classification is twofold. First, in the study of living forms we are forced by their enormous number to examine certain type species as examples of large groups. Also, in describing a newly discovered species, it is highly desirable to place it in a group of closely related species. The second purpose is to show by similarities and differences of anatomical structure the evolutionary position of any species in respect to all other species.

In speaking of classifications, therefore, we not only refer to the relationship of individual species to other species, but we refer to the relationship of the species to all other living things.

History of Classification.—The first chapter of Genesis records the ancient Hebrews' idea of differences between living things. Fowls and fish were created on the fifth day, "cattle, and creeping things and beasts of the earth"—and man—on the sixth day. "And Adam gave names to all cattle, and to the fowls of the air, and to every beast of the field." There is of course no idea of relationship among these animals—all were created independently. It is of interest to note that even in such a broad grouping as "fowls, fish, creeping things, and beasts," whales, which are mammals and closely related to the cattle, should be "created" with the fish on the fifth day. This is a good example of classification according to analogy.

The Greeks, though believing in the theory of spontaneous generation and the therefore independent origins of living things, attempted a grouping of forms according to similarities as well as one according to differences. Aristotle, who may be called the first comparative anatomist, and who incidentally recorded the

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[1] Osborne, H. F., "From the Greeks to Darwin," p. 24, New York, 1908.

first hint of the theory of organic evolution, divided all animals into two groups based on the presence or absence of blood. The bloodless animals were again divided into four subgroups: the insects, the molluscs, the crustacea, and the testacea. The animals with blood were divided into four groups: the fish, the amphibians, the birds, and the mammals. The last-named group, with the addition of the reptiles, resembles our modern grouping of the vertebrates. The use of blood as a distinguishing characteristic was incorrect, however, as blood is not a constant differential character, and many species of Aristotle's "bloodless" group are actually animals *with* blood, not without it. But Aristotle gave the first scientific attempt at classification.

From the days of Aristotle until about the time of the American Revolution, practically no progress was made in the scientific classification of living things. Then, after nearly two thousand years came a man who in a series of papers worked out a true basis for grouping living things, and proposed a system of naming plants and animals which is today used by the entire scientific world.

Carl von Linné, better known as Linnæus, was a Swedish botanist and physician who lived from 1707 to 1778. In his "Systema Naturæ" he classified all plants and animals which were known at his time. His orderly system, his concise descriptions, and, above all, his uniform use of specific names have been of the greatest value to all the biologists who have succeeded him.

The Modern Method of Classification.—In modern biological classification, a certain few fundamental anatomical or structural characteristics are used as a basis for a primary broad division of living things into groups. First, all living things are divided into two great groups or kingdoms: plants and animals. In the animal kingdom what is taken as the broad fundamental characteristics are the possession of a spinal column; of an external jointed skeleton (crabs, insects, etc.); of body segmentation (round or flat worms); of a body cavity; etc. By these characteristics all animals may be divided into about twelve great groups, called *phyla*. Now, in the case of each phylum a few outstanding anatomical characteristics are selected and the phylum is again divided into a few lesser groups. If the phylum Chordata is taken, it can immediately be divided into two *subphyla* on the basis of whether the dorsal column is cartilaginous or bony. The subphylum Vertebrata (those animals possessing a bony spinal column) is divided into five groups on the basis of suckling of the young (Mammalia); the possession of wings; method of obtaining oxygen; etc. These divisions of the phylum are called *classes*. Now in the same way each class is subdivided. In the case of the mammals the basis of division is the method of carrying the young (pouched animals, *e.g.*, the kangaroo); the formation of the hand or hoof, the teeth, etc. The subdivisions of the classes are called *orders*. In like manner the orders are subdivided into *families*, families into *tribes*, tribes into *genera*, and genera into *species*. Very frequently, because of the fact that many animals have ceased to exist on the earth, a genus, tribe, or even family may consist of a single species.

The system may also be looked at from the opposite viewpoint. A single kind of animal, like man, or the dog, or the cat may be taken—animals in which dissimilarities between individuals are so slight that gradations may be easily found among them—and that kind is called a *species*. Sometimes the species is for convenience divided by specialists in the study of a particular group like the *races* of mankind or the *varieties* of dog (greyhound, Airedale, etc.). If man is taken as an example of a species of animal, modern man and ancient man (paleolithic man) may be placed in a larger group—the genus. Certain of the higher apes very closely resemble man in his anatomical structure, so modern man, ancient man, and the monkey together form a still larger group. Again, in an important characteristic—the suckling of their young—other animals are similar to man, and there results a still larger group containing man, monkey, cat, dog, horse, etc. Once more it is found that in the possession of a spinal cord, birds, fish, frogs, and snakes are similar to man, and dissimilar to all other animals; and a still larger group is formed. In this way biologists have divided all animals and plants into a few great divisions, each with its progressive subdivisions, just as an army is divided into divisions, brigades, regiments, companies, and squads.

It must be clearly understood that the bases of these divisions are purely arbitrary. They are the result of a great amount of intensive study, but they are, after all, man-made classifications and may be changed at any time that our growing knowledge of nature makes them obsolete.

Nomenclature.—In naming a species (not "specie") two words are employed. The first is the generic or genus name and the second the specific name. Thus: *Homo sapiens*, modern man; *Felis leo*, the lion; *Felis tigris*, the tiger; *Eberthella typhi*, the typhoid germ. It will be noticed that the generic name is capitalized but the specific name begins with a lower-case letter.

Tree of Life.—One often sees in textbooks a so-called "tree of life" which shows diagrammatically the relationships among living things. Such a diagram may be quite confusing and lead to an entirely erroneous idea, if the fundamental basis of this "tree of life" is not fully understood. It should never be taken as a plan of ascent or progression from a lower form to a higher. (The bees and ants are probably as highly specialized as man.) Nor should it be taken to show how one form developed into another. The diagrammatic tree of life must be looked upon as a genealogical tree in which each branch is entirely divergent and independent of other branches. As a branch of a family tree may die out, so dead branches representing extinct forms appear on the tree of life. The great branches divide and subdivide into genealogical classes, orders, families, and genera, until we get to the ultimate twigs which represent species. The relationship between two species is found by going back to a common branch; there are no "by passes" leading from twig to twig or branch to branch. Two twigs may be individual shoots of a single, small branch; in this case they are closely related and would represent two species of the same genus. In another case two twigs may be on opposite sides of the tree; in which case, in order to show their relationship, it would be necessary to go back along larger and larger branches until the main trunk was reached, and then outward along smaller branches until the tracer came to the second twig. Thus, to show the relationship between two species, it may be necessary to go through genus, tribe, family, order, class, and phylum, and then backward through phylum, class, order, family, and genus to the second species.

The Place of Bacteria in Nature.—Quite divergent views are held even today as to where bacteria should be placed in the classification of living things. Some scientists take the point of view that, while it is true that bacteria as a whole class resemble plants more closely than they do animals, many species are closer to the animal than to the plant kingdom. If it is considered that all plants and animals have sprung from a common ancestor—the primitive cell—a group of living creatures too low in the scale of life to be definitely classed as a member of either the plant or the animal kingdom can easily be conceived of. It is in this group that bacteria would be placed.

On the other hand, there are workers who are not willing to accept the "lowest-form" theory, and, while agreeing that they are extremely low forms of life, would place bacteria in a group known as *Fungi imperfecti*, or degenerate fungus forms. Bacteria more closely resemble the Cyanophyceæ among the plants, and the Flagellata among the animals, although they bear no generic relationship to either. The cell wall of a bacterium is as closely related to the chitin of animals as it is to the cellulose of plants. On the other hand, certain species of bacteria can split up carbon dioxide without the aid of chlorophyll and sunlight, a property possessed by no other organisms, plant or animal.

It may be said that bacteria resemble the plants in morphology, in their power to form filaments, and, in a few species, in their ability to obtain nitrogen from the decomposition of ammonia compounds and assimilate it as food.

Bacteria resemble animals in that most of them require organic food, in the possession of flagella, and also in the close similarity between sporulation of bacteria and encystment in certain Protozoa.

The earliest attempt to divide the bacteria into genera was that of Mueller in 1786. He placed them among the Protozoa and recognized two genera (Monas and Vibrio) including forty-five species.

Ehrenberg, in 1838, recognized five genera (Bacterium, Vibrio, Spirillum, Spirochæta, and Spirodiscus), also regarding them as animals.

In 1849, Leidy showed that the Protozoa had many characteristics that bacteria did not possess. Shortly afterward (1857) Naegeli proposed a classification in which the bacteria were placed in the vegetable kingdom and first used the class name "Schizomycetes" (or fission fungi). From this time on systematists have regarded these minute organisms as plants, belonging among the Thallophyta, or the lowest form of vegetable life, and advances in knowledge have been along the lines of generic groupings.

From the earliest attempts at the classification of bacteria by Mueller in 1786 up to the last few years, systematists have depended entirely on morphological differences and similarities.

Davaine (1868), Cohn (1875–1879), Magnin (1878), Burrill (1882), Fluegge (1886), Sternberg (1892), Fischer (1897), and Migula (1900) studied these organisms and classified them, accepting previous classifications, but each adding to or emending until a complete system was worked out.

Because it was the most complete classification at a time when the result of a large mass of research was being presented to bacteriological literature in this country, and also because of its acceptance by the author of the only manual of determinative bacteriology published in English at the time,[1] Migula's classification firmly established itself in American colleges, which were just beginning to teach the new science. The result has been that until the Society of American Bacteriologists adopted the present system of classification of bacteria, there was no really accepted classification. Several had been proposed, but, with the exception of Migula's, none had received wide acceptance. The result has been that bacterial classification and nomenclature have been in absurd confusion.

Following Chester's adoption of Migula's classification in 1901, nothing of importance was published by systematists until 1908, when Winslow attempted to classify the Coccaceæ according to biologic as well as morphologic characters. Orla-Jensen, in 1919, proposed an intensive classification of bacteria based on morphologic, biologic, and biochemic characters, but his system has not been accepted in this country. Buchanan, in 1915, and Castellani and Chalmers, in 1919, gave two classifications which are based on biologic as well as morphologic characters.

While, until very recently, there has existed no strictly scientific method of classifying bacteria, there has been no method whatever, scientific or otherwise, of naming, them. A species is properly designated only by a binomial Latin name, the first member being that of the genus, as Bacillus, Micrococcus, etc., and the second that of the particular species, as, Bacillus subtilis, Sarcina lutea, etc. If a third word is employed at all, it should designate the person who first described the particular species, as, *Leptotrichia buccalis* Robin. According to the older systems (or lack of systems) of nomenclature, there are such names as "Bacillus enteritidis sporogenes," which, if interpreted according to botanical usage, would mean the spore-forming variety of Bacillus enteritidis. As a matter of fact, the species is only

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[1] Chester, "A Manual of Determinative Bacteriology," 1901.

distantly related to Bacillus enteritidis. It belongs to the typhoid-dysentery group of bacteria (Eberthella enterica), while the latter belongs among the pathogenic anaërobes (Clostridium). Again, as in "Bacillus aerogenes sputigenus capsulatus," there was a quatrinomial for the name of a bacterium which is not related closely to any other species. This hit-and-miss method of naming bacteria has led to the greatest confusion, as many of the common bacteria, especially the pathogenic bacteria, have two or three names which are used indiscriminately. Thus, "Bacillus Welchii," "Bacillus aërogenes capsulatus," "Bacillus phlegmonia emphysematosæ," and "Bacillus perfrigens" all refer to a single species. In order, therefore, to obtain a complete description of this particular bacterium, it was necessary to be familiar with all its nicknames. Because of the great mass of valuable literature written describing bacteria by the old method, great care must be taken properly to interpret the names used.

The Bases of Bacterial Classification.—The differences and similarities among bacteria which are used as essential characteristics in dividing these organisms into orders, families, tribes, genera, and species are even today not entirely satisfactory. The reason for this is that these characteristics are not invariable in the same species. As will be brought out more fully in the following chapters, bacterial species are not stable forms of life like the oak tree or man, but are constantly undergoing changes in morphology and physiology with, of course, changes in characteristics which at times may be used as essential differential features. The best that can be done is to group these species according to the characteristics which they display under the usual methods of laboratory procedure, namely, their growth at definite time periods on standard culture media and under the conventional external conditions of temperature, oxygen supply, light, etc.

1. *Morphology.*—The shape or form of the organism was the basis for the earliest systems of classification. Today the six orders of class Schizomycetes are divided upon the basis of form. Among true or undifferentiated bacteria (Eubacteriales) this characteristic is used in separating two of the five families. It is also used quite frequently in separating species and genera, as in the two genera of the family Spirillaceæ.

2. *Motility.*—The power of some bacteria to move is used to differentiate certain species. This characteristic was considered

more important in the past than it now is. Migula separated all the rod forms into two genera, the motile and the non-motile rods.

3. *Capsules.*—The ability of certain species to form well-defined capsules is used as a basis of differentiation as in the case of the tribe Klebsielleæ.

4. *Spore Formation.*—The families Bacteriaceæ and Bacillaceæ of the order Eubacteriales are distinguished solely upon whether or not they form spores. This is one of the most stable of all bacterial characteristics.

5. *Method of Cell Division.*—The grouping of individuals after division is used as the basis of classifying the spherical bacteria (family Coccaceæ).

6. *Pigment Production.*—Colors produced by bacteria in mass growth or in the medium form a basis of classification. The tribe Chromobacterieæ is made up entirely of chromogenic bacteria.

7. *Parasitic Nature.*—Many genera and species are distinguished as to whether or not they are parasitic to man, to lower animals, or to plants. In addition to this, some groups are divided on the basis of their disease-producing power, independent of their parasitic nature. The classification committee of the Society of American Bacteriologists has been criticized for placing undue emphasis on this characteristic, as its introduction separates species which otherwise are very closely related, and because the characteristic itself is unstable.

8. *Oxygen Requirements.*—Some bacteria demand atmospheric oxygen for their growth, and others demand the complete absence of free oxygen. This is used to distinguish the two genera of the family Bacillaceæ, and to a limited extent other species.

9. *Energy Requirements.*—The family Nitrobacteriaceæ is divided into genera on the basis of the substances from which the organisms obtain their energy. Some oxidize methane, others hydrogen, others ammonia, etc.

10. *Physiology.*—Among the vast majority of species, differentiation is made on biochemical grounds, such as food requirements, the ability to ferment certain carbohydrates, to liquefy gelatin, to produce indol, to hydrolyze starch, to reduce nitrates to nitrites, etc. While this method would seem the most logical, from our generally accepted point of view of using homologous characteristics, it is a method which unfortunately shows the

greatest variations among individuals of the same species and is most readily influenced by varying conditions of growth.

As knowledge of the life processes of bacteria becomes more complete, radical changes will probably be made in the bases of classification. In the light of present knowledge, however, it is better to accept an imperfect classification with its apparent errors than to continue with the hodge-podge of the past.

S. A. B. Classification.—At the annual meeting of the Society of American Bacteriologists in December, 1919, a Committee on the Characterization and Classification of Bacteria presented a report which summarized present-day opinion on classification.[1]

Later, a new committee of this society, headed by Dr. David H. Bergey, made a few changes and additions and published the data under the name of Bergey's "Manual of Determinative Bacteriology" (Baltimore, 1923, revised in 1925 and 1930). This committee places the bacteria (this term is dropped as a generic name and applied only in the general way of applying to all of these microörganisms as a synonym of germ, microbe, etc.) in the class Schizomycetes, phylum Thallophyta of the vegetable kingdom.

The Schizomycetes are defined as:

> . . . Typically unicellular plants, cells usually small and relatively primitive in organization. The cells are of many shapes, spherical, cylindrical, spiral, or filamentous; cells often united into groups, families, or filaments; occasionally in the latter showing some differentiation among the cells, simulating the organization seen in some of the blue-green, filamentous algæ. Multiplication typically by cell fission. Endospores are formed by some species of the Eubacteriales, conidia by some of the filamentous forms. Chlorophyll is produced by none of the bacteria (with the possible exception of a single genus). Many forms produce pigments of other types. The cells may be motile by means of flagella; some of the forms intergrading with the protozoa are flexuous, a few filamentous forms (as Beggiatoa) show oscillatory movement similar to that of certain blue-green algæ (as Oscillatoria).

The class Schizomycetes is divided into six orders: Spirochætales, Myxomycetales, Thiobacteriales, Chlamydobacteriales, Actinomycetales, and Eubacteriales. The first four of these orders will not be studied in detail here, but will be taken up in a later chapter. The fifth, Actinomycetales, and the sixth,

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[1] This report was published in the *Journal of Bacteriology*, vol. 5, pp. 191–229, 1920.

Eubacteriales, will be studied together. Some species, like the organism causing diphtheria, are now classed with the Actinomycetales, which, according to Migula's classification, were placed among the Eubacteriales.

The order Actinomycetales includes only a few species which are of interest. One, Leptotrichia buccalis, is a common inhabitant of the mouth of man. Mycobacterium tuberculosis is the germ causing tuberculosis. Under the old classification, this organism was known as "Bacillus tuberculosis," or Koch's bacillus. Corynebacterium diphtheriæ is the diphtheria germ. It was known as "Bacillus diphtheriæ," or the Klebs-Loeffler bacillus. One of the bacteria found in Vincent's angina (trench mouth) also belongs in this order.

The order Eubacteriales, the true bacteria, includes the simplest and least differentiated forms. "The cell metabolism is not primarily bound up with hydrogen sulphid or other sulphur compounds, the cells in consequence containing neither sulphur granules nor bacteriopurpurin (differing from the order Thiobacteriales)." The cells may be united into gelatinous masses (*e.g.*, Zoöglœa) but never form pseudoplasmodia nor develop a highly specialized cyst-producing fruiting stage, such as is characteristic of the Mycobacteriales. They are never protozoan-like as are the Spirochætales, nor are they sheathed like the Chlamydobacteriales. The Eubacteriales are distinguished from the Actinomycetales in not being mold- or plantlike and, while the undifferentiated cells may form in chains, they never form true filaments, nor are they branched.

The order Eubacteriales is divided into five families. Two of these, the Bacteriaceæ and the Bacillaceæ, are rod shaped; one, the Spirillaceæ, is curved; and one, the Coccaceæ, is spherical. The Nitrobacteriaceæ are usually rods, though they may be nearly spherical. They are distinguished from the other families by the fact that they obtain their food from the simplest of compounds. The other rod forms, the Bacteriaceæ and the Bacillaceæ, need more complex foods, and are distinguished from each other by their ability to form spores.

The family Nitrobacteriaceæ includes the forms which are capable of securing growth energy by the oxidation of carbon, hydrogen, and nitrogen, or simple compounds of these elements. They are obligate aërobes and never form spores. They are usually water or soil forms. The family is divided into nine genera, distinguished from one another by their source of energy and food.

The family Bacteriaceæ is comprised of non-spore-forming, rod-shaped cells. They are usually Gram-negative. They may be non-motile, or motile by means of peritrichous flagella. The metabolism is complex, their food consisting of amino acids and, generally, carbohydrates. There are thirteen tribes, comprising twenty-four genera. One of these tribes, the Erwiniæ, comprises a number of species of plant pathogens. Another, the Bactereæ contains a number of species important to the sanitarian. The tribe Bactereæ is composed of seven genera of Gram-negative, motile, or non-motile rods, which are usually intestinal parasites in the higher animals. They generally attack carbohydrates, forming acid and often gas (H_2 and CO_2). One genus (Alcalgenes) does not form acid or gas from any of the carbohydrates. The other genera (Escherichia, Aërobacter, Proteus, Salmonella, and Eberthella) are distinguished from each other by their action on various carbohydrates. The genus Shigella is distinguished from the genus Eberthella on the basis of motility. Escherichia coli (B. coli), Aërobacter aërogenes (B. lactis aërogenes), Proteus vulgaris (B. proteus vulgaris), Salmonella schottmulleri (B. paratyphosus B), Salmonella paratyphi (B. paratyphosus A), Eberthella typhi (B. typhosus), Shigella dysenteriæ (B. dysenteriæ) and Alcaligenes abortus (B. abortus, Bang) are common species of the tribe Bacterieae.

The family Bacillaceæ includes those rods which form spores. They are usually Gram-positive. There are two genera. The organisms of the genus Bacillus are aërobic. They are mostly saprophytic and they liquefy gelatin. Those of the genus Clostridium are anaërobic. These organisms are often parasitic. The rods are frequently swollen at sporulation.

The family Spirillaceæ includes the more or less spirally curved rods. They are usually without spores, and are motile by means of polar flagella. They are typically water forms, although some species are intestinal parasites. There are two genera. The genus Vibrio includes the short, comma-shaped rods. These may occur singly or united into spirals. They are motile by means of one to three short polar flagella. The cells are usually Gram-negative. The genus Spirillum comprises long, rigid spirals, having a tuft or polar flagella.

The family Coccaceæ comprises the spherical bacteria. Division may take place in one, two, or three planes. Motility is rare and spores are absent. Metabolism is complex. A pigment is often formed. The family is divided into three tribes and eight genera, four of which are parasitic and four saprophytic. Some of the most severe diseases of man are due to members of this family. Meningitis, pneumonia, gonorrhea, scarlet fever, boils, carbuncles, and various other diseases are caused by different species of the spherical bacteria.

The following diagram shows the relation between a few of the common species of bacteria. It will be seen that Alcaligenes melitensis and Alcaligenes fecalis are closely related, belonging to the same genus. These organisms in turn are related to Escherichia coli in the same tribe, to Lactobacillus acidophilus in the same family and to Neisseria gonorrhœæ in the same order. Again, Mycobacterium tuberculosis and Corynebacterium diphtheria belong to the same family but are only related to Eberthella typhi or to Treponema pallidum in that they belong to the same great class, Schizomycetes.

In the same way, the relationship between any two species of bacteria may be shown. Again, it must be emphasized that by "relationship" is meant an artificial classification and that there is no attempt to show or indicate any phylogenetic relationship.

Species	Genus	Tribe	Family	Order	Class	Sub-phylum	Phylum
Es. coli	Escherichia	Bacterieæ	Bacteriaceæ	Eubacteriales	Schizomycetes	Fungi	Thallophyta
A. melitensis	Alcaligenes						
A. fecalis							
L. acidophilus	Lactobacillus	Lactobacilleæ					
B. subtilis	Bacillus		Bacillaceæ				
N. gonorrhœæ	Neisseria	Neisserieæ	Coccaceæ				
M. tuberculosis	Mycobacterium		Mycobacteriaceæ	Actinomycetales			
C. diphtheriæ	Corynebacterium						
T. pallidum	Treponema		Spirochætaceæ	Spirochætales			

CLASSIFICATION OF BACTERIA[1]

- Class: Schizomycetes.
  - Order 1. Eubacteriales
    - Family 1. Nitrobacteriaceae.
      - Tribe 1. Nitrobacterieae.
        - Genus 1. Hydrogenomonas.
        - Genus 2. Methanomonas.
        - Genus 3. Carboxydomonas.
        - Genus 4. Nitrosomonas.
        - Genus 5. Nitrosococcus
        - Genus 6. Nitrobacter.
        - Genus 7. Acetobacter (**Acetobacter acetus**).
        - Genus 8. Thiobacillus.
      - Tribe 2. Azotobacterieae
        - Genus 9. Azotobacter.
        - Genus 10. Rhizobium (**Rhizobium radicicola**).
    - Family 2. Coccaceae.
      - Tribe 1. Streptococceae.
        - Genus 1. Diplococcus (**Diplococcus pneumoniae**).
        - Genus 2. Streptococcus (**Streptococcus pyogenes**).
        - Genus 3. Leuconostoc.
      - Tribe 2. Neisserieae.
        - Genus 4. Neisseria (**Neisseria gonorrhoeae**).
        - Genus 5. Gaffkya.
      - Tribe 3. Micrococceae
        - Genus 6. Staphylococcus (**Staphylococcus aureus**).
        - Genus 7. Micrococcus (**Micrococcus freudenreichii**).
        - Genus 8. Sarcina (**Sarcina lutea**).
        - Genus 9. Rhodococcus.
    - Family 3. Spirillaceae.
        - Genus 1. Vibrio (**Vibrio comma**).
        - Genus 2. Spirillum (**Spirillum rubrum**).
    - Family 4. Bacteriaceae.
      - Tribe 1. Chromobacterieae.
        - Genus 1. Serratia (**Serratia marcescens**).
        - Genus 2. Flavobacterium.
        - Genus 3. Chromobacterium.
        - Genus 4. Pseudomonas (**Pseudomonas aeruginosa**).
      - Tribe 2. Protominobacterieae.
        - Genus 5. Protominobacter.
      - Tribe 3. Cellulomonadeae.
        - Genus 6. Cellulomonas.
      - Tribe 4. Achromobacterieae.
        - Genus 7. Achromobacter.
      - Tribe 5. Erwineae.
        - Genus 8. Erwinia.
        - Genus 9. Phytomonas.

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[1] Based on Bergey, "Manual of Determinative Bacteriology," 3d ed., 1930.

Tribe 6. Lactobacilleae.
  Genus 10. Lactobacillus (**Lactobacillus acidophilus**).
Tribe 7. Propionibacterieae.
  Genus 11. Propionibacterium.
Tribe 8. Kurthieae.
  Genus 12. Kurthia.
Tribe 9. Pasteurelleae
  Genus 13. Pasteurella (**Pasteurella pestis**).
Tribe 10. Klebsielleae.
  Genus 14. Klebsiella (**Klebsiella pneumoniae**).
Tribe 11. Hemophileae.
  Genus 15. Hemophilus (**Hemophilus influenzae**).
  Genus 16. Dialister.
Tribe 12. Bacterieae. Tribe Bacterieae
  Genus 17. Escherichia (**Escherichia coli**).
  Genus 18. Aerobacter.
  Genus 19. Proteus.
  Genus 20. Salmonella (**Salmonella schottmülleri**).
  Genus 21. Eberthella (**Eberthella typhi**).
  Genus 22. Shigella (**Shigella dysenteriae**).
  Genus 23. Alcaligenes (**Alcaligenes faecalis**).
Tribe 13. Bacteroideae.
  Genus 24. Bacteroides.
Family 5. Bacillaceae.
  Genus 1. Bacillus (**Bacillus subtilis**).
  Genus 2. Clostridium (**Clostridium tetani**).
Order 2. Actinomycetales.
Family 1. Actinomycetaceae.
  Genus 1. Actinobacillus.
  Genus 2. Leptotrichia (**Leptotrichia buccalis**).
  Genus 3. Actinomyces.
  Genus 4. Erysipelothrix
Family 2. Mycobacteriaceae.
  Genus 1. Mycobacterium (**Mycobacterium tuberculosis**).
  Genus 2. Corynebacterium (**Corynebacterium diphtheriae**).
  Genus 3. Fusiformis.
  Genus 4. Cytophaga.
  Genus 5. Pfeifferella (**Pfeifferella mallei**).
Order 3. Chlamydobacteriale
Order 4. Thiobacteriales.
Order 5. Myxobacteriales.
Order 6. Spirochaetales.
  Genus 5. Treponema (**Treponema pallidum**).

CHAPTER IV

MORPHOLOGY OF BACTERIA

The term morphology means, literally, "the study of form." The morphology of bacteria, then, is that branch of bacteriology which treats of the form or structure of bacteria.

Bacteria are one-celled organisms. Each bacterium possesses in a single cell all the properties which make it a living creature. In man there are a large number of different kinds of cells—nerve cells, muscle cells, bone cells, blood cells, etc.—uncountable numbers of each kind, arranging themselves into tissues, organs and systems. The individual bacterium is just one cell.

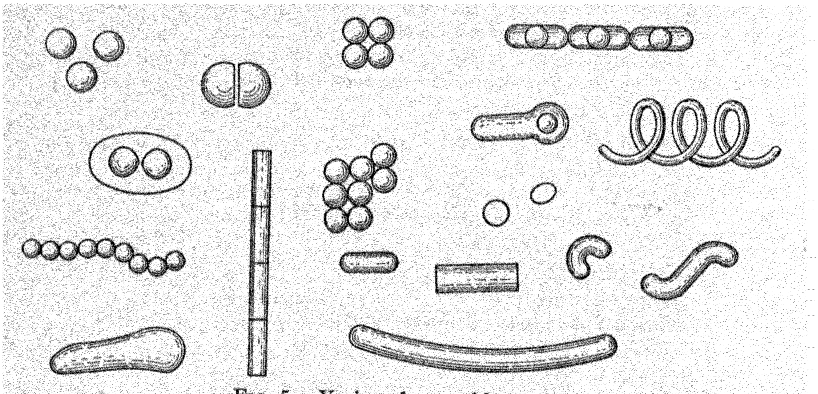

FIG. 5.—Various forms of bacteria.

Until the middle of the nineteenth century, bacteria were of interest solely as biological forms, and the shape of the cells received considerable attention. With the researches of Pasteur, Koch, and their followers, the students of bacteriology suddenly became interested in what bacteria do rather than in what they are, and the medical and chemical aspects of the science became of paramount importance. Recently the pendulum has started its return swing, and once again bacteriologists are becoming interested in biology.

The Usual Shape of Bacteria.—When bacteria are examined under the microscope, three general forms or shapes may be identified: spheres, straight rods, and spirals. Many modifications or variations of these general forms are found, and the arrangement of the individual cells may differ widely. Thus, the spheres may appear singly, in pairs, in chains, in regular planes, or in the form of cubical packets or irregular packets. The spheres may be flattened at the side where they are in contact with other cells, or they may be elongated at their proximal sides. Rods may be long or short, filamentous, fusiform, irregular, or straight, and arranged either singly or in chains. Spirals may vary from a slightly curved rod like a comma to a long corkscrew form of fifteen to twenty loops. These forms may also appear singly or in chains of many individual cells.

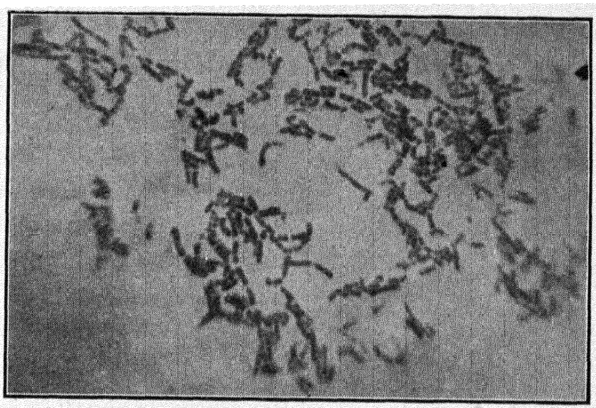

Fig. 6.—Lactobacillus acidophilus. Usual form.

In general the form and arrangement of the cells of any particular species are fairly constant, and bacteriologists have become accustomed to describe the morphology of a species in rather definite terms. For example, Escherichia coli is described as a short rod, occurring singly, in pairs, or in short chains; Sarcina lutea as a sphere occurring in regular packets; and Neisseria gonorrhœæ as a sphere occurring in pairs with the sides flattened where they are in contact. Thus we have built up a definite vocabulary for describing bacterial shapes, based on the fundamental idea of the immutability of the bacterial form.

In the early days of the science of bacteriology quite a debate occurred as to the constancy of form among bacteria. Billroth[1]

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[1] Billroth, Th., "Untersuchungen über die Vegetationsformen von Cocco-bakteria septica," Berlin, 1874.

took the stand that bacteria differ considerably in form according to circumstances, principally that of the abundance and the nature of the food supply. He placed all bacteria under a single species which he called "Coccobacteria septica." These might occur as spheres or rods, as single cells, pairs, chains, or filaments.

Taking the other side of the question, Cohn[1] believed that all bacteria having a particular form (*e.g.*, spheres, rods, or spirals) and having particular enzymic action should be regarded as distinct species until the fact of identity should be proven. In other words, every time a bacterium is found which possesses a form or physiological activity not already described as belonging to some other species, that organism should be considered a new species.

Naegeli[2] was convinced that the bacteria " . . . cannot be grouped in accordance with their action as ferments and their exterior forms," and, granting the possibility of certain definite species, he thought that they " . . . had little in common with the genera and species admitted today, and of which each runs through a cycle of determined forms sufficiently numerous." Thus, a single species is capable of many and diverse forms, chemical activities, and biological behavior.

Hueppe,[3] as late as 1896, said:

> The existence of rigid form-species, which not only the earlier observers, but even Cohn, Schroter, and Koch assumed, can be upheld no longer. The adaptability of bacterial forms to changing conditions of nutrition is not so boundless as Naegeli and Billroth supposed, but it is considerably greater than was once held to be compatible with the conception of the existence of constant species.

By the beginning of the twentieth century, Koch and his school had succeeded in convincing most bacteriologists of the correctness of the monomorphic view, and the classification of Migula[4] was generally accepted.

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[1] COHN, F., "Untersuchungen über Bakterien," *Beitrage zur Biologie der Pflanzen*, bd. 1, heft 3, p. 141, 1875.

[2] NÆGELI, CARL VON, "Die niederen Pilze in ihren Bezeilungen zu den Infectionskrankheiten und der Gesundheitspflege," Munich, 1877.

[3] HUEPPE, FERDINAND, "Naturwissenschaftliche Einfuhrung in die Bakteriologie," p. 15, Wiestaden, 1896.

[4] MIGULA, H., "System der Bakterien," bd. 1, 1897; bd. 2, 1900.

In this country a system of classification by Chester,[1] based on Migula's work, was universally adopted by the American pioneers in the science.

This author says:

> That typical forms or species of bacteria do exist no one can deny. These typical forms furthermore present certain definite morphologic, biologic, cultural, and perhaps pathogenic characters which establish the types, independent of minor variations.

Migula's classification was published in Germany at the time when the German school, headed by Koch, had gained the ascendency over the French following the death of Louis Pasteur. Chester's classification, based on Migula's, appeared in America just as the science was being introduced into American universities, and the results of American bacteriological research were becoming part of the literature. These two works may be said to mark the absolute ascendency of the dogma of the immutability of bacterial form.

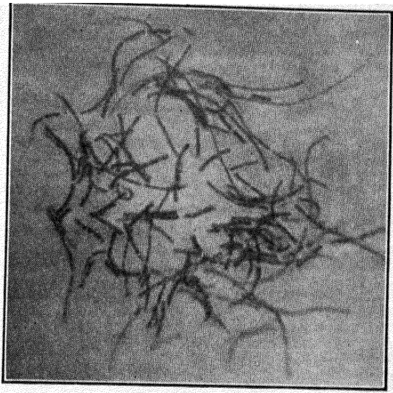

FIG. 7.—Lactobacillus acidophilus. (Note Figs. 6 and 8.)

Polymorphism, Pleomorphism, Involution Forms.—If we go back to the literature of the nineteenth century, we find constant reference to the terms "polymorphism" and "pleomorphism." The term "polymorphism" was used mainly to describe the different forms of bacteria during the processes of sporulation and germination. (At this time, the endospore was considered to be the germ of the bacterium.) The term was also used however, as synonymous with the term "pleomorphism," which designated an irregular development of a species, usually due to variations in external or environmental conditions. Thus, different media were supposed to bring about a variety of morphological phases. For example, in an old culture of Mycobacterium tuberculosis we find threads, granular filaments,

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[1] CHESTER, F. D., "A Manual of Determinative Bacteriology," 1901.

drumsticks, and diplococcal forms which are quite different from the morphology in young or freshly isolated cultures.

Along about 1900, these two terms were generally discarded, and the term "involution form" was adopted in their place. Says Newman:[1]

> Neither pleomorphism nor polymorphism is fully understood, and many bacteriologists find shelter from both in the term involution form. What we do know is that a species may take on divers forms when placed under different conditions.

This latter term, "involution form," soon took on a new meaning under the influence of our "immutability of species" dogma. While the size of the individual members of a species of bacteria varies more or less, under ordinary favorable conditions of life the shape was supposed to remain constant. Some species, however, if placed in an unfavorable medium, such as food containing lithium salts, or food containing a large amount of their own excretions, show marked variations in form. This is supposed to be due to a modification or degeneration of the protoplasm causing a distortion of the cell membrane, a phenomenon due to plasmolysis or the extraction of a portion of the water from the protoplasm. We know that a cell treated with a solution of certain salts, like barium chloride or sodium chloride can be plasmolysed at will. These salts which plasmolyse bacterial cells pass through the capsule of the cell but not through the cell wall. Instead, the substance extracts water from the

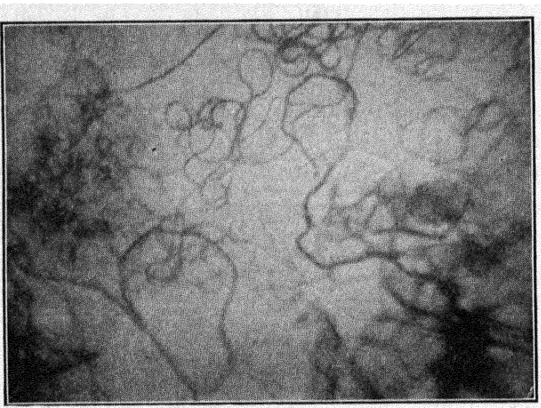

FIG. 8.—Lactobacillus acidophilus. (Note also Figs. 6 and 7.)

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[1] NEWMAN, GEORGE, "Bacteria," p. 12, New York, 1899.

inner parts of the cell. The cell contents surrounded by protoplasm therefore diminish in volume and the protoplasm recedes from the cell wall. This phenomenon is described by the term "involution form." The fact that a bacterium could thus be caused to change its shape led to the idea of pleomorphism or polymorphism. This definition of course carries with it the idea that if a bacterium is thus modified from its normal form, it will resume its former shape when placed in its proper environment. While this viewpoint is now generally accepted, Stitt,[1] in 1923, revived the old definition:

> In the prosecution of his work on localization of bacteria in the spinal cord, Rosenow found that the streptococci under observation developed very small forms, capable of passing through the pores of test filters, which he regarded as identical with the globoid bodies of Flexner and Noguchi, generally accepted as the cause of poliomyelitis. The development of these minute forms from streptococci of normal size is an example of pleomorphism, which may be defined as the assumption at different times of various distinct forms by a single organism or species. Pleomorphic changes are observed in young, actively growing cultures and are more or less permanent, usually persisting so long as the environment in which they arise remains unchanged.
>
> Pleomorphic forms are to be differentiated from degeneration and involution forms which appear not uncommonly in old cultures and under unfavorable conditions in environment, such as accumulation of waste products, unsuitable reaction, or osmotic tension, and the presence of harmful chemicals or specific antagonistic substances.

Life Cycle of Bacteria.—In 1916 Negri[2] described a number of different forms which the organism of "malignous granuloma" assumes. Because of a constantly recurring budding ("blastomycetes") form he placed this bacterium among the "Fungi imperfecti." During the same year Lohnis and Smith[3] fully described the morphological changes which they observed in the course of cultivation of Azotobacter and other bacteria. These authors for the first time used the term "life cycle," in describing cyclic changes in cultural development. They discarded the old

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[1] STITT, E. R., "Practical Bacteriology, Blood Work, and Animal Parasitology," 7th ed., p. 590, Philadelphia, 1923.

[2] NEGRI, E. E. A. M., "Untersuchungen zur Kenntnis der Corynebacterien, gleichzeitig ein neuer Beitrag zur Aetiologie des malignen Granuloms," *Folio Microbiol.*, Jahrg. 4, heft 2, pp. 119–187.

[3] LOHNIS, FELIX, and N. R. SMITH, "Life Cycles of Bacteria," *Journal of Agricultural Research*, vol. 6, 18, pp. 675–702, 1916.

familiar terms "involution forms," "bacterial polymorphism," and "pleomorphism," and stated that:

> The development of bacteria is characterized not by the irregular occurrence of more or less abnormal forms but by the regular occurrence of many different forms and stages of growth connected with each other by constant relations.

Fourteen distinct states were described in this life cycle, three of them—"conjunction," "budding gonidia," and "symplasm"—being means of reproduction augmenting, in the species history, binary fission.

The "symplasm" or symplastic state is a new idea. In this the bacteria appear under the microscope "as either unstainable or as a readily stainable mass without any visible organization, which later gives birth to new regenerated forms frequently of very characteristic and unusual appearance."

Changes in Cell Morphology during Growth.—Reproduction of bacteria will be discussed more fully later, but, as there are certain relationships between the growth of bacteria and their shape, it is necessary to mention some phases of this subject here. If a single bacterium be placed in a suitable environment, it will multiply rapidly, and, in the course of a few days, there will be millions of offspring from this one cell.

The fact that it has been a usual laboratory procedure to examine these subcultures after one or more days' incubation has had a distinct bearing on the "monomorphic" viewpoint. Recently, however, investigators have been interested in studying bacteria at frequent intervals during the growth period, and some rather interesting observations have been made.

While all species of bacteria do not follow the same mode of progression in their descendent cultural development, the following general plan is typical of what may be called "growth phases."

Most of the individual cells of Eberthella typhi taken from a 24-hour-old culture will be of a uniform size and correspond to what is usually described as the "typical" Eberthella typhi. If a single cell be removed from this culture and transplanted to a favorable medium and incubated at body temperature, after a few hours multiplication will begin. Now, if the daughter cells are examined at the end of 6 hours it will be found that they are considerably larger than was the parent cell, with a relatively greater increase in length than in thickness. After about 8 hours there is a gradual diminution in size of the cells being produced until at the end of 24 hours there appear once more the "typical" Eberthella typhi cells. This does not mean that the individual cells are large at the time of reproduction and then gradually become smaller. The variation is in the size of the daughter cells as they are produced.

Thus it will be seen that bacteriology today is undergoing a renaissance or revival of learning regarding the form of these microörganisms. These new ideas are not confined to form alone but have to do with every phase of the biological or life activity of bacteria.

Size of Bacteria.—Bacteria vary greatly in size. This is true not only of the cells of different species but among individual cells of the same species. Bacteria are so small that a special unit of measurement is employed. The unit is one-thousandth part of a millimeter (1/25,000 inch), called a micromillimeter or micron. The Greek letter μ (pronounced "mōō") is the symbol for this unit. Among the forms of bacteria which we are accustomed to see in the laboratory, the spherical forms average about ½ to 1 micron in diameter, and the rods and spirals have about the same diameter, with a length of from 2 to 4 microns (0.5μ to 1.0μ by 2μ to 4μ). Among all bacteria, the range is from the ultra-microscopic to about 60μ in length (*e.g.*, B. butschlii). In looking at stained specimens of bacteria, they appear, because of their extreme smallness and the monocular microscope usually used, to be flat. This, of course, is not the case. Bacteria are like minute rods or sticks, and have three dimensions.

Structure of the Bacterial Cell.—The form of the minute structure of the bacterial cell is hard to determine. A stained bacterium presents the appearance of a finely granular or almost homogeneous cell. By the use of different kinds of stains, by subjecting the cell to solutions of great densities, or osmotic pressures, and by *analogous inference*, it is possible to draw a diagrammatic picture of what the investigator *thinks* is the structure of the typical bacterial cell.

Capsule.—The protoplasm of the individual organism is surrounded by an outer margin called the "capsule." There has always been considerable doubt as to whether or not all bacteria possess capsules. Certainly, in some species, this outer layer of the cell is so thin that it cannot be demonstrated. In other species, it may be demonstrable only under special conditions of growth, such as is the case of the Pneumococcus isolated from fresh sputum. In still other cases, the capsule may be many times the size of the cell and by absorbing an enormous amount of water become gelatinoid, causing the individual cells to adhere, and forming a jelly-like mass incorrectly termed zoöglea, or animal glue (*e.g.*, Leuconostoc). A good example of this phenomenon is that of the so-called "mother" of vinegar.

The capsules of different species of bacteria differ not only in staining qualities, in size, and in physical structure, but also in chemical composition.

The capsules of the Friedlander bacillus and of Rhizobium radicicola consist of protein-free polysaccharides of galactose and dextrose, respectively. Those of Bacillus anthracis consist of a glycoprotein—a substance resembling mucin.

There is every reason to believe that some relationship exists between capsule formation and virulence. In cases of pneumococcus infection, the more severe the disease, the greater the likelihood of isolation of a capsulated strain of the Pneumococcus. This observation has also been made in infections with Bacillus anthracis and the Streptococci. Moreover, if the virulence of a strain of Pneumococcus be increased by passage through mice, it will quite frequently be noted that as virulence increases, the capsules become more easily demonstrable. The opposite effect is also apparent. When a freshly isolated capsulated strain is grown on artificial culture media, loss of virulence and loss of capusles take place at the same time. It would seem that capsules form a protective layer to the bacterium, guarding the organism against the action of the animal-body cells and blood fluids.

Some observers, though in a minority, still look upon capsules as the bacterial cell wall, corresponding to the cellulose of the plant cell.

Cell Membrane.—Although a definite cell wall has been demonstrated in the case of only a few bacteria, it is generally considered that inside the capsule there is a delicate membrane which forms a periphery or envelope for the cell protoplasm. When bacteria are plasmolysed by being placed in a strong salt solution, the cell contents are condensed and drawn away from the wall. Again, during the process of sporulation the cell contents retract from the wall. Finally, the appearance of "shadow forms" in old cultures seem to represent the cell wall remaining after the protoplasm has disappeared.

That the cell wall and capsule of bacteria are not the same is indicated by a difference in chemical structure. The cell membrane is highly nitrogenous, whereas the capsule is more nearly carbohydrate. The cell walls also preserve the individuality of bacteria held together by their capsules (*e.g.*, Streptococci).

The supposed protoplasmic nature of the cell membrane has led some bacteriologists to the opinion that this structure represents cytoplasm or the ectoplasmic zone in the morphology of the bacterial cell.

The so-called "cell membrane" may, therefore, be one of three things: it may be the capsule in the case of what we call "noncapsulated forms," it may be a distinct part of the cell morphology, or it may be the cytoplasmic layer of the cell protoplasm.

Cell Protoplasm.—The endoplasm, or cell substance, of the bacterium is the living part of the cell. Chemically, like the protoplasm of all other living cells, it is composed of proteins, complex combinations of hydrogen, carbon, oxygen, and nitrogen together with varying but small amounts of many other elements (see p. 3). The physical structure of the bacterial protoplasm presents problems on the solution of which bacteriologists cannot agree.

Nucleus.—The main controversy regarding the nature of bacterial protoplasm is about what constitutes the karyoplasm or nucleus. Under ordinary staining methods, the bacterial cell presents a homogeneous appearance in which *no* denser or more readily stainable portion, such as is found in other animal or vegetable cells, can be demonstrated. This fact has led to two widely divergent viewpoints, one, that bacteria are composed entirely of cytoplasm without any nucleus, and the other that, except for a thin layer of cell membrane, the cell consists entirely of karyoplasm. The latter view, because of the fact that the bacterium is readily stained by basic or nuclear stains, and also because biologists have come to accept the nucleus of the cell as essential in reproduction, is more generally accepted.

Still another idea is that the nuclear material of the bacterial cell, instead of forming a well-defined morphological unit, is scattered throughout the cytoplasm, either as a network of chromatin or as innumerable granules. Such a structure would account for the staining qualities and also for the rapidity of reproduction.

Paravincini[1] in 1918 obtained, by means of an iron hematoxylin stain, pictures showing bodies which he recognized as nuclei quite distinct from the chromatin granules.

Since then, other workers have claimed that a differentiation can be made between the cytoplasm and karyoplasm of the bacterial cell-substance. Churchman[2] has recently shown that Bacillus anthracis possesses a morphological nucleus which may be demonstrated by staining methods. Up to the present time no one has been able to demonstrate nuclear division, such as karyokinesis or mitotic cell division in all stages.[3]

Other Cell Constituents.—A large variety of organic and inorganic substances are found in the bacterial protoplasm. These may be in the form of globules or granules, or they may be crystalline in form.

Most species of the sulphur bacteria, Thiobacteriales, contain globules of free sulphur. The family Chlamydobacteriales usually contain inorganic iron compounds in the form of granules.

Mycobacterium tuberculosis contains within the cell protoplasm numerous droplets of a waxy substance, which, especially in older cultures, often unite to form large drops, occupying a considerable portion of the cell. This is the substance which gives the tuberculosis germs and those of a few other species their "acid-fast" characteristics.

Corynebacterium diphtheriæ contains readily stainable bodies called "Babes-Ernst granules" or "metachromatic granules." In some strains, they are located at the ends of the cell (polar granules); in others they are in rows across the cell at the short axis, and in still others, in irregular groups scattered throughout the cell. Considerable discussion has taken place as to the nature of these metachromatic granules. Formerly, they were considered as morphological nuclei. Later, this idea was given up, but it was thought that they had some bearing on reproduction.

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[1] "Zur Frage des Zellkernes der Bakterien," *Centralblatt für Bakteriologie, Parasitenkunde, und Infektionskrankheiten,* abt. 2, bd. 48, pp. 337–340, 1918.

[2] Churchman, J. W., *Proceedings of the Society of Experimental Biology and Medicine,* 24, p. 737, 1927.

[3] Mencl and Guilliermond have independently described different stages of mitosis.

At present, the generally accepted idea is that these granules are simply collections of reserve food material. Chemically, they are nitrogenous in nature. They have been grouped under the term "volutin."

Bodies known as Much granules are present in the protoplasm of Mycobacterium tuberculosis and other bacteria. Their significance is uncertain. They may represent a filter-passing stage of the bacillus (regenerative bodies), they may be gonidia (not the bodies called spores by bacteriologists), they may be a sort of protective layer surrounding diffused nuclear material, or they may simply be degenerative bodies.

A number of bacteria contain coloring matter in their walls or inside the protoplasm. Many bacteria contain a substance which imparts to them an ability to retain Gram's stain.

Organs of Motility.—Many, but not all, species of bacteria have the power of motion. This ability is due to the possession of extremely delicate and fragile lashes called "flagella" (singular form, flagellum).

The true nature of flagella is still under discussion. Some bacteriologists believe that they are simply projections of the cell capsule. Others claim that they are formed from the ectoplasm or outer layer of cell substance. Still others look upon these structures as extrusions of true protoplasm, together with elastic fibers corresponding closely to the organs of locomotion of some of the Protozoa. By immunological studies, it is clear that flagella possess antigenic properties which differ from those of the cell protoplasm. On the other hand, they can only be stained by true protoplasmic dyes.

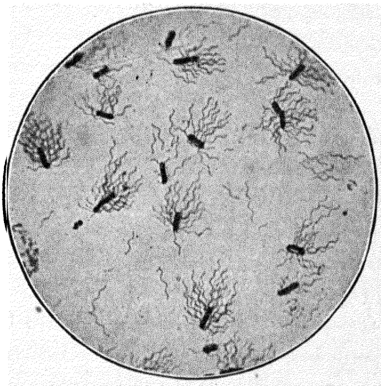

FIG. 9.—Flagella. Proteus vulgaris. (*Lutman.*)

Structurally, flagella are quite long, often being three or four times the length of the cell, and they are extremely slender. The diameter has been reckoned at less than 0.02μ. Motility is furnished by an undulating movement of the flagellum.

Flagella cannot be seen by ordinary microscopic methods in unstained specimens, and can only be demonstrated by special staining procedures.

The number and position of flagella are constant among individuals of the same species but differ in different species. Thus Vibrio comma (the spirillum of Asiatic cholera) has a single flagellum at one end of the cell. Pseudomonas aëruginosa (Bacillus pyocyaneus) and other rods of the family Bacteriaceæ move by means of a tuft or bundle of flagella at one end of the cell. Many species of the genus Spirillum have a tuft of flagella at each end of the cell (*e.g.*, Spirillum rubrum). Finally, a large number of rod-shaped organisms (*e.g.*, Eberthella typhi) are completely surrounded by flagella.

A classification of bacteria based on the number and arrangement of their flagella is sometimes used:

- Gymnobacteria or Atrichia, possessing no flagella
- Trichobacteria, possessing flagella
  - Monotrichia, possessing a single polar flagellum
  - Lophotrichia, possessing a tuft of polar flagella
  - Amphitrichia, possessing tufts of flagella at both poles
  - Peritrichia, flagella around the entire periphery of the cell.

The rate of motion by a bacterium does not seem to depend on the number of flagella present. Some of the organisms possessing a single polar flagellum move more rapidly than those in which flagella are peritrichic. The rate of movement of Vibrio comma has been calculated at about 18 centimeters per hour, that of the Eberthella typhi at only 4 millimeters per hour. It is necessary to realize that these speeds are magnified approximately 1,000 times when watching the bacteria move under the microscope.

The observation that the number and size of flagella have no bearing on rapidity of motion has led to the idea that these organs play some other, even though minor, rôle in the physiological economy of bacteria. It has been suggested that they may have something to do with food absorption.

Among the "higher" orders of bacteria, motility may be due at times to other means than flagella. Some of the members of the order Spirochætales have contractile ridges or "crista" running lengthwise along the cell, which, together with a flexuous body, give them an undulating motion. Others, like the Leptospira icteroides, the germ of yellow fever, have a rotary spinning motion which can be translated into a forward or backward movement.

Motility among the Eubacteriales is generally considered to be confined to the rod and spiral forms, although Migula has photographs of spheres showing flagella, and David Ellis claims that, under certain circumstances, all cocci are motile.

With the recent conception of bacterial dissociation, the question of motility takes on a new significance or lack of significance. It may be that the possession of flagella by an organism simply represents a stage in the cyclic development of that organism.

Brownian Motion.—When bacteria are examined in liquid suspensions for motility, it is necessary to take into account two entirely unrelated factors which may lead to false interpretations. The first of these is the flow of the liquid in which the bacteria are suspended. This is usually caused by a slow evaporation of the liquid from one side of the cell in which the suspension is being examined. In this case, all the bacteria move in the same direction. The movement is not due to the bacteria themselves but is analogous to that of a number of logs carried by the flow of a river.

The second factor is also due to forces acting upon the bacteria. When immotile bacteria are suspended in water, they appear under the microscope to be incessantly vibrating, with sometimes a slow, and sometimes an astonishingly rapid motion, yet never changing to any extent their relative positions. Anyone who has witnessed a game of pushball between two evenly matched teams can easily draw a mental picture of the motion of these minute particles of matter. From a given spot the ball is pushed to the right, back again, forward a little, back again, to the left, back again, and so on as either side gains a slight temporary advantage. In fact, if the ball alone were seen, and not the players who are forcing its change in position, it could easily be imagined to be an enormously enlarged moving picture of a bacterium in a liquid medium, as seen under the microscope.

The explanation of the fact was understood as a physical phenomenon long before very much about bacteria was known. In 1827, Robert Brown, an English botanist, showed that the cause of this peculiar motion was in the liquid itself. According to the kinetic theory of atoms established upon Brown's observations, the molecules of water are in perpetual movement in all directions. When a number of these molecules moving in the same direction strike a bacterium, there is sufficient force to drive the bacterium in their direction and to continue so to drive it until it is acted upon by a sufficient force in another direction. And so a bacterium is buffeted to and fro, up and down in the liquid. The same phenomenon may be demonstrated with pollen grains, with which Brown's original observations were made, with finely powdered carmine, or any other minute, insoluble particles suspended in water. As would be expected, the incessant movements become less vigorous the larger the particles. Even rapidly motile bacteria exhibit Brownian motion, which gives a zigzag character to their progression.

Spores.—One of the most interesting phenomena observed in the study of bacterial morphology is that called "sporulation." The word "spore" literally means "a seed," and this term accurately expressed the idea of the early bacteriologists as to the nature of the bodies observed in bacteria. Pasteur believed that spores (which he called *noyaux*) were reproductive bodies *i.e.*, seeds. The term "germ," which we now use as a popular synonym for bacterium, originally conveyed the idea that bacteria themselves were simply reproductive bodies of higher plants. Later, when these microscopic plants were looked upon as phylogenetically independent species, the term "germ" became synonymous with "spore." The two words, one Latin and the other Greek, have the same meaning. For example, Trouessart[1] in describing the anthrax bacillus speaks of "nuclei" observed in old cells, and then remarks " . . . these nuclei are the spores or germs of the microbes, which germinate when placed in the infusion, become elongated, and reproduce fresh bacilli."

Most bacteriologists today look upon spore formation as a resting stage of the organism somewhat analogous to the encystment of amœba and other protozoa, having no relation to reproduction. Various reasons have been suggested to explain why bacteria sporulate, and all have to do with external conditions. Buchner thought that the exhaustion of the food supply caused the change. Klein, believing that sporulation was confined to bacteria which cannot live in the presence of free oxygen, thought that exposure to air was the sole cause of it. Temperature seems to bear some relation to the phenomenon of sporulation, since it takes place at the temperature of most rapid

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[1] Trouessart, E. L., "Microbes, Ferments, and Moulds," p. 135, New York, 1886.

vegetative growth. Most species, like Bacillus anthracis, entirely lose their power of forming spores at abnormal temperatures.

If all the factors which seem to favor sporulation are analyzed it is generally found that they are the same factors which, in young cultures, favor vegetative growth. If sporulation is due to external or environmental factors, and not to physiological or internal stimuli, such as changes in the life cycle of the organism, it is highly probable that the principal factor in sporulation is the presence in the medium of bacterial excretions. It is readily observed that in a given culture a majority of the individual bacteria will sporulate at approximately the same time. This can be explained by the fact that when the conditions are most favorable for rapid vegetation, the greatest amount of harmful matter will be excreted. When the saturation of these excretions reaches a certain point, all the organisms in the culture are affected at the same time.

In the light of observations on the life cycle of bacteria, it is difficult to say that external conditions are solely responsible for sporulation. They may present the necessary stimulus, it is true, since it is possible by frequent transplantings into fresh media entirely to suppress spore formation. But the removal of these stimuli may be simply an artificial means of delaying temporarily what is a natural step in the cyclic development of a bacterial culture.

If a culture of bacteria is examined under the microscope at the time of sporulation, it is easy to observe the phenomenon. While an even progression of events takes place, it is possible to note certain points in the gradual development of the spore to illustrate the sequence. Naturally, the more points taken, the more complete is the picture.

When a bacterium starts to sporulate, the homogeneous protoplasm takes on a more granular appearance. The granules become larger and gradually collect toward one part of the cell. This location may be at one end of the cell (polar spore), partway between the end and the center (eccentric spore), or at the center of the cell (central spore). It is always the same in any particular species. As the granules coalesce, they become more refractile to light than the rest of the cell. Eventually, they form a highly refractile body which is surrounded by a dense membrane formed by a substance excreted by the spore material. This spore wall is called the "exosporium." The shape of the spore is spherical or elliptical. It may or may not be larger than the diameter of the vegetative cell. If elliptical, its long axis may be parallel or at right angles to the long axis of the original cell. As in the case of the position of the spore in the cell, these characteristics are constant for any particular species.

For a time after the appearance of a well-defined spore, the original cell may be motile. This would indicate that not all the protoplasm of the original cell is used to form the spore, for otherwise the flagella would not be functional. It would seem that the process is one of simple contraction of part of the cell into a dense mass, with a squeezing out of most of the water in the original cell substance. Each bacterium forms but one spore. A cell containing a spore is spoken of as a "sporangium."[1]

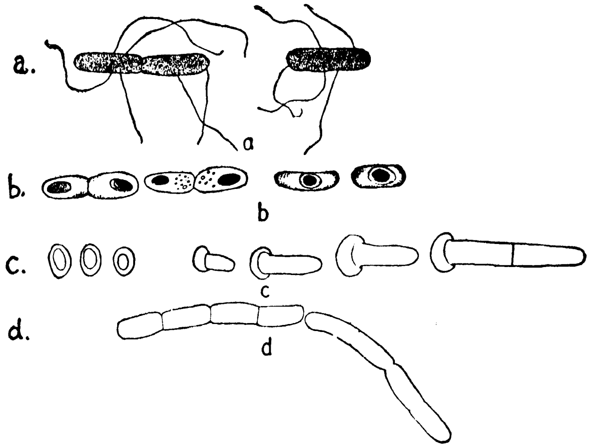

Fig. 10.—Spore formation and germination. B. vulgatus. (*Lutman.*)

The next step is the liberation of the spore from the original cell by the disintegration of the bacterial cell wall. Often practically complete cell walls., from which a spore has been liberated are found. These forms are only faintly stainable and are known as "ghost" or "shadow" forms.

Bacterial spores are highly refractile, dense masses of protoplasm, with definite shapes according to the species. They possess a much greater resistance to heat, dessication, staining, and all other external factors than do the cells from which they

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[1] This term was given prior to the present understanding regarding bacterial spores. In all higher forms of life, the sporangium holds reproductive bodies.

were formed. Few bacteria are able to resist the temperature of 80°C., but spores are unharmed at that temperature. Spores have been known to resist the temperature of boiling water for three hours. It is to kill off all spores in culture media that high-pressure steam sterilization is resorted to. Spores have been found alive after remaining in dried cultures for more than twenty years.

When transplanted to a suitable medium and placed under proper conditions of temperature, the spores germinate and again become vegetative cells. This is accomplished by the absorption of water and the consequent swelling of the protoplasm to the normal vegetative size. The spore wall ruptures, and a new cell membrane is formed. In the case of elliptical spores, the position of the rupture seems to be constant with species. Thus, the new vegetative cell of Bacillus subtilis splits the spore wall at the side and grows out at right angles to the long diameter of the spore. Clostridium butyricum, on the other hand emerges from the end of the spore. For a time after germination, the old spore wall is carried as a cap by the new cell. In some species, the spore wall may disintegrate as the new cell is formed. It is not believed that the spore wall ever becomes the new vegetative cell membrane. Vegetative cells always assume the same form on germination as they had before sporulation.

The ability to form spores is by no means common among bacterial species, although most of the common soil bacteria do so. With possibly a very few exceptions, sporulation is confined to the rod forms. On the basis of the present classification all the spore-forming rods of the order Eubacteriales have been grouped in the family Bacillaceæ.

Fortunately for man, spore formation among disease-producing bacteria is very rare. Clostridium tetani, the bacterium that causes lockjaw, and Bacillus anthracis, the cause of anthrax, are the principal spore-forming pathogens. The ubiquitous soil spore formers, however, are extremely important to man because of their constant presence in foodstuffs. Enormous expense in equipment and personnel is necessary in the canning industries to be sure that all spores are killed. Otherwise, in time, the spores would germinate, causing spoilage.

Arthrospores.—Since there is a considerable variation in resistance to unfavorable conditions among individuals of the same species, some bacteriologists have been led to the conclusion that there is a resting stage for bacteria not associated with endogeneous sporulation. These individuals were called, by Heuppe, "arthrospores" (joint spores). They are always spheroidal in form, and form the somewhat larger links in a chain of bacteria. Descriptions of "arthrosporulation" are generally included in discussions of the Streptococci. These so-called arthrospores are killed at a temperature below 80°C. Most bacteriologists agree that these forms are without significance as resting stages.

Conidia.—Some of the filamentous bacteria reproduce by means of special cells known as "chlamydospores," "swarm cells," or "conidia." These forms are similar to the ascospores of molds, and while they are somewhat resistant to external conditions, their principal function is that of reproduction. They will be discussed further under that head.

CHAPTER V

PHYSIOLOGY OF BACTERIA

As bacteria are living organisms, they demonstrate all of the characteristics or properties of living matter. The cell structure has been discussed from the morphological viewpoint, and now the study of the physiological or biochemical evidences of life, as exemplified by these single-celled plants, will be pursued.

Protoplasm, it will be remembered, is a physical concept. Chemically, protoplasm is a suspension of many different proteins in water. When a living organism is thought of, all parts of the body are likely to be conceived of as possessing life. This, however, is not correct. Many parts of the human body—the outer layers of the skin; the hair; the enamel of the teeth; the fingernails, etc.—are made up of substance which is not living. So it is with the bacterial cell. Metachromatic granules, volutin particles, waxy cell membrane, chitinous capsules, and other portions of the cell are inanimate. The observer would therefore expect to find, and does find, non-protein substance in bacterial protoplasm.

Chemistry of Bacterial Protoplasm.—When masses of bacteria are analyzed chemically they are shown to consist of from 75 to 85 per cent water. The dry substance remaining yields on ignition anywhere from 2 to 30 per cent ash. This ash consists mainly of phosphates, sulphates and chlorides of potassium and calcium. The burnable, dry substance consists of organic compounds, mostly proteins, but also varying amounts of carbohydrates and fats.

In discussing the general chemical composition of these cells, it must be understood that great variations exist. Species differ from each other enormously, and even in the same species great irregularities in composition occur in both kinds and amounts, according to the age of the culture and the medium upon which it has been grown. Here again is an illustration of bacterial instability.

Proteins.—The protein content of the dry weight of the bacterial cell varies between 23 and 90 per cent. The proportionate amount varies with species, becoming lower in the case of the capsulated organisms and those containing a large amount of some other organic compound. Thus the tubercle bacillus with it's high lipoid content and the encapsulated diplococcus of pneumonia show a proportionately low protein content. The bacterial proteins are chiefly nucleoproteins together with some globulins and proteoses. Other nitrogenous substances may be present either as amorphous material or as formed bodies. the metachromatic granules of the diphtheria germ consist of a nitrogenous reserve substance called volutin and are made up of compounds of nucleic acid.

Carbohydrates.—Between 4 and 30 per cent of the dry weight of the bacterial cell consists of carbohydrates. The gums, including dextrans, levulans and cellulans, together with polysaccharides and the closely related nitrogenous substance called "chitin," present in capsulated forms, accounts for most of the carbohydrate content. Starch is frequently present in the form of granules, and sugars and alcohols are often present in small amounts. Unlike other vegetable forms, the bacterial cell very rarely contains cellulose, and when this carbohydrate is present it is there in small amounts.

Fats.—Fats[1] usually consisting of the fatty-acid esters of the higher alcohols (as cetyl alcohol) and known as waxes, are present in most bacterial cells. The amount is generally quite small, as low as one tenth of one per cent. In some organisms, however, like the tubercle bacillus, waxes represent as high as 40 per cent of the dry cell weight.

As the chemical nature of the bacterial cell varies, naturally the elementary composition cannot be stated in exact quantitative proportions. Mathews[2] gives the following, which is probably a fair average analysis:

	Percentage		Percentage
Nitrogen	16	Oxygen	22
Carbon.	50	Phosphorus	0.4
Hydrogen	7	Sulphur	0.3

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[1] By the term "fat" is usually meant a neutral fat consisting of the trihydric alcohol (glycerin) ester of a fatty acid, such as stearic. Fats composed of the esters of monohydric alcohols of high molecular weight are called "waxes."

[2] MATHEWS, H. P., "Physiological Chemistry," p. 109, New York, 1922.

Metabolism.—As bacteria are living things made up of the chemical elements carbon, nitrogen, oxygen, hydrogen, and smaller amounts of phosphorus, sulphur, potassium, calcium, and iron, they must be able to get these elements if life is to be sustained. The anabolic process of taking compounds containing the necessary elements and changing them into bacterial protoplasm is extremely complex, and involves both analysis and synthesis. Some phases of the process have been studied in observing the results of the action of extracellular bacterial enzymes. Other phases are intimately connected with the life of the cell, and are obscure. What we do understand is that bacteria are able to manufacture living substance from lifeless material.

Pabulum.—The substances which supply the necessary elements to bacteria are bacterial foods. Bacteria assimilate food by osmosis through the cell wall. To be available for bacteria, therefore, the pabulum must be in a liquid state. Food is used to manufacture protoplasm and to supply the energy necessary for the chemical syntheses involved. Energy is also dissipated by some bacteria in the form of motion. A few bacteria transform energy into light energy, and many forms give off appreciable heat during growth.

The substances which may constitute bacterial food range from very simple elements to the most complex organic compounds. A few genera of bacteria (of the family Nitrobacteriaceæ) can exist on carbon dioxide, nitrogen, phosphorus in the form of simple phosphates, and water, which supplies the hydrogen and some of the oxygen. As bacteria contain no chlorophyll, most of them are unable to assimilate atmospheric carbon dioxide, although some aërobic and facultative bacteria grow well in the absence of oxygen, when carbon dioxide is present.[1]

The higher plants absorb carbon dioxide, and through photosynthesis build up starch, which is later utilized in making tissue. Such plants are said to be "holophytic." In regard to nutrition, it has long been supposed that a sharp distinction should be drawn between the chlorophyll-bearing and the non-chlorophyll-bearing plants. The ability of the nitrifying bacteria[2] and others

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[1] Rockwell, G. E., "A Study of the Gaseous Requirements of Various Bacteria," *Journal of Infectious Diseases*, vol. 28, pp. 352–356, 1921.

[2] Winogradsky, *Annales de l'Institut Pasteur*, 1891.

described by Kaserer,[1] and by Beijerinck and Van Delden[2] to develop in very simple mineral salts without the presence of organic matter of any kind, indicates, as is brought out by Jordan,[3] that a complete synthesis of organic matter may be effected by certain bacteria independently of the presence of pigment and the action of the sun's rays. For such bacteria, it is evident that some form of energy is necessary and this is obtained from the oxidation of inorganic ammonium salts and carbon monoxide. These bacteria lie at one end of the scale. At the other end lie the hemophilic bacteria, which require hemoglobin in their food (genus Hemophilus) and the obligate parasitic bacteria (as Neisseria gonorrhœæ) which will not develop anywhere but in the living body.

Bacteriological literature contains a great many descriptions of "special media" for the growth of particular bacteria. When it is considered how pathogenic bacteria find conditions most suitable in definite parts of the body (the diphtheria germ in the throat, the gonococcus in the urethra and the eyes, the typhoid bacillus in the intestinal tract, etc.), it is easily understood how they may respond by growth on selective media. Some special media have been prepared in which large groups of bacteria show better growth than in ordinary culture media. Tryptic digests of meat muscle and casein have been found especially favorable for the growth of some pathogenic bacteria. There seems to be little doubt now that the value of these particular media depends on the amount of amino acids present. The same is true of a commonly used prewar peptone. Recently, it has been demonstrated that the value of all media is markedly enhanced by the presence of the growth-producing, water-soluble vitamin B. Many bacteria themselves synthesize this accessory.

Food Requirements.—Bacteria may be grouped according to their food requirements into three classes. The first are those bacteria which are able to utilize the simplest kinds of food, such as the bacteria which "fix" free nitrogen (Azotobacter), called the *prototrophic bacteria.* The prototrophic bacteria are able

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[1] Kaserer, *Centralblatt für Bakterien, Parasitenkunde, und Infekionskrankheiten,* abt. 2, pp. 16, 681, 1906.

[2] Beijerinck and Van Delden, "Über eine farblose Bakterie, deren Kohlenstoffnahrung aus der atmospharischen Luft herruhrt." *Centralblatt für Bakterien, Parasitenkunde, und Infekionskrankheiten,* abt. 2, pp. 10, 33, 1903.

[3] Jordan, "General Bacteriology," 8th ed., p. 85, Philadelphia, 1924.

to manufacture their own food from inorganic substances. The chemical processes which go on in the cells of these organisms are, as a whole, synthetic; that is, the final products formed in their metabolism are more complex than the substances used as food. This group includes the whole family Nitrobacteriaceæ.

Most bacteria, however, are broadly katabolic in their physiological functions. They require organic food, either in the form of dead animal or vegetable matter, or in the form of living substance. The largest of these groups comprises the second main class, the *metatrophic bacteria,* which includes the bacteria causing fermentation, decay, and the decomposition of fatty substances. The metatrophic bacteria are able to assimilate different kinds of foods, and they accommodate themselves to different conditions according to the nature of their food supply. In this group amino acids and the simpler proteoses and polypeptids serve as the most common source of nitrogen. Here again, the scale is broad. Some can use a simple amino acid, such as asparagin, while others need complex proteins.

The third class is known as *paratrophic.* These bacteria prefer living food, and, while numerically they are the smallest group of bacteria, they are of the greatest importance, since they include most of the pathogenic, or disease-producing, bacteria. In the study of the pathogenic bacteria in the laboratory, it is necessary to furnish them with food which is as nearly as possible like that which they obtain from a living host.

A slightly different grouping of bacteria, according to their food and energy requirements, is given by Waksman:[1]

> As a major division, the bacteria may be separated into two large groups: (1) autotrophic, and (2) heterotrophic forms. Living organisms that require for their nutrition substances which have been built up by other organisms are called heterotrophic. The heterotrophic saprophytic bacteria consume, for their energy and for the building up of their protoplasm, the organic compounds of plant and animal bodies. Organisms like the green plants and certain bacteria that can thrive on purely inorganic substances and obtain their carbon from the carbon dioxide of the atmosphere are called autotrophic. But while the green plants derive their energy photosynthetically, the autotrophic bacteria derive their energy from the oxidation of purely inorganic substances, or chemosynthetically.

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[1] WAKSMAN, SELMAN A., "Principles of Soil Microbiology," p. 4, Baltimore, 1927.

Amount of Food Required.—The amount of food required by bacteria differs greatly with the species. When the minute size of the organism is considered, it can be realized that, all other conditions being favorable, starvation is practically impossible. Some studies have been made on the effect of depriving bacteria of food. Braun states that when Proteus vulgaris is grown on agar poor in nutriment, the rods lose their motility and the cells become much shorter, resembling the Coccaceæ. Eberthella typhi, under similar conditions, lose their motility and show atypical serological relationships. Deficient nourishment affects both the endoplasm and the ectoplasm of the bacterial cell.

By-products of Metabolism.—The metabolism of bacteria plays an essential part in the life of all other organisms. Were it not for the metatrophic bacteria—especially the saprogenic or protein-splitting bacteria—the earth long ago would have been covered to such a depth with dead matter that no living thing could reach it for the necessities of life. Were it not for the prototrophic bacteria, the supply of fixed nitrogen which is essential to life would have long since ceased to be available. The study of these bacteria and the mechanism of their action will be taken up in detail later.

The final katabolic or excretory products of bacterial life are usually simple chemical substances. In the process of respiration, bacteria (with the exception of the anaërobes which obtain their oxygen from organic compounds, mainly sugars) like other plants take in oxygen and give off carbon dioxide. Other excretory products are ammonia, free nitrogen, hydrogen sulphide, methane, hydrogen, and water.

In the process of metabolism, bacteria may produce various substances by which a particular species may be easily recognized. For instance, certain bacteria produce pigments, others are characterized by phosphorescence or light production, others by their own growth are able to cause temperature changes in the medium in which they are growing.

Thermogenic Bacteria.—As just remarked, heat is generated during the metabolism of certain bacteria. These organisms are called "thermogenic bacteria." This phenomenon usually occurs among the thermophilic bacteria (see p. 97) but not all the thermophilic bacteria are thermogenic. The thermogenic bacteria play an important part in the rise in temperature which occurs in hay and cotton waste. Bacteria are not able to cause spontaneous combustion, however, as the ignition point is far above their thermal death point. Free oxygen is essential to the growth of the thermogenic bacteria.

Photogenic Bacteria.—Certain species of marine bacteria, especially those which live on decaying fish, are able in the process of respiration, or combustion of food material, to produce phosphorescence. These bacteria are called "photogenic bacteria." The production of light seems to have no importance physiologically, but is simply due to the oxidation of substances produced in the life process of these organisms. Gerretsen[1] describes the process as the oxidation (by means of an enzyme) of an intracellular phosphorescent substance, photogen, produced in the bacteria by the action of an enzyme (photogenase).

Aromatic Bacteria.—Other substances produced by the metabolism of bacteria, such as indol, skatol, and mercaptan, usually grouped together as "aromatic substances," are capable of chemical analysis. Many unpleasant and disagreeable odors are given off by these bacteria, which are known as "aromatic bacteria." These odors are from the bacteria themselves and their metabolic products, and are not to be confused with the odors of fermentation and putrefaction which are due to the fermenting or decaying material acted upon.

Chromogenic Bacteria.—A few species of bacteria are able to produce coloring matter while growing. Almost all the colors of the solar spectrum are represented in the products of different species of bacteria. These bacteria, known as the "chromogenic bacteria," may be grouped in various ways.

First, they may be divided into three groups, according to the location of the pigment granules in the bacterial cell. These are: (1) the chromoparous bacteria. These organisms excrete the coloring substance, which is scattered throughout the medium upon which the bacteria are growing. (2) The chromatophorous bacteria. In these bacteria, the pigment granules are combined with the protoplasm of the cell and, probably, in some species, play a part in the anabolism of the cell. (3) Where the pigment granules are contained in the cell wall of the bacterium, the organism is known as a "parachromatophorous bacterium." Except in the relatively unusual case of the

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[1] Gerretsen, "Über die Ursachen des Leuchters der Leucht Bakterien," *Centralblatt für Bakteriologie, Parasitenkunde, und Infektionskrankheiten*, abt. 2, vol. 52, pp. 353–373, Jena, 1920.

chromatophorous bacteria, pigment production is without significance. In this case, however, the pigment aids the bacteria in utilizing carbon dioxide from the atmosphere in somewhat the same way as chlorophyll aids the green plants. Light, oxygen, and high temperature affect chromogenic bacteria in different ways, some bacteria producing pigments under conditions that absolutely preclude pigment production by others. Except in very rare cases, oxygen is necessary for the production of pigments.

Second, Migula divides bacterial pigments into three classes, according to their solubility: first, the pigments produced by Pseudomonas aëruginosa (B. pyocyaneus), Pseudomonas fluorescens, etc. are soluble in water; second, those produced by Serratia marcescens and most other chromogenic bacteria are soluble in alcohol but not in water; third, a few pigments, such as that of Micrococcus citreus, and Micrococcus flavus, are insoluble in either alcohol or water.

A third method of grouping the bacterial pigments is on the basis of the color, regardless of its chemical composition, solubility, or part of the cell where it is produced. This would seem to be the least scientific method possible, yet it is the one which is used whenever pigment production is recognized as a characteristic on which classification is based. For example, Bergey groups the genera of the tribe Chromobacterieæ on the basis of the *color* (red, yellow, violet, and green) produced by the rods.

The best classification of pigments is that given by Buchanan and Fulmer[1] in which location of the pigment, color and solubility are all used. The following diagram briefly summarizes their classification.

A. Pigments not producing a color change in the medium. They may be diffused through the protoplasm, incorporated in granular globules, in the cell wall, or in capsules.
  1. Pigments soluble in chloroform:
    a. Soluble in alcohol (Examples: the red pigment of the genus Rhodococcus, the yellow pigment of Staphylococcus aureus, the red pigment of Serratia marcescens)

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[1] Buchanan, R. E., and E. I. Fulmer, "Physiology and Biochemistry of Bacteria," vol. 1, pp. 122–135, Baltimore, 1928.

- 2. Pigments insoluble in chloroform:
  - *a.* Soluble in water
  - *b.* Insoluble in water
    - (1) Soluble in alcohol
    - (2) Insoluble in alcohol (Example: the yellow pigment of Micrococcus flavus)
- *B.* Pigments producing a color change in the medium
  - 1. Pigments soluble in water:
    - *a.* Soluble in chloroform (Example: the blue pigment of Pseudomonas aeruginosa)
    - *b.* Insoluble in chloroform (Example: the green pigment of Pseudomonas aeruginosa)
  - 2. Pigments insoluble in water: (Example: the brown pigment produced by Azotobacter chroöcoccum)

Enzymes.—The chemical changes which bacteria bring about are due to substances produced by the cell. The rapidity with which putrefaction takes place indicates that the process, though a result of bacterial action, cannot be due primarily to the cells themselves. The process is carried out by substances called "enzymes," which are produced by the bacteria, and are able to cause chemical changes in amounts far in excess of their own volumes. The essential concept of an enzyme is that of a catalytic agent, that is, a substance which hastens chemical change without itself entering into the reaction. The products formed often exert a germicidal action on the bacteria producing the enzyme, and also an unfavorable action on the enzyme itself. If, however, these products of change are removed, the action of a given amount of enzyme is practically unlimited.

Enzymes, in their relation to environmental factors, present a great similarity to protoplasm. Gautier[1] claimed that enzyme possess two fundamental properties of living substance: metabolism and propagation. The fact, however, that each enzyme is a single definite chemical substance which will react with other enzymes disproves this theory.

Various means of classifying enzymes have been proposed. Effront[2] and others have elaborated excellent ones. While all living things produce enzymes, these classifications will be

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[1] Gautier, A., "Les toxins microbiennes et animales," Paris Société d'editions scientifiques.

[2] Effront, "Enzymes and Their Application," translated by Prescott, 1909.

limited to the enzymes produced by bacteria. Bacterial enzymes may be divided into five classes, according to the types of substances upon which they act. These are: the oxidizing and reducing enzymes of the nitrifying and denitrifying bacteria in the soil; the amylolytic enzymes of the zymogenic bacteria, which cause the fermentation of carbohydrates; the proteolytic enzymes of the saprogenic bacteria, which cause putrefaction or decay; and the steatolytic (or lipolytic) enzymes which split fats into fatty acids and glycerine.

Enzymes are usually autolytic, that is, they are poisonous to the bacteria which produce them. Enzymes are said to be "isolytic" when they are poisonous to other bacteria. This fact is of great importance in the destruction of pathogenic bacteria in water and sewage, and is known as "antagonism" of bacteria. At other times, the enzymes or the results of their action are very beneficial to other bacteria. Many examples of this can be given. An anaërobe grows well if mixed with a strong aërobe under diminished oxygen tension. When mixed with Staphylococci, the tetanus organism is more apt to cause lockjaw. In these cases, only one of the species is benefited. The mutual dependence of two organisms, known as "symbiosis," is rare.

Toxins.—Toxins are poisonous substances closely allied to enzymes. They are the result of the metabolism of the pathogenic bacteria. Toxins are divided into two classes, according to whether they are or are not diffused into the medium in which the bacteria are growing. A true toxin is a substance which, when injected into a living animal, stimulates the production of an antitoxin. These substances will be discussed more thoroughly later (Chap. XIV, Pathogenic Bacteria).

Irritability.—Bacteria respond by a direction of movement to external stimuli. Light, heat, oxygen, and other factors influence the movement of motile bacteria. Some chemicals attract bacteria and others repel them. This phenomenon of the attraction which certain substances have is known as "chemotaxis" (or "chemotrophism"). The alkalies and alkaline earths (magnesium, calcium, etc.) and most organic substances are chemotaxic, while alcohol and most of the inorganic acids (nitric, hydrochloric, sulphuric, etc.) produce a repellent effect or are negatively chemotaxic. This attraction or repulsion of bacteria by chemical agents is not to be considered as simply a chemical effect on the bacterial cell, but as a response to a stimulus (irritability) which is analogous to instinct in the higher organisms.[1] The phenomenon of chemotaxis may be demonstrated by immersing a capillary tube filled with a substance like urea, in solution, in a drop of water containing bacteria. As the solution of urea diffuses out of the tube, the bacteria collect in the tube. Chemotaxis is of great importance in the study of immunization against disease.

Growth of the Individual Cells.—Usage has given the term "growth" as applied to bacteria a different meaning than when applied to man or tree. In speaking of the growth of a man, increase in size, weight, height, etc. is understood. Among bacteriologists, the same term is applied, not to the individual, but to the mass growth or multiplication of bacteria in a culture. In order to avoid confusion, in speaking of the growth of the individual cell, its enlargement from daughter to adult will be what the writer has in mind. As in the case of all living things, every bacterium has a fairly definite size, and this size is the maximum for its species. As is the case with all living matter, increase in size of the bacterial cell occurs by intussusception through the building up of cell substance out of suitable pabulum.

Reproduction.—Among the bacteria, reproduction is usually asexual. Under favorable cultural conditions, the vegetative adult cell divides in two, and each half grows until it becomes an adult, whereupon the process is repeated. This method of reproduction has given bacteria the name of "Schizomycetes," or fission fungi. A bacterium about to divide becomes slightly larger than normal and, in the spherical forms, elongated, giving it an oval appearance. The cell protoplasm becomes aggregated at opposite ends of the cell and a circular constriction takes place midway between these accumulations, at right angles to the long axis of the bacterium. The constriction deepens and a thin, gelatinous membrane divides the cell in two. The gelatinous layer soon hardens and the two new cells separate, though sometimes the daughter cells may remain united for a considerable length of time, forming chains or masses of bacteria (*e.g.*, Streptococci). The time required for a bacterium to reach maturity and divide varies with the species. Under optimum conditions of

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[1] JENNINGS, H. S., "Behavior of the Lower Organisms," p. 39.

temperature and pabulum, a single cell of Bacillus subtilis will reach maturity and divide in 30 minutes. Vibrio comma (the bacterium which causes cholera) will reach maturity and divide in 20 minutes. Should Bacillus subtilis multiply unhindered for 24 hours, a single organism at the end of that time would have a progeny of about 300,000,000,000.

As the average length of these organisms is about 3.5μ and the average diameter about 0.6μ, each individual would weigh (assuming their specific gravity as 1) 0.000000000001 (1×10^{-12}) gram. Thus, the descendants of a single individual at the end of a day would weigh about an ounce, and at the end of a week would form a mass equal in bulk to the entire earth. Of course, uninterrupted growth at this rate is absolutely impossible. Aside from the fact that the food supply would soon give out, the excretory products of the cells themselves inhibit multiplication beyond very small limits.

Complex Phases of Reproduction.—The idea that bacterial reproduction is not a simple mechanism is not new. The concepts of pleomorphism, polymorphism, and sporulation all carried with them suggestions of reproductive processes. Recently, bacteriologists have again devoted their energies to this subject, especially with the thought of explaining changes in function and form, adaptations and other biological problems associated with reproduction.

Hort among others has pointed out that even such "stable" organisms as the typhoid bacillus at times reproduce by budding, by branching, and by a process quite similar to the symplasm and reproductive body stages described by Lohnis.

Even binary fission cannot be as simple as the oft-used term, "simple fission," implies. If it were just a splitting, like breaking a match stick in half, how could the fact that each daughter cell gets exactly the same inheritable characters from the parent cell be accounted for?

Philip Hadley[1] in 1928 said:

> What is the deeper meaning of these phenomena concerned with the separation of bacterial cultures into distinct components whose nature and behavior we have now briefly reviewed? It can mean only one thing: that those living cells that we have commonly regarded in past

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[1] HADLEY, PHILIP, "The Dissociative Aspects of Bacterial Behaviour," in "The Newer Knowledge of Bacteriology and Immunology," edited by Jordan and Falk, p. 100, Chicago, 1928.

years as among the simplest of plant forms, and characterized by a correspondingly simple reproductive apparatus, possess in reality a highly complex genetic mechanism, which enables them to reveal, in cultures, pictures of morphological and physiological diversity with which our old and limited notions of "reproduction by simple fission" are utterly unable to deal. Although we may not yet be justified in accepting Enderlein's view of actual sexual reproduction among the bacteria, we must accept the fact that the nuclear equipment and reproductive behavior of bacteria are highly complicated matters. We can no longer doubt that the hereditary mechanism in bacterial cells makes provision for amphimixis, so long denied to these forms; nor can we hesitate in accepting phenomena of gonidia formation, zygospore formation, and perhaps a kind of budding, as common methods of bacterial reproduction. In all of these matters bacteriologists as a class have combined in denying the existence of things that they have not been willing to take the trouble to search for.

Gonidia.—Among the specialized or differentiated forms of bacteria, such as the Chlamydobacteriales, reproduction by a process similar to sporulation by the molds has long been recognized. Within the last few years, different workers have described this phenomenon among the undifferentiated forms of Eubacteriales, such as the typhoid bacillus. Cohn described two forms of bacterial gonidia: first, rather large bodies formed in the filament (macrogonidia); and second, minute bodies (microgonidia). The latter are frequently so small that they can pass through Berkefeldt filters. Both macrogonidia and microgonidia may develop flagella and become "swarm spores." They are looked upon as true spores or reproductive bodies of bacteria.

FIG. 11.—Abnormal reproduction in Escherichia coli. (*Lutman, after Mellon.*)

Zygospores.—A number of different observers have described a method of cell division among bacteria which resembles gametic reproduction among the algæ and some protozoa. Among the filamentous forms, the walls between contiguous cells of the same filament disappear and the cell protoplasm takes on a granular appearance. These granules are the gametes, which ultimately unite in pairs forming a zygote or zygospore. The zygospore is a resting stage of the organism, and is quite similar to the endospore.

Budding.—Some organisms, like the diphtheria bacillus, (Corynebacterium diphtheriæ) demonstrate a form of budding quite like that of the yeasts. Reasoning by analogy, it may be presumed that before the bud appears, the nucleus of the cell divides, and a portion of it travels to the periphery of the cell, where the bud forms.

Symplasm.—One of the most interesting observations upon the reproductive forms of bacteria is that made by Lohnis and Smith[1] which they describe as a symplasm or symplastic stage. The symplasm consists of the protoplasmic content of a number of bacteria from which the cell walls have been dissolved or disintegrated. Various writers have described this material (which is difficult to stain) as zoöglœa, as capsular stuff, as disintegrated or dead bacterial material, or simply as dirt, grease, or artifacts. According to Lohnis and some more recent observers, this material is living, and represents material out of which new morphological cells are organized. An interesting feature of the problem is the possibility of the existence of life without form. If the conclusions drawn are correct, there is here a biological analogy to the chemist's idea of the atom, and the biologist must give up his unit, the cell, and think of amorphous living substance.

Heredity.—As soon as one gets away from the simple fission idea of bacterial reproduction, and considers a nuclear involvement, he immediately sees the possibilities of mutations. The mechanism of bacterial heredity will have to remain obscure until at least some of the stages may be seen.

A British biologist, Stewart,[2] has recently pictured a phase in the life cycle of bacteria as resembling segregation (Mendelian) in higher forms.

> Shortly before vegetative growth stops, the second phase of the life cycle begins, in which a few out of the great number of bacteria in a colony (either on solid or in liquid nidus) go through segregation, auto-

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[1] Lohnis, F., and N. R. Smith, "Life Cycles of Bacteria," *Journal of Agricultural Research*, vol. VI, no. 18, p. 680, 1916.

[2] Stewart, F. H., "Segregation and Autogamy in Bacteria," p. 93, London, 1927.

> gamic conjugation and, under certain circumstances, variation. In spore-bearing races, the zygote forms the spore. Segregation in bacteria is the same as in the higher forms; in it, allelomorphic couples of the organism divide.
>
> We know little about the mechanism of autogamy, but it seems not unlikely that before segregation takes place each allelomorphic couple is represented in the "anterior" and "posterior" halves of the bacterium and that, after segregation, and if nothing disturbs them, the "right-hand" allelomorphs of one half of the body unite with the "left-hand" allelomorphs of the other . . . But if a definite external stimulus is at that moment bearing on a heterozygous bacterium, then, in the one pair (anterior and posterior) of allelomorphic couples, which is concerned with the stimulus, the comitant allelomorphs are dissipated (as primitive polar bodies), the recessives come together, and the bacterium varies.

Still other writers, notably Neisser, Massini, Bernhardt, and Weil have attempted to apply the DeVriesian idea of mutation to bacterial variance. Mutations, or sports, are due to changes of unknown cause in the germ plasm, the hereditary substance of the germ cells. These changes are to be clearly distinguished from fluctuating or Darwinian variations (environmental), which are due to variations in the somatic, or body, cells. The latter are not inheritable.

Now, we are dealing with inheritance units. All modern theories of heredity assume the presence of ultra-microscopical units or bodies in the germinal protoplasm, which, by their many combinations, are responsible for the morphological structures in the adult. Various names have been given to these units by different authors; they are the "physiological units" of Herbert Spencer, the "gemmules" of Darwin, the "plastidules" of Elsberg and Haeckel, the "ultra-cellular pangenes" of DeVries, the "plasomes" of Weisner, the "idioblasts" of Hertwig, the biophores and "determinants" of Weismann, the "genotypes" of Johannsen.

Originating suddenly, mutants

> . . . are not connected with the parent species by intermediates, and have no period of slow development before they reach the full display of their characters. They do not always arise, but only from time to time. A parent species may produce its offspring separately at intervals, or in larger numbers during distinct mutating periods. After this reproduction, the old species is still the same as it was before, and it subsists in the midst of its children (DeVries).

Regarding the justification of the term "mutation" as applied to bacteria, it would seem that unless a sexual form of reproduction can be demonstrated, the word is being used in a sense never intended by botanists.

Recently Philip Hadley[1] of the University of Michigan has summarized the mutation theory as applied to bacteria, and has coined the new term, "microbic dissociation," to describe the phenomenon.

Hadley emphasizes the changes in morphology which bacteria undergo and includes under his theory of dissociation such changes as colony variations, loss or gain of capsules, variations in virulence, in toxin production, serological properties, spontaneous agglutinability, gelatin liquefaction, and numerous other phenomena. In his general conclusions, Hadley says:

> From the varied assortment of data on experiment and observation which I have brought together in this review, I believe that it becomes clear that many important problems in modern bacteriology and pathology have their roots in the phenomenon of bacterial instability, and especially in that aspect which has been termed microbic dissociation. We begin to appreciate, moreover, that this phenomenon, far from being a sign of chaos, is in reality merely the necessary manifestations of certain more or less orderly processes that are correlated with a physiologic and reproductive mechanism, the nature and significance of which we are just beginning to observe and, perhaps, to a limited extent, to understand.

Mellon combines the life-cycle idea of Lohnis with the mutation idea of Neisser and his followers, and concludes that the biogenetic law of Haeckel is capable of application to the genetic history of bacteria. To him, the various phases of the pleomorphic cycle represent the ontogeny of a strain; when the phases are stabilized through mutation they collectively represent the phylogeny of this strain. He condemns the bacterial classification that widely separate the spherical forms (Coccaceæ) from the rods (Bacteriaceæ) as not having genetic support, for the reason that group and specific agglutinogens have been demonstrated between stable species of these families.

Mellon also claims that there is a considerable biologic diversity among the various phases of the pleomorphic cycle which,

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[1] HADLEY, PHILIP, "Microbic Dissociation," *Journal of Infectious Diseases*, vol. 40, no. 1, 1927.

he thinks, explains the heterogeneity of pure-line cultures. This latter idea may be best made clear by the work of Johannsen, with beans.

When the progeny of a single bean of a common garden variety were separated from the rest, it was possible to isolate pure lines which differed in average weight. By selection, he was able to break up the bean population into a number of such pure lines, but selection *within* the pure line failed to shift the mode of the variation curve in either a plus or a minus direction. He concluded that the general population of beans showing variation in weight—in much the same way as a population of men shows a variation in weight—is really made up of a number of pure lines. The difference between pure lines rests upon an hereditary basis, the variation within the pure lines upon non-inheritable factors.

Until recently, bacteriologists have not concerned themselves with these principles of genetics. A new phase of study is now presenting itself, and many scientists of the future will attack the research problems of a "pure science" rather than those of the applied bacteriology of the past.

CHAPTER VI

THE CULTIVATION OF BACTERIA

When the growth of a man is mentioned, his physical development from infancy to maturity is meant. Bacteriologists, however, use the term "growth" in an entirely different sense. They speak of bacterial growth as the development of a mass of offspring obtained from one or a few germs in reproduction. These growth masses are usually distinguishable by the unaided eye. The same idea is conveyed by the farmer in speaking of the growth of a stand of wheat.

Mass Groupings.—Any material in which bacteria find nourishment and in which they can reproduce is called a *culture medium.* The growth or crop of organisms obtained in such a medium is spoken of as a *culture.* When the bacteria in a culture are all of the same species, the culture is called a *pure culture;* and, conversely, when there are two or more different kinds of bacteria growing in a medium, the culture is called a *mixed culture.* As a rule, bacteriologists work with pure cultures, and are accustomed to think of a culture as being pure, unless a mixed culture is definitely designated. If a culture accidentally contains more than one species of bacteria, it is called a *contaminated culture.*

Culture media may be liquid or solid. Media containing gelatin or agar, both of which are solid at ordinary laboratory temperatures, but which may be melted at higher temperatures, are spoken of as liquefiable solid media. Both of these kinds of media are useful for obtaining cultures of bacteria, and each presents certain desirable properties in the study of bacterial growth.

Pellicle.—When grown in liquid media, certain bacteria, like the tuberculosis germ, form a thick skin, or *pellicle,* on the surface of the liquid. The pellicle consists of millions of bacteria held together by their membranes, together with their excretions and the substances derived from the medium. The size of the pellicle depends on the surface area of the medium, since it usually grows until it covers the entire surface. The fact that all of the bacteria which form pellicles demand atmospheric oxygen led to the idea that pellicle formation was for the purpose of securing more oxygen than could be found in the depth of the medium.

Recent experiments have shown that bacteria which can live in a limited amount of oxygen can form pellicles. Larson[1] believes that pellicle formation is an indication of the inability of the liquid to wet the surface of certain bacteria.

It is obvious, therefore, that bacteria growing on the surface of a liquid are supported in this position by some force. Since aërobiosis does not constitute a force, we must look elsewhere for the explanation. It is undoubtedly the tension in the surface of the medium which supports the bacteria in this position. An analogy may be found in the floating steel needle. If a steel needle is coated with a film of oil and carefully placed upon the surface of water, it will remain supported upon the surface. Here there can be no question of aërobiosis. In the language of the physicist, the needle is not "wet" by the water; its weight is not sufficient to break the water surface. It is thus supported by the surface tension of the liquid. The addition of a little soap or other surface-tension depressant causes the needle to sink promptly. There is little doubt but that pellicle formation is a property of the surface tension of the medium, although the character of the bacterial surface is also an important factor.

In the case of organisms containing a high percentage of fats or waxes, the attraction of water molecules for each other is greater than the attraction of the water molecules for the bacteria, and the bacteria float.

Zoöglœæ.—Some capsulated bacteria, when grown in liquid media, form masses of gelatinous material. These masses, miscalled "zoöglœæ (animal glue) are irregular lumps of seminitrogenous jelly containing bacteria. The "mother of vinegar" is an example. Such also is the origin of ropy milk, and to these masses is due the efficacy of the slow-sand filter used in purifying municipal water supplies.

Colonies.—Pellicles and zoöglœæ are mass formations of bacteria in liquid media. When bacteria are sown on a solid medium, and so scattered that each individual germ is well separated from others, each will multiply into a definite mass of its

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[1] LARSON, W. P., "The Effect of Surface Tension on the Menstruum upon Bacteria and Toxins," in JORDAN and FALK'S, "The Newer Knowledge of Bacteriology and Immunology," p. 181, Chicago, 1928.

progeny. These masses, which represent the offspring of one, or at most a very few, bacteria are called "colonies." Under usual laboratory conditions, the type of colony is characteristic for each species and is not an accidental formation.

These differences in colony formation by species of bacteria may be likened to the characteristic differences which distinguish the villages of Neolithic man, the American Indian, and the lake dweller.

Since the progeny of each bacterium placed on a medium in which they cannot become freely distributed form a colony, it is possible to obtain a pure culture of any particular species by separating the individual bacteria in a mixture and allowing each individual to form a colony. For exact research work, bacteriologists are unwilling to risk the possibility, small though it may be, that a colony is the combined progeny of two or more bacteria (possibly of different species, sticking together) so they prepare their pure cultures by actually picking out a single germ from a liquid and growing it on a suitable medium. This method, known as "single-cell technique," is unnecessary in ordinary laboratory procedures.

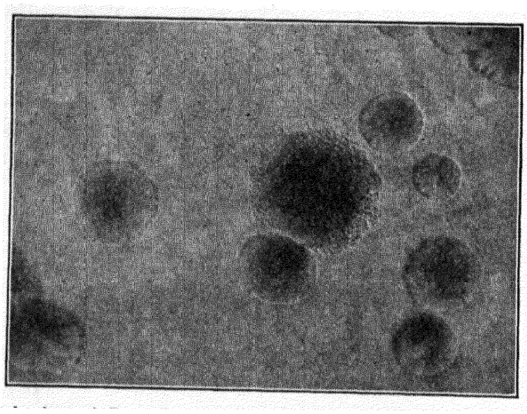

FIG. 12.—Colonies of Lactobacillus acidophilus. (Refer to Figs. 6 and 7.)

Dissociation Aspects of Colony Formation.—The recent work of Hadley and others shows that what has been said regarding the specificity of colony formation is not always true. While, in general, it may be justifiable to speak of a "typical Escherichia coli colony," or to distinguish a Staphylococcus colony from a Bacillus mycoides colony—the one being circular with an unbroken edge, the other very irregular, with root-like projections—this is only the case when dealing with the *majority* of colonies formed under what has been adopted as the standard laboratory conditions of culture media, temperature, time of incubation, etc.

The instability of bacterial morphology, the variations in physiological activities and the deviation in methods of reproduction from the usual binary fission have been pointed out. The same general considerations are necessary in the study of colony formation.

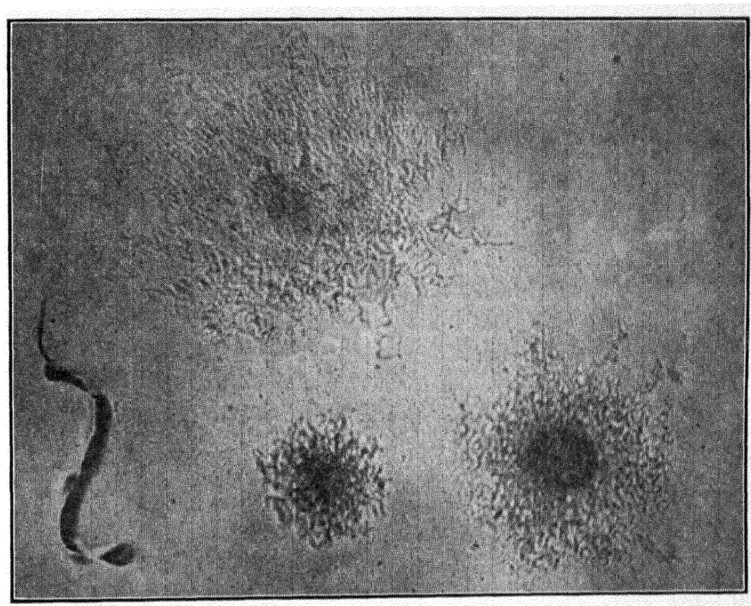

FIG. 13.—Colonies of lactobacillus acidophilus. (Refer to Fig. 12.)

For ease in explaining the phenomenon of colony variation, Hadley has distinguished two general types: the one, round and smooth, with a glistening surface; the other, flat, irregular, and uneven, with rhizoid branchings. The former he calls the S or smooth type, and the latter the R or rough. He recognizes an intermediate type O as a transitional form.

An example frequently cited is that of the colony differences between Bacillus subtilis and Bacillus anthracis. Usually Bacillus subtilis forms a smooth, regular colony S and Bacillus anthracis a rough, rhizoid colony R; but it is well known that

Bacillus subtilis may at times form a rough colony so similar to the Bacillus anthracis colony that the two are frequently confused. There is every reason to believe also that Bacillus anthracis forms a smooth colony which is similar to the usual Bacillus subtilis colony.

In the same way variations in colony formation have been noted with numerous other species. It is quite probable that these variations are simply symptoms or indications of fundamental cyclic variations in the life history of the bacteria.

Growth Curves of Bacteria.—In the last chapter it was said that under optimum conditions of growth bacteria divide every 20 to 30 minutes. Theoretically, if bacteria are planted in a favorable medium, it would be expected that they would begin multiplying immediately, and that the increase in numbers would be in geometrical ratio with the time. In other words, a curve representing the increase in numbers with time would be a straight line. This, however, is not the case. When a culture of bacteria is transplanted to a new medium, there is always (except where repeated inoculations have been made at short intervals) a period of stagnation, or readjustment in which there is very little or no growth. This interval is called the "lag" period. It is variable with the species of bacteria and with the age of the culture from which the transplant was made. Other factors, such as type of medium, temperature, etc. also influence the length of the lag period.

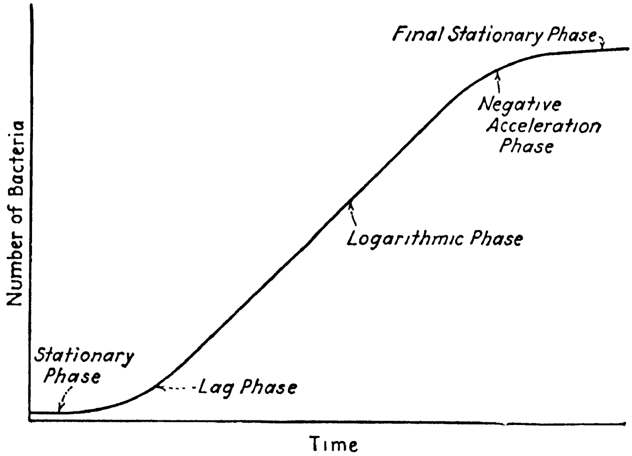

FIG. 14.—Growth curve of bacteria.

Following the lag period, the growth curve becomes a straight line. Now the bacteria are multiplying in geometrical ratio to the time. This period is known as the "logarithmic phase" of growth.

As soon as the conditions begin to be unfavorable for maximum growth, mainly because of an accumulation of excretory products of the bacterial life, the curve becomes progressively flattened. Increase in numbers is no longer in proportion to time, and active growth rapidly ceases. This period is called the phase of "negative acceleration." Finally, a stationary phase ensues, in which no multiplication takes place.

If broth cultures of bacteria are examined, it is easy to follow the different growth phases. On solid media, the most striking effect is the gradual increase in the size of colonies until a maximum is reached.

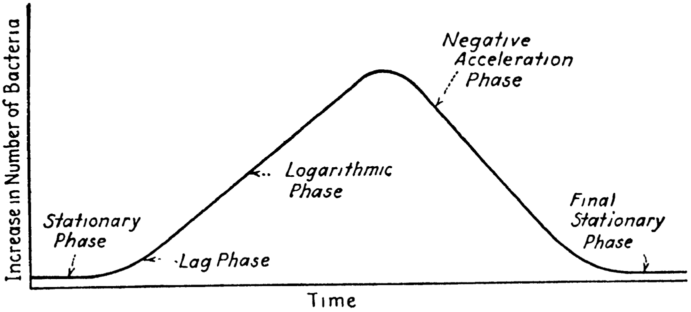

FIG. 15.—Growth curve of bacteria.

The length of the lag period of the subculture is determined by the growth phase of the parent culture at the time of transplanting. If repeated transplants are made at the time of the logarithmic phase, the lag period can, in certain species, be cut to zero.

The Distribution of Bacteria.—The distribution of bacteria in nature depends almost entirely on the conditions which are necessary for their growth. At what period in the earth's history bacteria first developed, it is impossible to say. From a study of organic evolution it would seem that they were among the first of the living creatures on the earth. That proper conditions have existed for bacterial life since the Carboniferous and Devonian periods is clear from their presence in fossils.

Walcott[1] believes that bacteria played a part in the precipitation of the Algonkian and Precambrian limestones of Montana. Paleontologists believe that little or no life existed prior to these periods.

In an examination of thin sections of various Jurassic and Cretaceous rocks, collected for the study of borings for ironstone, David Ellis devoted special attention to microörganisms that might be associated with iron deposits. Hyphæ, sporangia, and spores of a fungus form were identified in the fossils from the Frodingham ironstone of Lincolnshire. Branching hyphæ of a second fungus were found in the ironstone of Raasay (Northwest Scotland). Ellis also described a species of Actinomyces, and several bacteria from the same or similar rocks. The organism from the Frodingham district, which he called Phycomycitis frodinghamii, seems to have had a chemiotactic affinity for iron compounds, and may have enriched the deposits where it was found. All these fossil fungi were found in the cells of plant tissues that were in a rotting condition when the fossilizing processes took place.[2]

Evidences of bacteria have been found in the oldest fossil-bearing rocks. Disease germs evidently attacked forms of life long before man came on the earth. From the Carboniferous down through the Permian age, as shown by their presence in fossil fishes, through the long Mesozoic period or the Age of Reptiles, bacteria have left their indelible traces in the rocks.

Since the vast majority of bacteria are saprophytic (that is, require dead organic matter as food), the natural habitat for most bacteria is in the upper layers of the soil. With the exception of the parasitic bacteria (for example, the pathogenic or disease-producing bacteria), which require a living host, nearly all bacteria could find in the soil the conditions necessary for growth.

But the soil is not the only place where these conditions are fulfilled. Bacteria are washed from the soil into rivers, lakes, etc. in large numbers, and obtain their food from the material

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[1] WALCOTT, C. D., Smithsonian Miscellaneous Collections, vol. 64, no. 2 pp. 94–95, Washington, 1914.

[2] ELLIS, DAVID, "Fossil Microörganisms from the Jurassic and Cretaceous Rocks of Great Britain," *Proceedings of the Royal Society of Edinburgh*, vol. 35, pp. 110–112, 113–132, 1915; A. L. S , in *Journal of the Royal Microscopic Society of London*, pp. 249–381, 1919; E. B. F., in *Abstracts of Bacteriology*, vol. 4, p. 233, 1920.

which is washed into the water with them. Milk affords an ideal food for bacteria, and, once inoculated, it soon is swarming with them. The air contains varying numbers of bacteria, blown by the wind from the dry surfaces of the earth. Here, however, there is little or no food for bacteria, and little multiplication takes place.

Environmental Factors.—In speaking of the effects of various external conditions upon bacteria, it must be clearly borne in mind that bacteria vary greatly in their environmental requirements for existence. In no other group of living things is this even approximately true. The great differences among bacteria in their food and energy requirements have already been spoken of, and now a few of the factors which influence germ life will be reviewed.

Drying.—Bacteria secure their food by absorption through the capsule and cell wall; the food, therefore, must be in solution. In the vegetative state, bacteria vary greatly in the ability to withstand drying. Some organisms, for example, Eberthella typhi, are unable to withstand drying for even a few hours, while others, like Mycobacterium tuberculosis, can withstand complete desiccation for a considerable length of time. (Sputum which had been dried for two months was found to contain virulent tuberculosis organisms.) Spores are able to resist drying for a much longer time than vegetative cells. Anthrax spores have been known to resist drying for 25 years. Since very few pathogenic bacteria form spores, however, this group of harmful germs is unable to withstand desiccation, and, for this reason, infection through the air is uncommon.

It is probable that the actual extraction of water from the protoplasm during drying is relatively unimportant as a *lethal* process. Desiccation is germicidal, because, when bacteria are dried, they cannot replenish the cell substance used up by katabolism.

Direct sunlight always inhibits bacterial growth, and to many of these organisms, especially the pathogens, it acts as an immediate poison. Weinzirl[1] found that when Mycobacterium tuberculosis, Corynebacterium diphtheriæ, Eberthella typhi, etc. were placed on glass or paper, and exposed to the direct rays of the sun without any covering, they were killed in from 2 to 10

[1] Weinzirl, *Journal of Infectious Diseases*, vol. 4, sup. 3, p. 126, 1907; *American Journal of Public Health*, vol. 4, pp. 969–974, 1914.

minutes, but that the time required to kill spores by this method varies from 2 to 8 hours (or about sixty times that required to kill non-spore formers). With one exception (the purple bacteria, Thiobacteriales), bacteria prefer darkness to even diffused light. The fact that ultra-violet rays have the greatest germicidal effect, and that infra-red rays have practically no effect, leads to the conclusion that the action is purely chemical. Victor Henri[1] claims that the mechanism of the action of the ultra-violet rays consists in a direct photochemical reaction on the cellular contents (protoplasm) and not in an indirect action, such as the formation of nascent oxygen or other chemical poison. It may well be that a molecular rearrangement of proteins is effected by the light stimulation and thus life destroyed. The rays from an arc light act like direct sunlight, while X-rays have no germicidal powers. The author was unable to show any germicidal power in the radiations from radioactive substances.

Electricity, passed through a bouillon culture in a continuous current, will ultimately kill all bacteria in the culture, both vegetative bacteria and spores. The electric current itself has no germicidal properties but destroys bacteria by the production of substances by electrolysis (*viz.*, nascent oxygen). Abbott[2] has shown that, with a weak current, cultures of certain bacteria gather at the cathode, but if the bacteria are grown in an acid medium they gather at the anode.[3]

Oxygen.—While all bacteria require oxygen as a part of their food supply, they vary greatly in their need of free (uncombined) oxygen, *i.e.*, oxygen in the air, or that dissolved in water. Most bacteria cannot live without the presence of some atmospheric oxygen, which they use as a source of energy or for building protoplasm. There are, however, bacteria which can live in the complete absence of free oxygen, and others which soon die in the presence of atmospheric oxygen. Those bacteria which demand the presence of free oxygen are called "aërobes"; those which demand the absence of free oxygen are called "anaërobes." Many bacteria grow well in various diminished concentrations of oxygen. These are called facultative anaër-

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[1] Henri, Victor, *Comptes rendus Société de Biologie*, vol. 73, p. 323, Paris.

[2] Abbott, *Science*, p. 910, June 12, 1908.

[3] The results of a long series of experiments on the "Effects of Direct Alternating, Tesla Currents, and X-rays on Bacteria," were published by Zeit in the *Journal of the American Medical Association*, Nov. 30, 1901.

obes, or simply *facultative bacteria.* This group is sometimes divided into those which prefer the presence of free oxygen but can live without it, and those which are preferably anaërobes but can become aërobes. The aërobic bacteria can tolerate the absence of oxygen for some time, and most anaërobes can tolerate a certain small amount of oxygen. Corynebacterium diphtheriæ is an example of the aërobes, Eberthella typhi of the facultative anaërobes, and Treponema pallidum of the anaërobes.

Nascent oxygen is a pronounced germicide. This fact is utilized in the purification of drinking water with ozone. Manufacturers have attempted to introduce ozone apparatuses for disinfecting sickrooms in which there had been a communicable disease. Results of experiments, however, entirely discredit the claims of these manufacturers.[1]

Temperature.—The temperature limits between which bacterial life is possible are probably absolute zero (−273°C.) and 160°C. The range of temperature in which bacteria can grow and carry on their physiological activities is much narrower, the limits being 5 and 80°C.; and the range for each particular species is still narrower. It is possible to recognize a maximum and a minimum temperature (the highest and lowest temperatures of growth) for each species, and to classify bacteria according to their optimum temperature (the temperature at which they grow most rapidly).

Bacteria of one class grows best at about 10°C. These are called *psychrophilic* (cold-loving) bacteria. They are usually water bacteria and are found in the depths of the ocean, in springs, ètc. The decay of foods while in cold storage is largely the result of the activities of psychrophilic bacteria.

Another class grows best at a relatively high temperature. The *thermophilic* (heat-loving) bacteria prefer a temperature of about 55°C. In ensilage, decaying heaps of manure, and hot springs, these bacteria are sometimes found growing at a temperature as high as 80°C.

In the research laboratory of the National Canners' Association, a study was made of bacteria which resisted the usual sterilization processes and caused spoilage of canned goods. The organisms causing spoilage were facultative and obligate anaërobes. In many cases, growth occurred at a temperature of

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[1] *Journal of the American Medical Association,* Sept. 27, 1913; *Engineering News,* vol. 70, pp. 1092, 1096; *Engineering Record,* vol. 68, pp. 645–646.

80°C., and in some cases the organisms resisted boiling temperature for 17 hours. One organism, Bacillus stearothermophilus, has been isolated from spoiled corn which had been processed at 118°C. for 75 minutes.[1] It was formerly thought that thermophilic bacteria were the cause of the high temperatures in ensilage and manure heaps, but as the temperature in these deposits sometimes reaches a point far above that at which these bacteria can live, that is hardly likely. It is more probable that hydration and pressure are responsible for these temperatures.

The optimum temperature of the great majority of bacteria lies between these two extremes, or between 20 and 40°C. Such organisms are called the *mesophilic* bacteria. Mesophilic bacteria may really be divided into two groups, the *oikophilic* bacteria, which have an optimum temperature of about 20°C. (room temperature), and the *somatophilic* bacteria, which grow best at about 37.5°C., or blood heat. In the first class belong the saprogenic and other bacteria found in the soil; in the second class belong the pathogenic and other parasitic bacteria which have warm-blooded animals as their hosts.

The influence of the various temperatures on the different bacteria finds an application in every line of bacteriological study. Since each species has a different optimum temperature, and even pure cultures give different products at different temperatures, the value of temperature regulation in fermentation industries is apparent. In butter and cheese making, the wine industry, tobacco curing, and, in fact, in every industry which depends on the action of bacteria, the temperature at which the process is carried on is of the greatest importance.

From a sanitary standpoint, the chief interest is in the extremes of heat and cold which bacteria, especially the pathogenic bacteria, can stand. For purposes of comparative study bacteriologists define the thermal death point of bacteria as that temperature at which all bacteria in a fresh (vegetative) culture are killed by the application of moist heat for 10 minutes. The exact knowledge of the thermal death point of the various pathogenic bacteria is applied in the production of vaccines, antitoxins, and in certain pathological laboratory tests.

The resistance of bacteria to dry heat is much greater than to moist heat. While most vegetative cells are killed by 10 min-

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[1] DONK, P. J., "A Highly Resistant Thermophilic Organism," *Journal of Bacteriology*, vol. 5, pp. 373–374, 1920.

utes' exposure to boiling water, 100°C., it takes more than an hour at 150°C. dry heat to attain the same end.

Bacteria are not nearly so sensitive to cold as to heat. In a frozen medium, for example, ice, most spores lie dormant but they germinate as soon as the temperature rises. The number will gradually decrease, but spores of bacteria have been found living in ice which had been frozen for nearly a year. Sedgwick and Wilson[1] have found that as soon as typhoid organisms are frozen the greater number of them are destroyed. Experiments conducted in the author's laboratory prove that Eberthella typhi can resist freezing for no longer than three weeks.[2]

These facts are of practical value in the use of "natural ice" and of considerable interest in presenting a new point of view regarding water-carried infection. The historic Plymouth epidemic has often been cited as an example of the resistance of the typhoid germ to freezing. It is far more likely that fresh and vigorous organisms found their way into the water from fæces deposited within a few days of the time the infected water was consumed.

How the ordinary saprophytic bacteria in milk will resist freezing is shown in the following table.[3]

MILK KEPT AT −3 TO −7°C.

	Bacteria per Cubic Centimeter
Freshly frozen	200,000
After 1 day	105,500
After 2 days	72,300
After 3 days	62,000
After 4 days	46,400
After 7 days	44,000
After 14 days	40,500
After 21 days	30,300
After 35 days	22,500
After 49 days	14,200

This evidence clearly shows that it is impossible to sterilize milk by freezing. Frozen milk, however, will not be decomposed

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[1] SEDGWICK and WILSON, *Centralblatt für Bakteriologie, Parasitenkunde, und Infektionskrankheiten*, bd. 27, p. 684.

[2] THOMAS, STANLEY, "The Resistance of the Typhoid Bacillus to Freezing," *Science*, vol. 60, pp. 244–245, 1924.

[3] MARSHALL, "Microörganisms," after Bischoff, p. 158, Philadelphia, 1912.

by these bacteria, which are simply in a dormant state and not capable of obtaining food or causing fermentation.

Surface Tension.—The effect of certain substances, such as soaps, bile salts, etc., upon the growth of bacteria is out of proportion to any possible beneficial or germicidal properties which they might possess in themselves. This effect is explained by the fact that such substances diminish the attraction of water molecules for each other and thus depress the surface tension of the culture medium, thereby increasing the attraction of the bacterial surface for the medium. Larson[1] suggests that it is the operation of the laws of surface energy which make it possible for bacteria to obtain food:

> The nutrient material in culture media must be concentrated at the bacteria-water interfaces, which make them immediately available to the bacterial cells. It will perhaps not be considered too theoretical to assume that the rate of growth is influenced by the effect the most immediate surface-tension depressants have upon the organism. If it happens to be an ideal food for the particular bacterium, it constitutes an ideal culture medium. If, on the other hand, the surface-tension depressants are toxic, there will be little or no growth. The use of bile in culture media may illustrate the point in question. It has been known for many years that bile salts, when added to culture media, stimulate the growth of the colon-typhoid bacteria, whose normal habitat is the intestinal tract. The pneumococci and streptococci, on the other hand, are very sensitive to bile salts and soluble soaps.

Albus and Holm[2] have suggested that the bile salts present in the intestinal tract have an effect on the implantation of the aciduric bacteria. Kopeloff and Beerman[3] systematized a method for surface-tension determinations and for growing the Lactobacilli at varying surface tensions. They demonstrated that the two species, Lactobacillus acidophilus and Lactobacillus bulgaricus could be differentiated by this means.

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[1] LARSON, W. P., "The Effect of the Surface Tension of the Menstruum upon Bacteria and Toxins," in JORDAN and FALK'S, "The Newer Knowledge of Bacteriology and Immunology," p. 182, Chicago, 1928.

[2] ALBUS, W. R., and G. E. HOLM, "The Effect of Surface Tension upon the Growth of Lactobacillus acidophilus and Lactobacillus bulgaricus," *Journal of Bacteriology*, vol. 12, no. 1, pp. 13–18, 1926.

[3] KOPELOFF, N., and P. BEERMAN, "Surface Tension Studies with Lactobacillus acidophilus and Lactobacillus bulgaricus," *Journal of Infectious Diseases*, vol. 40, pp. 656–666, 1927

Pressure.—Bacteria are able to resist very great mechanical pressure. Most bacteria will resist pressures of 45,000 pounds to the square inch for a considerable length of time. Bacteria are found living at the bottom of the ocean, where, of course, an enormous pressure is exerted upon them. A few years ago an attempt was made to commercialize a method of sterilizing canned fruit by pressure. The process, however, met with no success. It is still the hope of the canning industry, however, to perfect a method of sterilization without heat.

Mechanical agitation seems to have no effect on the life of bacteria. Agitation is important, however, when used with germicides, as it exposes the bacteria thoroughly to the action of the substances which will kill them. In the purification of water by ultra-violet light, ozone, etc., and in the disinfection of sewage, agitation plays an important rôle.

Reaction.—Bacteria are sensitive to the "reaction," or acidity or alkalinity, of the medium in which they are growing. Some grow well in a slightly alkaline medium and others in a slightly acid medium. Most pathogenic bacteria prefer a reaction of from pH 6.8 to 9.6. This reaction has always been a source of dispute among bacteriologists and it was not until the use of the hydrogen-ion potentiometer and the various indicators introduced by Clark and Lubs with the employment of control buffer solutions that this very important requirement was understood.

Chemicals.—Different concentrations of chemicals in solutions have entirely different effects on bacteria, although a minute amount of salts is always necessary to produce the proper osmotic pressure. A very weak solution of mercuric chloride will stimulate the growth of some species, while a slightly stronger solution will kill them.

Work undertaken by the U. S. Bureau of Fisheries to study the cause of loss due to red coloration in salted fish brought out some interesting facts about two species of bacteria. These organisms, one a spirochæte and the other a bacillus, live in symbiosis. They are strictly aërobic and the optimum concentration seems to be the point of saturation of sea salt. No growth could be obtained on culture media containing less than 16 per cent sea salt.[1] Some chemicals attract bacteria and

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[1] Reported by William W. Browne, *Abstracts of Bacteriology*, vol. 4, pp. 11–12, 1920.

others repel them. This phenomenon of the attraction which certain substances have is known as "chemotaxis."

The most important effect of chemicals on bacteria, however, lies in their destructive action. As germicides, disinfectants, antiseptics, and preservatives, chemicals have a most important rôle in every phase of the study of bacteriology.

Professor E. G. Hastings[1] has recently shown, as a result of his experiments on anthrax spores and a member of the coli-aërogenes group, that:

> . . . it seems probable that observations as to the resistance of an organism to physical and chemical agents made on cells from artificial cultures may have little or no value in indicating the resistance of cells of the same organisms as it occurs in nature.

Concomitant Growth.—The effect of the growth of one species of bacteria upon that of another species is frequently very striking. True *symbiosis*, where two different species mutually benefit each other, is quite rare. The symbiotic relationship of Rhizobium and the leguminous plants is an example of benefit to both associates. More frequently, the growth of one species is benefited by another without itself aiding the second. The growth of Hemophilus influenzæ in the presence of Streptococci, and various anaërobes in the presence of Bacillus subtilis are two of the many examples that might be cited. The term *metabiosis* is sometimes used for this coexistence where the benefit accrues to but one of the associates and that only after the growth of the other has continued for a time. The third case of concomitant growth is still more common. This is where the growth of one species is harmful to another. It is called "antagonism." The overgrowth of Lactobacillus acidophilus in the intestinal tract of man, crowding out Escherichia coli, the destruction of Corynebacterium diphtheriæ by Staphylococcus aureus, the inhibitory effect of the aciduric Doderlein's bacillus[2] (Lactobacillus acidophilus) on the gonococcus in the human vagina are examples of bacterial antagonism. The direct cause of antagonism may vary in each case—acid production, inhibitory excretions or what not—but the result is the same.

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[1] HASTINGS, E. G., *Journal of Infectious Diseases*, vol. 33, no. 6, pp. 526–530, 1923.

[2] THOMAS, STANLEY, "Doderlein's Bacillus—Lactobacillus acidophilus," *Journal of Infectious Diseases*, vol. 43, pp. 218–227, 1928.

CHAPTER VII

BACTERIAL ENZYMES

In the chapter on the Physiology of Bacteria it was seen that certain substances, called enzymes, were produced as products of bacterial life to aid the organisms to assimilate the food necessary to build up their cell protoplasm. Thus, the function of these enzymes is exactly that of the enzymes of the human body, such as pepsin, pancreatin, etc. Because most of the changes in nature by which bacterial actions are recognized are due to enzyme action, the study of bacterial enzymes is an important part of bacteriology.

History of Enzymes.—As we have already learned, man recognized the activities of "ferments" centuries before he had any knowledge of what they were. From the beginning of the nineteenth century, with Lavoisier's conception of a reversible chemical reaction in alcoholic fermentation, $C_6H_{12}O_6 \rightleftarrows 2C_2H_5OH + 2CO_2$, to Liebig's theory of molecular agitation, propounded near the middle of the century, scientific literature records numerous attempts to explain complex organic change by simple chemical reactions.

The fact that inorganic catalysts were known, confused rather than helped to solve the problem. The observation that platinum could cause hydrogen peroxide to break down into water and oxygen led to the idea that life was not necessary in fermentation.

The spontaneous-generation controversy during the middle of the past century, however, brought forward such a mass of scientific data that today we have a fairly clear conception of the process of fermentation, even if we cannot produce ferments except through the agency of living matter.

In the confused state of knowledge regarding bacteria, their origin and relation to fermentation and disease, some believed that fermentation produced bacteria, others that bacteria produced fermentation, that is, that bacteria were ferments, and still others that bacteria produced substances which in turn caused fermentation.

Pasteur discarded the purely chemical and spontaneous-generation ideas and divided the ferments into two groups. In one case, organic changes are only effected by the living cell itself. These he called "organized ferments." In the other case, a soluble substance is secreted by the bacteria and may effect changes apart from the living cell. He called the latter "unorganized" or "unformed ferments."

Experiments soon showed that it was possible to extract a substance from the organized ferments which would cause fermentation apart from the cell. This fact obviated the necessity for Pasteur's distinction. Later it was recognized that fermentation and putrefaction were similar processes, only differing in the material acted upon. Today the term "ferment" has been discarded in favor of the Greek, *en zyme* (in leaven) which means the same thing but does not carry with it the confusion of ideas built around the older term.

Enzymes are biochemical catalysts, and possess certain essential properties which are characteristic of them as a class of substances. As catalysts, enzymes are capable of accelerating chemical changes, although they do not initiate them nor do they enter into the reaction. As someone aptly expressed it, "Enzymes are like the oil in machinery, they do not make it run, but they make it run more rapidly and smoothly." As a consequence a very small amount of enzyme may produce a great amount of chemical change. Enzymes must be considered apart from the bacterial cell, as they are soluble in water and glycerine and are capable of carrying out their reactions after removal from the cell which produced them. As Pasteur showed (although he was mistaken in his conception as to the nature of organized ferments), some enzymes *usually* act in the presence of the living cell and some are readily diffused through a liquid medium. Pasteur's classification has been revised and the former (organized) are called intracellular enzymes and the latter (unorganized) are called extracellular enzymes.

Nature of Enzymes.—While the exact nature of enzymes is unknown, they are evidently related chemically to the albuminoids.

Enzymes are usually assumed to be protein in nature, but they may be other substances not proteins, which, however, are so intimately associated with the cell proteins that it is impossible to separate them. Willstatter[1] considers the enzyme molecule as composed of two groups, the one colloidal in nature and nonspecific, the other, a specific group or radical carried by the first.

Enzymes show many properties similar to living bacterial cells. As is the case with bacteria, each enzyme has an optimum temperature. Cold diminishes the activity of enzymes, and heat above 70°C. completely destroys them. In other words, they possess what is analogous to the thermal death point of bacteria. Sunlight and, especially, ultra-violet light destroys the activity of enzymes, as it destroys the life of bacteria. Like bacteria, each enzyme has its specific optimum reaction. This in general falls within the same range as that of bacterial growth (pH 5 to pH 8). The mode of enzyme action is exceedingly complex. Evidently, organic colloids associated with them at least play a part in the chemical changes which the substance acted upon undergoes.

Classification of Enzymes.—Because of our imperfect knowledge of the nature of enzymes, it has been impossible for authorities to agree upon their classification. Oppenheimer[2] divides them into two groups, the hydrolases and the desmolases. The *hydrolases* are those which bring about hydrolytic decomposition of carbohydrates, fats, and proteins. With the addition of water groups into the molecule of the substance acted upon, cleavage takes place and the molecule splits into simpler substances. The *desmolases* are those which bring about molecular changes through the addition or subtraction of oxygen atoms. The hydrolases are usually extracellular and the desmolases intracellular.

Waksman,[3] one of the leading authorities on bacterial enzymes, divides these substances into four groups according to the nature of the reaction in which the enzyme plays a part:

> . . . (1) *Hydrolysis.* This involves the transformation of various polysaccharides into sugars, of proteins into amino acids, of amino acids into ammonia and oxy-acids, the transformation of insoluble organic substances (starch, fibrin) into soluble forms, and finally glycolytic decompositions, as the transformation of sugar into lactic acid ($C_6H_{12}O_6 = 2C_3H_6O_3$). (2) *Oxidation,* resulting in the liberation of energy. Some of the oxidation processes, such as the formation of acetic acid from alcohol, and citric and oxalic acids from glucose, are frequently

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[1] WILLSTATTER, R., *Journal of the Chemical Society,* p. 1374, 1927.

[2] OPPENHEIMER, C., "Lehrbuch der Enzyme," Leipzig, 1927.

[3] WAKSMAN SELMAN A., "Principles of Soil Microbiology," p. 371, Baltimore, 1927.

referred to as fermentations. These are distinguished from true fermentations, which result in the liberation of energy by the decomposition of complex organic compounds into simpler forms, without the intervention of free oxygen as in alcoholic and butyric-acid fermentations. (3) *Reduction.* Substances rich in oxygen are reduced to substances poor in oxygen, such as the reduction of nitrates, nitrites, and sulphates, and the oxygen may be used for purposes of oxidation. The coupled reaction of reduction and oxidation, whereby one substance is reduced and the other oxidized at the same time are common in microbiological reactions. (4) *Synthetic reaction,* including anhydride formation and condensation.

Since the principal interest is in the results of the action of bacterial enzymes, they may be divided, for convenience, into groups according to their action on the different kinds of substances which are transformed through their agency.

According to this plan, the bacterial enzymes may be arbitrarily grouped into five classes: oxidizing enzymes, reducing enzymes, amylolytic (carbohydrate-splitting) enzymes, proteolytic (protein-splitting) enzymes, and steatolytic (fat-splitting) enzymes. It must be understood that, according to the arbitrary grouping, certain enzymes which would be grouped together by some writers are here placed in different classes, and, conversely, some of the enzymes which are placed in the same group are separately classified by others.

Naming Enzymes.—Different methods for the naming of enzymes have been employed. Most of these make use of the suffix *-ase.* In some, the ending is added to the substance acted upon, as lactase, amylase. In others, the suffix is added to a term which expresses the type of reaction, as oxidase, reductase. In a few cases, the ending indicates the substance produced, as alcoholase. Finally, certain older terms without scientific origin such as trypsin, pancreatin, and pepsin have become common names for specific enzymes.

Bacteria and Enzymes.—Since in this study the interest is in the enzymes of bacteria, the enzymes will not be considered *per se,* but rather the kinds of bacteria which produce them. Bacteria may be divided into groups based on the kinds of enzymes which they produce. Those bacteria generating oxidizing enzymes are known as oxidizing bacteria; those generating reducing enzymes, as reducing bacteria; those generating amylolytic enzymes as zymogenic bacteria (zymos, "fermentation";

genus, "source"); those generating proteolytic enzymes as saprogenic bacteria (sapros, "rot"); and those generating steatolytic enzymes as glycerogenic (glukus, sweet, glycerine) bacteria.

The enzymes in any particular class do not act upon the same products nor is the change stimulated the same, even when the identical substance is acted upon. Thus, a bacterium may produce a proteolytic enzyme which will split the proteins of gelatin but will have no effect on egg albumin; or a bacterium may, by its oxidizing enzyme, convert ammonia into a nitrite, but not be able to change the nitrite into a nitrate, which process is also an oxidation and is accomplished by the enzymes of another species of bacteria. Again, one bacterium may ferment a hexose, forming lactic acid, and another organism may ferment the same sugar with acetic acid as the end product. A single species of bacteria, moreover, may produce more than one kind of enzyme. Escherichia coli, for instance, secretes a reducing enzyme which reduces potassium nitrate to potassium nitrite, and also a proteolytic enzyme which decomposes trytophane, giving off indol as one of the split products. At the same time, it produces an amylolytic enzyme which will convert lactose into lactic acid, carbon dioxide,.and hydrogen.

Oxidizing Bacteria.—Bacteria that produce oxidizing enzymes are of the greatest economic importance to the agriculturist. They are mostly soil forms, though a few species may be considered as natural inhabitants of water. The oxidizing bacteria are generally autotrophic and always prototrophic. They always require the presence of atmospheric oxygen, and they secure their energy through the oxidation of simple inorganic substances as carbon, hydrogen, nitrogen, sulphur, etc. Bergey classifies this whole group in the family Nitrobacteriaceæ of the order Eubacteriales.

Pasteur, in 1862, suggested that the production of nitric acid and its salts in the earth might be due to the activities of bacteria. Winogradsky, by a series of researches, has demonstrated conclusively that bacteria are responsible to a great extent for the production of those nitrogen salts in the soil which are necessary for plant life.

The process of nitrate formation is not carried out by a single species of bacteria. There are several steps involved, each of which may be due to a number of different bacteria. Animals are not able to use pure atmospheric nitrogen, and the amount that the green plants can utilize is negligible. There is, however, a group of bacteria, the nitrogen-fixing bacteria, which are able to bring about the oxidation of free nitrogen and "fix" it with other elements into combinations which can be assimilated by plants. Other bacteria found in the soil are able to oxidize ammonia (NH_3) into nitrites, and others are able to oxidize nitrites into nitrates, according to the equations:

$$2NH_3 + 3O_2 = 3H_2O + N_2O_3$$
$$H_2O + N_2O_3 = 2HNO_2$$

and

$$N_2O_3 + O_2 = N_2O_5$$
$$H_2O + N_2O_5 = 2HNO_3.$$

Still other members of the family Nitrobacteriaceæ are able, through their oxidizing enzymes, to oxidize other simple compounds. Thus, the genus Hydrogenomonas will convert molecular hydrogen into water:

$$2H_2 + O_2 = 2H_2O.$$

The genus Methanomonas can convert methane into carbon dioxide and water:

$$CH_4 + 2O_2 = 2H_2O + CO_2.$$

Sulphur and hydrogen sulphide may be oxidized by bacteria into sulphites and sulphates:

$$2H_2S + O_2 = 2H_2O + S_2$$
$$S_2 + 3O_2 + 2H_2O = 2H_2SO_4.$$

The sulphuric acid always combines with some alkaline earth to form the sulphate

$$H_2SO_4 + CaCO_3 = CaSO_4 + H_2O + CO_2.$$

In the same way, arsenites may be oxidized into arsenates, phosphites into phosphates, carbon monoxide into carbon dioxide, etc. All these chemical changes may be due to the oxidizing enzymes of the oxidizing bacteria.

Reducing Bacteria.—Reducing enzymes are usually produced by anaërobic bacteria. As these organisms are unable to thrive in free oxygen, they must get the oxygen necessary for life from compounds containing that element. This is not always the case, however. One class of the reducing bacteria which reduce inorganic nitrogen compounds and are, therefore, called "dentrifying bacteria," demands some atmospheric oxygen.

The reducing bacteria thrive best in the presence of easily split organic substances such as lactic acid. Unlike the oxidizing bacteria, they are unable to live in the complete absence of organic matter and hence are heterotrophic. The bacteria which produce these reducing enzymes are found most commonly in the soil, although they may be present in the animal body, manure, and in water.

As in the case of the oxidizing bacteria, our examples of reduction may be taken from among the nitrogen compounds. The splitting of proteins and other organic nitrogenous substances is carried out by the saprogenic bacteria. The term reduction will here be limited to the inorganic compounds, such as nitrates and nitrites, the process being the extraction of oxygen from the molecule for the use of the bacteria as a source of energy.

A large number of bacteria are capable of reducing nitrates to nitrites. Escherichia coli, Bacillus subtilis, Bacillus mycoides, Serratia marcescens, and other common laboratory cultures all possess this property. Some, like Bacillus subtilis, can carry the process still further and reduce the nitrite to atmospheric nitrogen. Other species can reduce the nitrate molecule directly to free nitrogen. Still others reduce nitrates to ammonia and others ammonia to nitrogen.

Inasmuch as carbon compounds are essential to denitrification, this process is sometimes looked upon as an oxidative change, since the carbon is oxidized:

$$4KNO_3 + 5C + 2H_2O = 4KHCO_3 + 2N_2 + CO_2$$
$$4KNO_2 + 3C + H_2O = 2KHCO_3 + 2N_2 + K_2CO_3$$
$$C_2H_5OH + 2N_2O_3 = 2N_2 + 2CO_2 + 3H_2O.$$

Bacteria are also responsible for the reduction of sulphur compounds in the soil. Anaërobic conditions are necessary, and the presence of an organic source of energy. Hydrogen sulphide is the end product of the reduction process, although, as was seen before, the hydrogen sulphide may be immediately oxidized to water and sulphur.

Hydrogen sulphide is also a disintegration product of proteins, when these contain sulphur.

Two interesting organisms have been described by Green and Kestell[1] and are called by them Bacillus arsen-reducens (a

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[1] GREEN and KESTELL, "Behavior of Bacteria toward Arsenic," *South African Journal of Science*, vol. 15, pp. 369–374, 1919.

member of the colon-typhoid group), and Bacillus arsenoxydans. The one reduces arsenates to arsenites, and the other oxidizes arsenites to arsenates. It is interesting to note that most bacteria are killed by arsenic in a dilution of 0.05 per cent of the acid.

As a general rule, the oxidizing and reducing enzymes produced by bacteria are intracellular, that is, they are usually confined within the bacterial cell and do not diffuse throughout the medium in which the bacteria are growing.

In the bacteria producing carbohydrate, protein, and fat-splitting enzymes, however, there is every reason for thinking that these enzymes are produced in larger quantities than can be used by the organisms in their own metabolism, and the excess enzymes are freed from the cell with their powers as enzymes unimpaired.

Saprogenic Bacteria.—From the economic standpoint, the bacteria which produce proteolytic enzymes are of the greatest importance. Were it not for these organisms, the earth long ago would have been uninhabitable for either animal or plant, because of the accumulated dead refuse. The saprogenic bacteria are the scavengers of nature.

A number of different processes are grouped under the term "protein decomposition." As the proteins comprise an enormous group of substances of varying but always great complexity, it may readily be seen that a large number of different chemical reactions are possible. In general, the changes involving the splitting of the protein molecule may be divided into two stages.

First, the proteins which made up the protoplasm of previously living animals and vegetation are hydrolysed into the simpler albuminoses, peptones, and amino acids.

Second, the intermediate amino acids, etc. are split, with the liberation of ammonia.

Sometimes, the degradation of the protein molecule may be accomplished through successive stages of oxidation and reduction, as in the formation of indol from tryptophane. In some cases, in addition to ammonia, the gases carbon dioxide and hydrogen sulphide are end products. Sometimes we find a single organism like Bacillus mycoides capable of producing ammonia directly from a protein. This does not mean that a single enzyme produces all the stages of decomposition, but that one organism produces several enzymes.

Some authorities still draw a distinction between two processes of protein decomposition: putrefaction and decay. Putrefaction is defined as being protein splitting by anaërobic bacteria in which offensive odors are given off. The term "decay" is used to designate the process of protein hydrolysis in the presence of oxygen. The offensive odors of decomposing animal material are due to the formation of a sulphur compound, mercaptan. The odor of rotten eggs is largely due to hydrogen sulphide. As these are non-nitrogenous substances liberated during the splitting of the protein molecule and do not at all indicate a type of chemical change, the differentiation of putrefaction and decay is not justified.

Zymogenic Bacteria.—Although it has been known from time immemorial that milk will sour on standing, until Pasteur's classical work on fermentation in 1857 the true cause of this change was not understood.

Pasteur showed that the lactose, or sugar of milk, was converted by certain bacteria into lactic acid. This acid, like all other acids, will coagulate the casein in the milk, producing the characteristic curd. Lactic fermentation has received a great deal of attention since the time of Pasteur, especially from Metchnikoff and his co-workers.

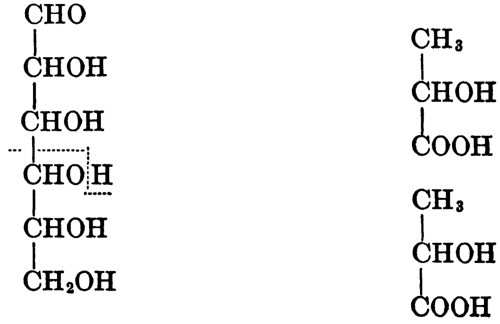
Two distinct processes of lactose fermentation are known. In one, carried out by certain anaërobic bacteria, the lactose is first inverted into two hexose molecules:

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6.$$

This reaction is carried out by an enzyme known as invertase.

The hexose molecule is then split up into two molecules of lactic acid:

$$C_6H_{12}O_6 = 2C_3H_6O_3 \quad (CH_3CHOHCOOH)$$



$$\begin{array}{l} \mathrm{CHO} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHO\vdots H} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_2OH} \end{array} \qquad\qquad \begin{array}{l} \mathrm{CH_3} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{COOH} \\ \\ \mathrm{CH_3} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{COOH} \end{array}$$

It will be noticed that one half of the hexose molecule is oxidized and the other half reduced.

A large number of bacteria produce these amylolytic enzymes. Lactobacillus bulgaricus and Streptococcus lebenis are examples.

The lactic acid thus formed may be acted upon by the enzymes of other zymogenic bacteria, as, for example, Clostridium butyricum or Clostridium amylobacter, and be converted into butyric acid, carbon dioxide, and free hydrogen:

$$2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + 2H_2.$$

Other aërobic bacteria utilize the sugar molecule as a source of energy and oxidize it directly into lactic acid, carbon dioxide and water:

$$C_6H_{12}O_6 + 3O_2 = C_3H_6O_3 + 3CO_2 + 3H_2O.$$

The above cases are simply types of carbohydrate splitting by bacterial enzymes. There are, of course, a great many organic compounds of carbon, oxygen, and hydrogen which fall under the general classification of carbohydrate. A large number of bacteria are involved in their splitting. Some bacteria can only ferment one or at most a few carbohydrates; some are able to decompose a great variety. Even when two or more bacteria split the same carbohydrate, the end products may be different. It is apparent, then, that the same zymogenic bacterium produces several different amylolytic enzymes.

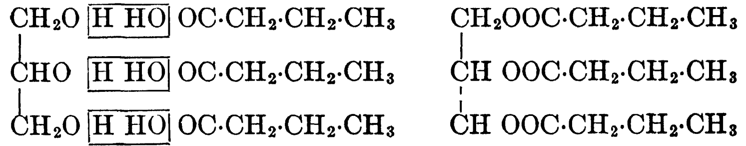
The following table gives an idea of this complexity:

Bacterium	Carbohydrates					
	Dextrose	Lactose	Sucrose	Maltose	Mannite	Inulin
Staphylococcus aureus...	Acid	Acid	Acid	Acid	Acid	—
Streptococcus pyogenes .	Acid	Acid	Acid	—	—	Acid
Neisseria intracellularis..	Acid	—	—	Acid	—	—
Neisseria gonorrhœæ.. ..	Acid	—	—	—	—	—
Escherichia coli... . .	Acid and Gas	Acid and Gas	—	Acid and Gas	Acid and Gas	—
Eberthella typhi.	Acid	—	—	Acid	Acid	—
Clostridium tetani.....·.	—	—	—	—	—	—
Clostridium botulinum..	Acid and Gas	Acid and Gas	Acid and Gas	Acid and Gas	Acid and Gas	Acid and Gas

In reading a description of a great many bacteria, one runs across the characterization, "sugar fermentations variable." The expression means that, even under standard laboratory conditions, certain strains of a given species may not react uniformly in fermenting carbohydrates. By varying conditions of growth it is possible to change most zymogenic bacteria in this respect.

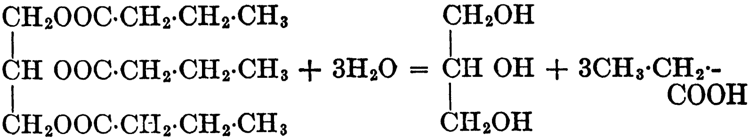
Glycerogenic Bacteria.—A few common bacteria produce steatolytic (lipolytic) enzymes, which split various fats into glycerine and fatty acids. Pseudomonas aeruginosa, Serratia marcescens, and several of the pyogenic Staphylococci belong to this group of glycerogenic bacteria. The process of fat splitting is so universally associated with protein splitting (putrefaction) that it is commonly classed with this process, and the glycerogenic bacteria are classified as a special group of the saprogenic bacteria. Inasmuch as no nitrogen is concerned in the operation, the process more closely resembles fermentation carried on through the agency of the zymogenic bacteria. Glycerine may be produced commercially by the fermentation of sugar by a yeast (p. 283).

The process of fat degradation may easily be carried out in the laboratory without the agency of bacteria. It is called "saponification," and is a hydrolytic action; that is, water is added to the fat molecule, and it is then split up into its fatty acid and glycerine.



$$\begin{array}{l} CH_2O\boxed{H\ HO}OC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \\ | \\ CHO\ \boxed{H\ HO}OC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \\ | \\ CH_2O\boxed{H\ HO}OC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \end{array} \qquad \begin{array}{l} CH_2OOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \\ | \\ CH\ OOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \\ | \\ CH\ OOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \end{array}$$

Glycerine Butyric acid three molecules Butter fat



$$\begin{array}{l} CH_2OOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \\ | \\ CH\ OOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \\ | \\ CH_2OOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3 \end{array} + 3H_2O = \begin{array}{l} CH_2OH \\ | \\ CH\ OH \\ | \\ CH_2OH \end{array} + 3CH_3{\cdot}CH_2{\cdot}COOH$$

Fat + hydrolysis = glycerine + fatty acid.

The fatty acid thus formed may be decomposed into carbon dioxide and water by bacteria.

The various processes carried out through the agency of bacterial enzymes may be summarized and the elements necessary to life may be followed in complete cycles.

Nitrogen Cycle.—Starting at any point, as with living animal protein (protoplasm), a nitrogen atom may take the following journey: The animal protein, upon katabolism in the living body, is given off as urea, uric acid, or a ureate. This substance is acted upon by the proteolytic enzymes of the saprogenic bacteria and broken down into ammonia. If the animal dies before the nitrogen is converted into urea, it will be acted upon directly as animal protein by the saprogenic bacteria and the result will be the same.

As a constituent of ammonia, the nitrogen atom may be acted upon by the oxidizing enzymes of a nitrifying bacterium and built up into a nitrite, and finally into a nitrate. As a nitrate, the nitrogen may be absorbed by a plant and through plant anabolism built up into plant protein. From here it may pass to animal protein through the digestion of the plant by an animal (animal anabolism), completing the cycle.

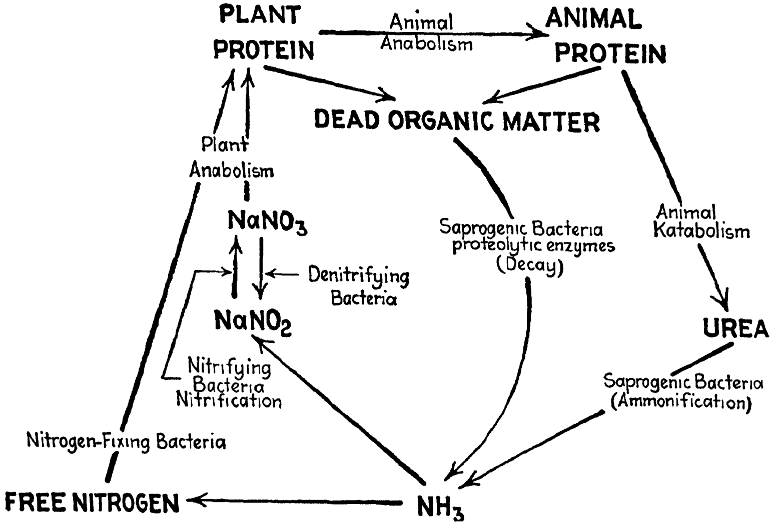


FIG. 16.—Nitrogen cycle.

The cycle may take other forms, however. In case the plant dies and is not utilized as food by an animal, the nitrogen atom may be acted upon by the saprogenic bacteria, and be converted again into ammonia. The ammonia may be oxidized to nitrate and then, instead of being taken up by plants, the nitrate may be reduced by the reducing enzymes of the denitrifying bacteria back to nitrite. The reduction of nitrates and nitrites may not stop with the formation of ammonia, but this may be further decomposed and the nitrogen atom may become free, or atmospheric, nitrogen. In this condition it can be acted upon by the nitrogen-fixing bacteria and become available for plants.

Carbon Cycle.—As in nitrogen, carbon goes through a "cycle" in nature. In this case bacteria do not have the essential rôle that they do in the nitrogen cycle, yet, as is seen from the fermentation of sugar, carbon dioxide is produced through bacterial agencies.

Nature has provided that plant photosynthesis and the respiration of animals be diametrically opposed as regards carbon.

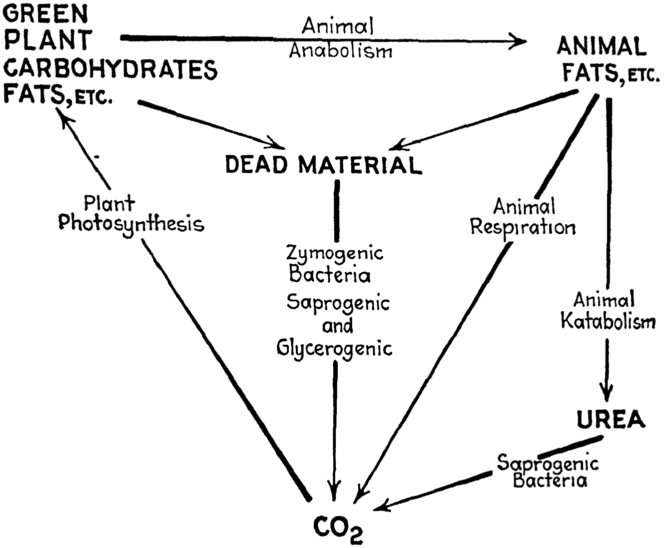


FIG. 17.—Carbon cycle.

Thus the green plants, with the aid of sunlight, absorb carbon dioxide and give off oxygen, using the carbon to build up their proteins and carbohydrates. Animals inhale oxygen and exhale carbon dioxide. Thus, a carbon "cycle" is mainly back and forth through plant and animal. Since carbon is present in proteins, carbohydrates, and fats, its compounds are acted upon not only by the zymogenic bacteria, but by all other metatrophic and paratrophic bacteria, and, therefore, a carbon cycle could not be complete without including the work done by bacteria.

As in the nitrogen cycle, an atom of carbon, in the form of animal protein, will be taken as the starting point. In this case, by animal katabolism, the carbon atom may be eliminated from the animal in the form of urea, or by animal respiration given off as carbon dioxide. In the latter case it may be taken from the air by plants in their metabolism. In the former case the journey of the carbon particle is longer. The saprogenic bacteria, through their proteolytic enzymes, may convert the urea into ammonia and carbon dioxide, and then the carbon in the form of carbon dioxide is available to plants.

In case the animal dies, the carbon atom may, through the decomposition of the animal protein, appear in the form of methane (CH_4). Here it may be acted upon by an oxidizing bacterium (Methanomonas methanica), and the methane changed into carbon dioxide and water.

Plant proteins may be split up in the same way. Carbohydrates are acted upon by the zymogenic bacteria, which through their amylolytic enzymes finally convert the carbon particle into carbon dioxide. Graphically, the carbon cycle may be indicated as in Fig. 17.

Hydrogen and oxygen, the two remaining elements essential for life, also go through "cycles," through the agency of bacterial enzymes. These, for all purposes, may be studied in the nitrogen and carbon cycles. Their principal form outside the living organism is in the compound, water. Free hydrogen formed, for instance, in the fermentation of a lactic acid may be reclaimed by the action of the "hydrogen bacillus," Hydrogenomonas pantotrophus. This interesting bacillus can oxidize hydrogen into water—solely, however, in the presence of carbon dioxide. Formaldehyde is formed as an intermediate product. Atmospheric oxygen is used in energy transformations by all plants and animals, with the exception of the anaërobic bacteria. These organisms obtain their necessary oxygen from organic compounds, usually carbohydrates.

Phosphorus Cycle.—The element phosphorus comprises from 5 to 10 per cent of nucleoproteins. It is thus present in the nuclei of all animal and vegetable cells. When these proteins are decomposed by the saprogenic bacteria, the phosphorus is split from the purine and pyrimidine bases, and from carbohydrates, and takes the form of phosphoric acid. Phosphine (PH_3) and other phosphorus compounds may be intermediate compounds. The phosphoric acid immediately unites with any alkali to form soluble or insoluble phosphates. If soluble phosphates are formed (Na_3PO_4, etc.), the element may be directly assimilated by plants, built up into plant nucleoproteins and the cycle completed.

In most cases, however, an insoluble phosphate, normal calcium phosphate ($Ca_3(PO_4)_2$) is formed. In animal refuse about 80 per cent of calcined bone consists of this salt.

The transformation of the insoluble phosphorus compound into one available to plant metabolism may be carried out in nature in three different ways, in all of which bacteria play a part. These three processes go on at the same time and should not be considered as independent actions.

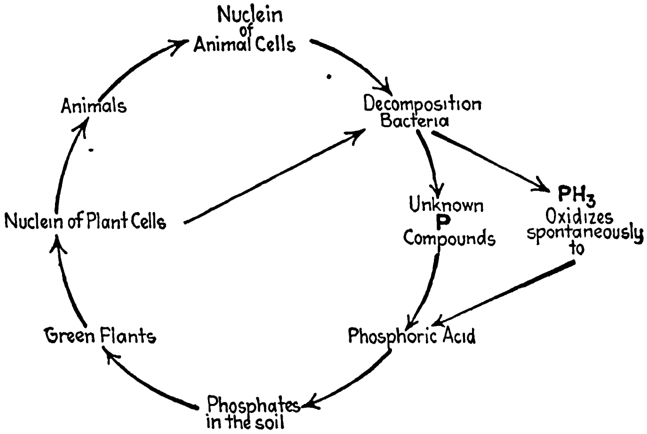


FIG. 18.—The phosphorus cycle. (*After Morrey.*)

First, certain bacteria secrete an intracellular enzyme which is capable of preparing the insoluble tricalcium phosphate for assimilation into bacterial protoplasm, and probably, plant protoplasm.

Second, when normal calcium phosphate is treated with water and carbon dioxide, it readily goes into solution. This is accounted for by the conversion of the insoluble salt into a soluble phosphate, thus:

$$Ca_3(PO_4)_2 + 4CO_2 + 5H_2O = Ca(H_2PO_4)_2 \cdot H_2O + 2Ca(HCO_3)_2.$$

Some of the carbon dioxide may have been washed into the soil from the air. The reaction may be accounted for entirely by bacteriological means when we remember that in both carbohydrate and protein degradation CO_2 may be produced.

Third, the insoluble phosphates may be transformed into soluble salts by organic or inorganic acids formed by bacteria.

$$Ca_3(PO_4)_2 + 4HNO_2 = Ca(H_2PO_4)_2 + 2Ca(NO_2)_2.$$

Sulphur Cycle.—In tracing the cycle of a sulphur atom in nature, the observer is struck by the similarity of its course to that of the nitrogen atom. In the splitting of dead animal proteins by the saprogenic bacteria, the sulphur in the protein molecule is liberated as hydrogen sulphide. No sulphur compound analogous to urea is excreted by the animal.

The hydrogen sulphide is oxidized by various oxidizing bacteria to sulphur and then to sulphuric acid. This acid immediately combines with the alkaline earths to form sulphates.

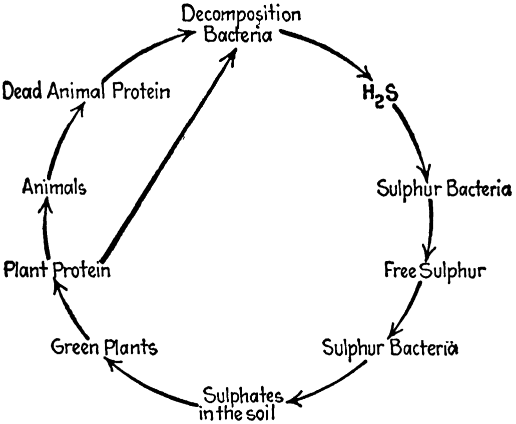


FIG. 19.—The sulphur cycle. (*After Morrey.*)

The sulphates are assimilated by plants and converted into plant protoplasm. As in the case of the nitrogen cycle, however, the oxidation reactions may be followed by a reduction process. The sulphate, instead of being taken up by the plants may be again reduced by certain reducing bacteria, especially in the presence of organic substances and under anaërobic conditions:

$$3CaSO_4 + 2CH_3CHOHCOONa = 3CaS + 2Na_2CO_3 + 2CO_2 + 3H_2S + 2H_2O$$

$$CaS + CO_2 + H_2O = CaCO_3 + H_2S.$$

These opposite oxidation and reduction reactions may be going on simultaneously in the same soil, oxidation at higher levels, and reduction in the deeper layers.

It will be apparent from these "cycles" that a number of different chemical changes are constantly taking place in nature, due to bacterial action. Moreover, it is impossible to look upon these various changes as separate processes. In the decay of a dead plant or animal, oxidizing, reducing, zymogenic, saprogenic and glycerogenic bacteria may all play a part, either simultaneously or consecutively. The oxidation of an organic compound may take place under the same conditions as the reduction of sulphates. So it is impossible to say where one process ends and another begins. Emphasis is again placed on the fact that bacteria, through their enzymes, are responsible for these essential transformations of elements which are necessary to life.

CHAPTER VIII

MICROÖRGANISMS OTHER THAN BACTERIA

The bacteriologist of today, whether he be primarily interested in agriculture, in sanitation, in disease, or in the fermentation industries, constantly has to do with microscopic organisms which cannot, biologically, be classed as bacteria. A few of the more important of these forms will be described.

Filterable Viruses.—In the study of diseases and their bacterial causes, several pathogenic conditions have been demonstrated to be due to living organisms, and yet no specific microörganism capable of producing the disease has been isolated. For example, if some of the material is extracted from the vesicles in the mouth or mucous membranes of an animal suffering from foot-and-mouth disease, and filtered through a diatomaceous earth filter, and then examined for bacteria by microscopic and cultural methods, it will apparently be sterile. Yet, if this filtered lymph is inoculated into a healthy animal, that animal will contract foot-and-mouth disease. By successive filtrations and inoculations, the disease can be transmitted through a long series of animals, proving that the filtrate contains not simply the toxins of the disease but some organisms capable of reproduction.

This and similar observations gave rise to the idea of the existence of an ultramicroscopic form of life which would explain the transmission of disease in which no bacterial agent could be found. Hog cholera, the various "poxes"—chicken pox, cowpox, smallpox, etc.—poliomyelitis (infantile paralysis), mumps, and measles are some of the more common human and animal diseases which are thought to be due to organisms of this kind.

The term "filterable virus" has been pretty generally adopted as applied to these forms. It is open to severe objections, as, properly speaking, some of these organisms are not "filterable." For example, the smallpox germ remains behind in a diatomaceous earth filter. Moreover, a great many of the true bacteria are probably "filterable" at some stage in their life cycle.

As summarized by Rivers,[1] a number of factors are involved in filtration. The amount of electrical charge on the virus or on the filter, temperature, pressure employed, and the amount and kind of other protein material with the virus all may influence filtration.

> Furthermore, sufficient attention has not been given to the possibility that some filters may not only hold back certain viruses but may also inactivate them in some manner so that they can never become active again.

The term "filterable virus," however, has come into general use, and, as it serves as a label for the causative agents in diseases in which contagion indicates some form of living but unknown virus, its use is justified.

The study of these ultramicroscopic living organisms has been confined to obligate parasitic forms. Even cultivation has so far been limited to growth on living tissue. It is not improbable, however, that many non-pathogenic organisms belong to this class, which cannot, because of inadequate technique, be classified as animal or vegetable.

Bacteriophage.—An interesting phenomenon was observed by Twort[2] during attempts to cultivate vaccine virus on artificial culture media. Colonies of Staphylococci, which are always present as a contamination in vaccine virus, were seen to become transparent and completely disappear. Twort thought that the cause of the lysis of the cocci was an enzyme with the power of growth, or else living protoplasm without form. This "substance" was later studied by d'Herelle[3] who named it "bacteriophage." Bacteriophage dissolves and completely consumes cultures of bacteria, either in vitro or in living tissues. d'Herelle believes that it is a parasite which lives on bacteria. Some workers think that there are a number of different bacteriophages, others that bacteriophage is non-specific, attacking all species of bacteria, although a specificity may be developed for particular organisms.

The habitat of this organism is the intestinal tract of animals. In every case of intestinal disease, according to d'Herelle, no

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[1] RIVERS, T. M., "Filterable Viruses," p. 10, Baltimore, 1928.

[2] TWORT, F. W., "An Investigation on the Nature of Ultramicroscopic Viruses," *Lancet*, ii, p. 1241, 1915.

[3] d'HERELLE, F., "Sur un microbe invisible antagoniste des bacilles dysenteriques," *Comptes rendus Academie de Sciences*, vol. 165, p. 373, 1917.

matter how mild, bacteriophage may be isolated from the fæces at the beginning of the illness. It can always be isolated from convalescent cases, but not from fatal cases.

In its relation to its environment, bacteriophage resembles both bacteria and enzymes. It loses its activity when dried; it is "killed" at about 70°C. in 1 hour; its optimum reaction, depending on specificity developed, is between pH 4.5 and pH 9.0.

Jules Bordet[1] and others are of the opinion that bacteriophage is not a living organism but an autolytic ferment produced by the bacteria after vitiation by leucocytes in their host.

Philip Hadley[2] conceives of the bacteriophagic reaction

> . . . as representing one of the mechanisms for maintaining the wide range of cell and culture changes which are presumably adaptive, which are a constituent part of the specific cyclogeny and which are, therefore, inherent in the biology of the species. The most fundamental aspect of the bacteriophagic reaction is not lysis and destruction but cell transformation.

Hadley thinks that bacteriophage represents a stage in the life cycle of the specific bacterium it "dissolves." He closes his monograph on "Microbic Dissociation"[3] with the following prophetic paragraph:

> . . . and, finally, if for a moment we abandon ourselves to speculation, though not perhaps without some evidence in fact—it may eventually be demonstrated, not that a filterable virus gives rise to dissociation and to autolysis in the d'Herelle sense, but, on the contrary, that the fundamental physiologic reaction, of which both microbic dissociation and transmissible autolysis are only different modes of expression, gives rise to the filterable virus.

Spirochætes.—In 1833, Ehrenberg gave the name Spirochæta to a flexible microörganism he found in water and which reminded him of a finely coiled hair. Since then a large variety of organisms, more or less resembling each other morphologically, have been grouped under the general term Spirochæte. The causative

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[1] Bordet, Jules, *Johns-Hopkins Hospital Bulletin*, vol. 32, pp. 302–303, Baltimore, 1921.

[2] Hadley, Philip, "The Twort-d'Herelle Phenomenon," *Journal of Infectious Diseases*, vol. 42, p. 421, 1928.

[3] Hadley, Philip, "Microbic Dissociation," *Journal of Infectious Diseases*," vol. 40, no. 1, p. 296.

agents of a number of diseases, *e.g.*, syphilis, epidemic jaundice, relapsing fever, and yaws, are placed in this group. Parasitic, but non-pathogenic forms are found in smegma, and in the alimentary canals of man and shellfish. Saprogenic Spirochætes are found in fresh and salt water.

Although placed in the class Schizomycetes, or bacteria, by the Committee on Classification of the Society of American Bacteriologists, there is considerable doubt as to whether all or any of these organisms are bacteria. In many ways, in fact, they more closely resemble animals than plants. Syphilis in its chronic stages closely resembles typical protozoan infections. The characteristics of flexuous body, axial filaments, elastic membrane, and peristaltic movement make these organisms decidedly unlike other bacteria. On the other hand, in their reproduction by transverse fission—usually binary—and in having no morphological nuclei, they resemble bacteria.

Except for a few species, the Spirochætes are corkscrew shaped, with a flexible, cylindrical body. The length of the saprogenic species is from 45 to 500μ, of the parasitic and pathogenic species, 8 to 15μ. They are quite thin, the bodies of the pathogenic forms averaging about 0.25μ. The number of spiral turns varies with the species, the shorter pathogenic forms being also more closely wound. Treponema pallidum has from 14 to 24 spirals.

The Committee on Classification of the Society of American Bacteriologists[1] have grouped these forms of the order Spirochætales all under the one family Spirochætaceæ, and have divided the family into six genera as follows:

1. *Spirochœta:* Non-parasitic, with flexible, undulating body and with or without flagelliform, tapering ends. Protoplasm is spirally wound around an elastic axial filament. Common in sewage and foul waters.
2. *Cristispira:* Giant forms with undulating body and peculiar flattened ridge or "crest," erroneously called an "undulating membrane," which runs the length of the body. Parasitic in molluscs.
3. *Saprospira:* Non-parasitic forms similar to Cristispira, but without the flattened ridge or "crest," which is either absent or is replaced by a straight columella or thickening of the periplast. Cross-section is regular.

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[1] Bergey's "Manual of Determinative Bacteriology," Baltimore, 1930.

4. *Borrelia:* A small, spiral, flexible body, with terminal filaments but no membrane. Spirals are large, three to five in number. Species of the genus are the cause of relapsing fever.

5. *Treponema:* Parasitic and frequently pathogenic forms with undulating or rigid spirilliform body, without crest or columella. With or without flagelliform tapering ends. Treponema pallidum, the causative agent of syphilis, is of this genus. This organism was first observed by Schaudinn and Hoffman in 1905.[1]

6. *Leptospira:* Parasitic forms. Sharply twisted cylinders with flagelliform tapering ends, one extremity being sharply curved into a "hook." Leptospira icteroides is the cause of yellow fever. Another species of this genus causes infectious jaundice.

"Higher Bacteria."—Among the older textbooks on bacteriology, the term "higher bacteria" was used for the group of more highly specialized form of the Schizomycetes. The term corresponds in a sense to Migula's Thiobacteria, and even more closely to Fischer's Trichobacterinæ. In a general way they include the species which we today place in the orders Myxobacteriales, Thiobacteriales, and Chlamydobacteriales. Some species of the Actinomycetales would also be included in this group.

The organisms belonging to this group are all devoid of chlorophyll. The individual cells are usually united by a strong sheath (closely allied to the capsules of the order Eubacteriales) which holds them in filaments. These filaments show either true or "false" branching. There may be a slight differentiation of function among the cells of this group of organisms, in that the cells at one end of the filament seem to supply the food, while at the other end the cells are set apart for reproduction. The method of reproduction is either by binary fission or by the formation of what is known as conidia (singular, conidium). Conidia are spores (not like the endospores of the Eubacteriales) similar to those produced by molds. The individual cells which make up the filament are capable of independent existence outside the filament.

The order *Myxobacteriales* includes those forms in which the vegetative cells are held together by a gelatinous secretion into

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[1] SCHAUDINN and HOFFMAN, *Arb. a.d. Gesundhtsamte*, vol. 22, p. 527, Berlin, 1905; *Deutsche med. Wochenschrift*, vol. 31, p. 711, 1905.

a pseudoplasmodium which resembles the plasmodium, or naked protoplasm, of the slime molds (Myxomycetes). These "higher bacteria" differ from the slime molds in that the individual cells do not fuse into a true plasmodium. The organisms are motile rods multiplying by fission. They are fairly constant in size, even among different species. Just before dividing, they average a little less than 15μ in length. Their life cycle is complex. They are common in manure, compost heaps, decaying wood, and on other moist surfaces.

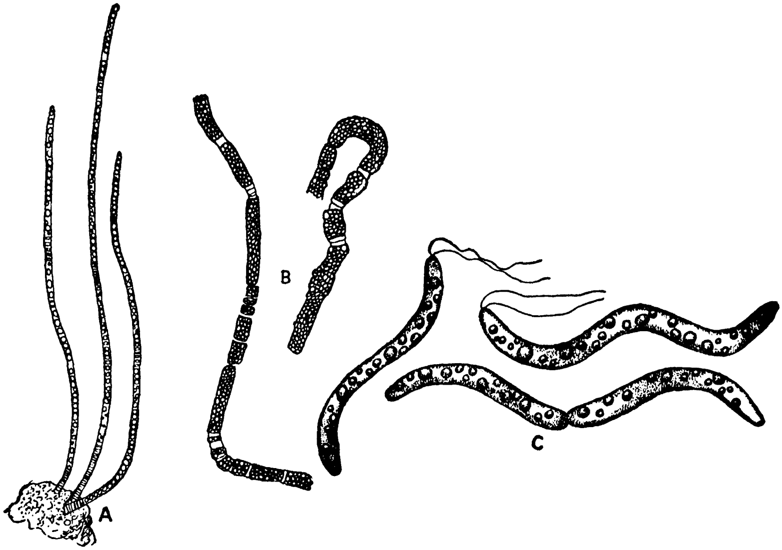

FIG. 20.—Forms of sulphur bacteria. *A* and *B*, thiothrix annulata. *C*, spirillum granulatum. (*Lutman.*)

The order *Thiobacteriales* includes the forms in which growth energy is obtained from the oxidation of simple sulphur compounds. The cells are free or united in long filaments. A pseudoplasmodium is never formed. The organisms usually contain granules of free sulphur or of bacteriopurpurin. Spores are rarely found. In masses, these organisms usually show a beautiful violet or red color. They are difficult to cultivate on artificial media. Sulphur deposits may be due to the activities of these organisms.

The *Chlamydobacteriales* form filaments like the Thiobacteriales, but sulphur and bacteriopurpurin granules are absent. They do not form pseudoplasmodia. Iron is frequently present in the sheath, from which fact they get the name "iron bacteria." They are typically water forms.

Other Thallophytes.—The "higher bacteria," like the Eubacteriales, comprise orders of the class Schizomycetes. The Schizomycetes in turn form one class of the phylum Thallophyta, the seedless, rootless, stemless plants.

While only distantly related to the bacteria, some species of the other classes of the Thallophyta are of great interest to bacteriologists. Others, no farther removed phylogenetically, cannot, because of their size, be considered as microörganisms.

Botanists divide the phylum Thallophyta into two subphyla: the Fungi and the Algæ. The Fungi are saprophytes or parasites like most of the bacteria. The Algæ manufacture their own food by the process of photosynthesis, possessing, like the higher plants, chlorophyll. Except in this particular, the Fungi and Algæ are quite similar. Many botanists believe that the Fungi are degenerate forms of Algæ, and that particular classes of the Fungi have been derived from corresponding classes of Algæ.

Especially in their means of reproduction do the Fungi and Algæ show close similarities. As has been seen, the bacteria reproduce in a number of ways. Among the other classes of Thallophytes there are many diverse and highly complex methods of reproduction. Even in the same species, several methods of propagation may be found. Like the bacteria, these organisms may reproduce by binary fission or by conidia formation. In addition, they form spores which, unlike the resting spores of bacteria, are true reproductive bodies. Some are equipped with organs of motility, and swim freely in water. Some species reproduce by sexual means in the union of two cells into zygospores (where two cells are alike) or oöspores (where the two uniting cells are different).

Fungi.—More than forty thousand species of Fungi have been described, ranging in size from microscopic forms to those several feet in diameter. A number of different methods of classifying the Fungi have been proposed. For the present purpose, the subphylum will be divided into three classes: the Phycomycetes, the algæ-like fungi or tube fungi; the Ascomycetes, or sac fungi; and the Basidiomycetes, or basidium (base) fungi. The method of reproduction, or, rather, the type of organ which carries the reproductive bodies is used as the basis of classification.

The class *Phycomycetes* consists of a group of algæ-like fungi which includes many of the common molds. The black bread mold (Rhizopus nigricans), and several species pathogenic to plants belong to this class. Among the latter, the "potato blight," "grape blight," and "onion mold" cause economic losses.

All of the members of this class are multicellular, the plant body consisting of a tangled network of web (called mycelium) of branched threads. The individual threads are known as mycelial hyphæ. The mycelium spreads along the surface of the medium on which the fungus is growing, and penetrates more or less deeply into the substratum.

The members of the class Phycomycetes are sometimes called "tube fungi," since the mycelial hyphæ consist of many irregular, ill-defined cells lying within a common wall and giving the appearance of a single tubular cell with many nuclei.

Reproduction may be either asexual or sexual. In the case of asexual reproduction, erect shoots or aërial hyphæ appear at various places along the mycelial hyphæ. The aërial hyphæ develop straight upward and at the tip of each appears a globular sporangium which at maturity contains thousands of spores. When the sporangium breaks, these spores are scattered by air currents. Each spore is capable of germinating into a new plant. In sexual reproduction, mycelial hyphæ fuse together, forming bodies known as zygospores or oöspores, depending on the similarity or dissimilarity of the uniting bodies.

The second and third classes of Fungi differ essentially from the Phycomycetes, especially in the multicellular forms. Here the hyphæ are made up of well-defined cells separated from one another by septa (singular, septum), each cell containing a single nucleus.

The class *Ascomycetes,* or sac fungi, includes a number of orders, certain species of which are quite important to man. Among those which are beneficial are the yeasts, the edible morels and truffles, Penicillium (the mold which gives Roquefort and Camembert cheeses their flavor), the ergot (Claviceps), and lichens which are used in medicine. Those Ascomycetes which are harmful to man are powdery mildews, the black knot of fruit trees, the chestnut blight, etc.

The term "sac fungi" is derived from the reproductive body, which is usually in the form of a sac.

The *yeasts* are unicellular "sac fungi" characterized by the formation of buds. The cells are spherical, ellipsoidal, or cylindrical, consisting of granular protoplasm (especially in old cells), surrounded by a thin cell wall which gradually grows thicker

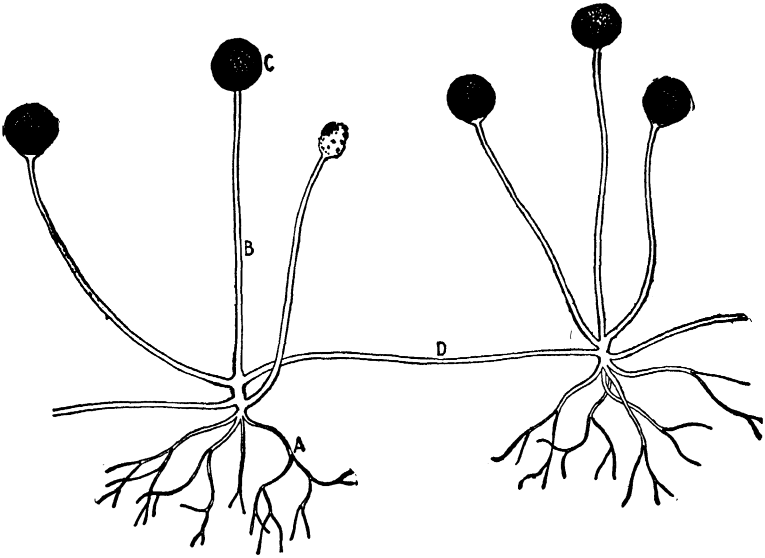

FIG. 21.—Bread mold. Rhizopus. *A*, mycelium. *B*, aerial hypha. *C*, sporangium. *D*, stolon connecting two groups of sporangrophores. (*Sinnott.*)

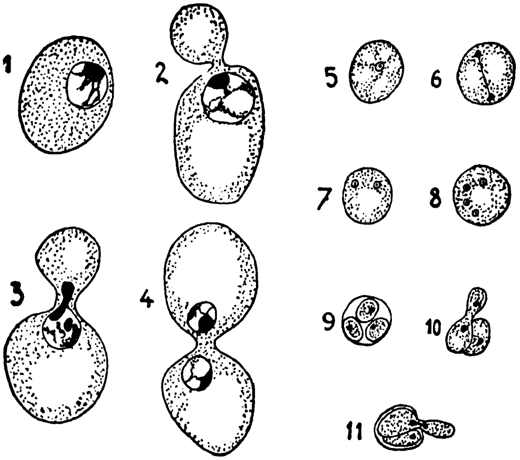

FIG. 22.—Yeast. Saccharomyces cerevisiae. 1–4 shows bud with division of nucleus; 5–11 sporulation. (*Dodge after Guillermond.*)

with age. The protoplasm contains a nucleus and one or more cavities called "vacuoles." Reproduction takes place by fission, by sporulation, or by budding. In the latter case, a slight swelling takes place on the periphery of the cell. This swelling slowly enlarges, becoming constricted at its base and forming a knoblike protrusion, or bud. The bud finally becomes completely separated from the parent cell and is then an individual cell. Often the daughter cell becomes an adult, and reproduces before it completely separates from its mother cell. The result is a chain of cells or a branching cluster. When reproduction takes place by fission, the nucleus divides first, forming two new nuclei, and a division of the cytoplasm follows.

When young, vigorous cells are placed in an unfavorable environment (as to food) they assume the form of asci or comparatively large cells in which new cells are developed. The ascus divides into four ascospores which, when placed in suitable food, enlarge and break free from the mother cell. In one genus (Saccharomycoides) the germinating spore and its daughter cell hold together, forming a promycelium.

Yeasts are divided into two families according to whether reproduction takes place by fission (Schizosaccharomycetes) or by budding (Saccharomycetes). The principal genus belongs to the Saccharomycetes and is known as Saccharomyces. Saccharomyces cereviseæ is the common bread yeast. It, like all other yeasts, produces an amylolytic enzyme which converts sugar into alcohol and carbon dioxide.

Recently, the use of yeasts for therapeutic purposes has received considerable attention. Beneficial results have been reported from its use in certain cutaneous and gastro-intestinal diseases, such as acne and constipation. It is thought by some workers that yeasts need an accessory growth substance besides pabulum for their growth and reproduction. This substance, vitamin D, or better, "bios," may be produced by the cells themselves, or at least is present in a medium in which yeasts are growing. There is no germicidal power in yeast and no stimulating effect on either leucocyte production or antibody formation.

Very few yeasts are pathogenic, although a repulsive skin disease (Blastomycosis) is caused by one species.

The *Basidiomycetes* comprise the third class of Fungi. Here the reproductive bodies are carried as naked spores on special cells at the end of hyphæ. Among the Basidiomycetes are found the smuts and rusts, two very destructive orders to agriculture, and the puffballs, toadstools, and mushrooms.

Algæ.—The Algæ, second subphpla of the Thallophytes, are distinguished from the Fungi by the presence of chlorophyll, and from the higher plants by the absence of roots, stems, and leaves. Because they are chlorophyll bearing, they are capable, like the higher plants, of synthesizing organic carbon compounds from carbon dioxide and water. The Algæ are primarily water forms. The subphylum is divided into four groups according to their color. All possess chlorophyll, but in all but the green algæ the color is obscured or masked by other pigments.

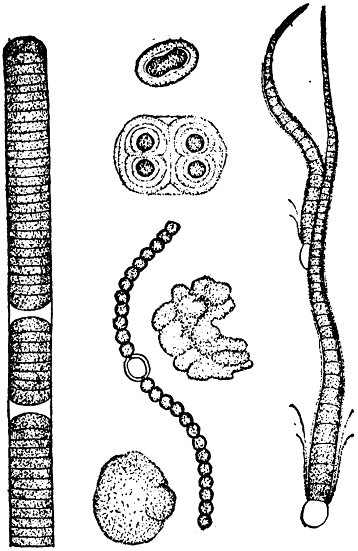

FIG. 23.—Cyanophyceae. (*Sinnott.*)

The Cyanophyceæ.—These are the blue-green algæ, and are the lowest forms of green plants. They are only loosely related to the other algæ, some scientists placing them in a third subphylum between the Algæ and Fungi. Some forms are practically identical with the bacteria morphologically, except that they are larger and possess chlorophyll and other coloring substances. Their color is never a distinct green, but may be blue-green, brownish, reddish, etc., depending on the balance of the different pigments. The coloring matter is diffused throughout the cell and not located in plastids, as in the green algæ. It is usually bluish green, due to the presence of phycocyanin, a blue pigment. The Cyanophyceæ possess characteristic odors and often cause trouble in water supplies. They are either unicellular, or exist as filaments. Oscillaria is one of the most common of blue-green algæ. Anabæna, Clathrocystis, Coelsphærium, and Aphanizomenon are the most troublesome of this group of plants.

The Red Sea gets its name from the presence of enormous numbers of a species of blue-green algæ (not from the red algæ, as might be expected). Many of the vivid colors of hot-spring deposits are due to the deposition of these forms.

The Chlorophyceæ.—Due to the presence of chlorophyll (usually there is no other masking color present) most of the Chlorophyceæ are bright green. The cell possesses a well-defined nucleus, starch grains, and very often a cell wall of true cellulose. The plant may be either one-celled, as the Protococcus, or many-celled, as the Spirogyra. Most are aquatic, usually marine, though a few forms find their natural habitat on damp soil. The Chlorophyceæ produce either asexually, by the formation of spores within the cell; or sexually, either through the union of two similar zoöspores, or by the fertilization of female cells by male cells. There are a great many species. A few of these species, taken as types, will answer for a description of the group.

The Protococcales.—One-celled, spherical forms. Sometimes small, irregular clusters are formed through the union of the thin cell membrane. The diameter of the cell varies greatly with the individual, but averages about 25μ. They may be motile or non-motile. The genera of this family which are to be found in water are the Pleurococcus and Volvox. Pleurococcus consists of a single cell, and reproduces only by division. It contains one large chloroplastid. Volvox is a term applied to a colony of one-celled, green, ciliated organisms which are placed among the Chlorophyceæ by many biologists. Considerable doubt exists, however, as to whether Volvox is a plant. Zoölogists classify this interesting organism as a Protozoan and place it among the Flagellata (Mastigophora). The colony is spherical and may consist of as many as 10,000 cells.

The Conjugales.—These are single-celled or unbranched, filamentous forms. They are non-motile or motile and may reproduce by sexual as well as by non-sexual means. An enormous number of beautiful forms make up this family. The most familiar genera are those grouped under the Desmids and the Spirogyra. The Desmids are characterized by a demarcation of the cell into symmetrical halves and by the projection of spines on the surface of the cells. Multiplication takes place by cell division and by sexual means. There are many species, some assuming importance as troublesome factors in public water supplies.

Spirogyra is a filamentous alga in which the chloroplastid is a spiral running around the cell. The filament is bright green and forms large masses which can easily be collected. Pond scums usually are composed largely of this genus.

The Brown Algæ.—With the exception of the group of diatoms, brown algæ are found exclusively in salt water. Different species of this group are extremely interesting. One species, Laminaria, the common kelp, is a source of iodin and potassium. It grows to 800 feet or more in length—more than twice the height of the giant redwoods of California —yet it has no roots, no stems, and no leaves.

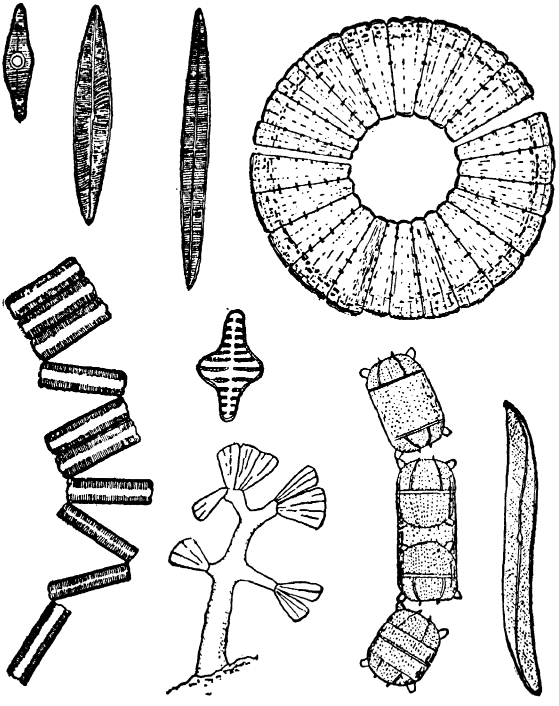

FIG. 24.—Diatoms. (*Sinnott.*)

The Diatoms possess a brown pigment (diatomin) in addition to chlorophyll. They have a hard cell wall, divided into two parts which fit together like a pill box and its lid. This wall contains silica and is marked in most wonderful ways. The Diatoms are usually motile and are found in abundance in fresh and marine waters. One genus, Asterionella, has been troublesome in public water supplies. The cells resemble long, small-headed dumbbells. They may be united into radially arranged groups resembling the rays of a star—hence the name *diatom* (a little star).

Deposits of "diatomaceous" earth, which consists of myriads of diatom shells, are often over a hundred feet in depth. The material, sometimes called Fuller's earth, is used to make bacterial filters. It is extensively used also as an abrasive in metal polishes, and sometimes unfortunately, in tooth pastes and powders.

The Red Algæ.—Like the brown algæ, these are almost always found in salt water, generally in warm climates. The agar-agar used in bacterial culture media is a red alga. Irish moss is another species.

Protozoa.—The Protozoa are unicellular, microscopic animals. As in the vegetable (yeasts, molds, etc.) and in the bacterial cell, the protozoan cell consists of a mass of protoplasm surrounded by a cell wall and containing a nucleus. These forms are sometimes solitary (as the Amœba) and sometimes united in colonies. They are either free living or parasitic. A few species possess chlorophyll and thus closely resemble plants. The Protozoa usually reproduce by binary fission, though not uncommonly by sexual means. The life cycle of many Protozoa includes both of these methods, as in the malarial parasite.

Separating the Protozoa into classes according to the presence or absence of organs of locomotion, and the nature of these organs when present, the group may be divided into four classes: (1) the Sarcodina, (2) the Mastigophora, (3) the Sporozoa, and (4) the Infusoria.

Of the *Sarcodina*, the Amœba is the most familiar example. It is common in the slime of ponds. It is a soft, colorless cell which constantly changes its shape by extending portions of its protoplasm to form pseudopods or "false feet." It ingests its food at any point on the surface of its body, accomplishing this by simply flowing around it. The food of Amœba consists usually of very small aquatic plants, but some species, as Endamœba coli, and Endamœba histolytica (Amœba dysenteriæ) are parasitic. Endamœba histolytica causes intestinal ulcers (Amœbic dysentery) which are frequently fatal to man. Hegner and Payne state that on the average about 9 per cent of all persons are infected with this protozoan. Until recently it was thought that pyorrhea was caused by a member of this genus.

The flagellates comprise the principal order of the *Mastigophora.* They differ from other Protozoa in the possession of one or more flagella at the anterior end of the body. Uroglena is a flagellate which, because of its oily odor, at times causes considerable trouble in public water supplies. The individuals are pear shaped, about 10μ in length. They have two flagella. Reproduction is by fission. The individuals are rarely found free; more often they are united into more or less spherical colonies just visible to the unaided eye (0.5 millimeter in diameter). As a rule, the colony is made up of a gelatinous mass with the individual Uroglena at the periphery. Dinobyron, Synura, Clenodinium, and Peridinium are other genera belonging to this class of Protozoa, which are troublesome in water supplies. Trypanosoma gambiense is an eel-shaped Flagellate about 1.5μ wide and 20μ long, possessing a single flagellum. It is a parasitic organism and causes a fatal disease in man known as "sleeping sickness." This organism is carried by the blood-sucking tsetse fly of Africa.

Leishmania donovani is a Hemoflagellate which causes a disease of the liver and spleen known as kala-azar. The organism is spherical or oval, varying from 2 to 4μ in diameter, and contains a relatively large nucleus.

The *Sporozoa* are Protozoa reproducing by spore formation and by sexual means. The best known and economically most important family of Sporozoa are the Plasmodia, which cause malaria. Malaria is transmitted to man solely through the bite of the anopheles mosquito. The Plasmodium has an extremely interesting life history. In man, the organism is asexual, reproducing by the formation of many spores. When the spores are freed, they are accompanied by a toxic secretion. A peculiar feature is that practically all the organisms present in a person suffering with malaria mature at the same time. Thus, the spores are liberated at the same time and also their accompanying toxin. This large amount of toxin produces a chill.

Three distinct types of malaria are distinguished during the time it takes the Plasmodium to develop from a freshly liberated spore to a mature organism ready to burst into its spores. Plasmodium vivax matures in 48 hours, Plasmodium malariæ in 72 hours, and Plasmodium falciparum irregularly, but always in less than 48 hours, and in more than 24 hours. The forms of malaria which these different organisms produce are called respectively, tertian, quartan, and quotidian (estivo-autumnal or malignant tertian).

In the anopheles mosquito, the Plasmodium goes through a sexual generation.

The *Infusoria* are Protozoa having cilia as organs of motion. Cilia also aid in gathering food for the organism. Although one celled, these animals have what may be likened to a rudimentary alimentary canal. Very few Infusoria are parasitic and only one (Balantidium coli) has been known to cause a disease in man.

CHAPTER IX

BACTERIA IN THE SOIL

Soil is that portion of the earth's surface on which plants can grow. The depth of the soil varies considerably, depending on geological formation, rainfall, and many other factors, both past and present. There is no soil on the bare rocks of mountain ranges, in the alkali wastes of New Mexico, nor in wide stretches of the Sahara Desert. In the deltas of great rivers or in alluvial plains, on the other hand, the soil may be a thousand or more feet in depth.

Composition of Soil.—In physical composition soil is a mixture of solids, water, and air. If a bit of soil is examined under the lens, it will be seen to consist of clumps of earth of different sizes and shapes more or less loosely packed together. Each minute clump is surrounded, except where it is in contact with other clumps, by a film of water containing dissolved mineral substances. This soil water keeps the atmosphere of the soil completely saturated. The interstices between the clumps consist of air spaces. Solids are both mineral and organic. The varying amounts of solid matter in relation to water and air, and the relative proportions of mineral and organic solids give rise to differences in physical and chemical properties of soils.

An idea of the comparative volumes of these constituents in soil may be obtained from the following table:[1]

Soil	Solid matter		Pore space	In pore space	
	Mineral	Organic		Water	Air
No. 1	62	4	34	23	11
No. 2	51	11	38	30	8
No. 3	41	12	47	40	7

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[1] Russell, Sir E. John, "The Microörg anisms of the Soil," p. 17, London 1923.

Soil No. 1 was arable, no manure applied to soil; No. 2 was arable, dung applied to soil; No. 3, pasture.

The *mineral* constituents of soil are the products of a long series of changes, both physical and chemical, which resulted in disintegration of rocks. This is usually accomplished through the agency of water, either by frost, by the grinding action of glaciers, or by actual solution and reprecipitation. Bacteria also play a part in some cases, as in the deposition of limestones, manganese, and iron rocks, the solution of silicates, zinc and aluminum compounds, and the transformation of phosphorus and sulphur into salts which are available for plant use. Some of these mineral constituents of the soil are essential plant foods, others are simply media in which the available materials are found.

The *organic* constituents of the soil are made available for the use of higher plants, chiefly through bacterial action. The richness of the soil depends on the amount of organic matter present, and on the rate at which this material is being acted upon by the bacteria.

Lipman[1] places the quantity of organic matter in light sandy soils at less than 1 per cent, with an increase to over 5 per cent in heavy silt or clay loams. "In muck or peat soils, the organic matter content may range up to 85 per cent."

Fertility of the soil means the availability of the elements necessary to plant growth. For agricultural purposes the fertility is maintained by the addition of salts which are lacking for the particular crop to be raised. Nitrogen, in the forms of nitrates, sulphates, phosphates, or potash has to be added at intervals, and the reaction, *i.e.*, acidity, or alkalinity, adjusted.

The part that bacteria and other microörganisms play in soil fertility has not been understood until recently, and even now the value of the study of soil bacteriology is not generally appreciated by farmers. A fertile soil is an excellent medium for bacterial growth. If the proper kinds of bacteria are present, they aid the plants still further by their activities. On the other hand, some kinds of bacteria are harmful to vegetation, through their chemical activities, and these forms, like weeds, also thrive in a fertile soil. The farmer's problem is not only to keep out undesirable plant life but also to keep the proper bacterial life in the ascendency.

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[1] Lipman, J. G., "Soil Fertility," in, "The Newer Knowledge of Bacteriology and Immunology," Jordan and Falk, p. 342, Chicago, 1928.

Numbers of Microörganisms.—The microörganisms that are natural inhabitants of the soil are bacteria, molds, and other fungi, algæ, and protozoa. The relative number of these organisms in any particular soil depends on certain environmental factors which aid them in their life processes or which limit their growth. In general, temperature, moisture, acidity, and the presence or the absence of the proper food or pabulum are the principal environmental conditions upon which they are dependent. The presence of other microörganisms which use bacteria as a source of food also has a bearing on the relative number of these organisms present.

For example, in different soils the microörganic population varies greatly, and even in the same soil the actual numbers of different kinds of organisms fluctuate constantly with external conditions such as rainfall, temperature, etc., which give rise to soil conditions more or less favorable to a particular type of life. The following table will give an idea of the average numbers of microörganisms present in a series of analyses of *one* soil.

NUMBER PER GRAM OF SOIL[1]

Bacteria	22,500,000	to	45,000,000
Fungi	700,000	to	1,500,000
Algæ	100,000		
Protozoa:			
Ciliates	100	to	1,000
Amœba	150,000	to	280,000
Flagellates	350,000	to	770,000

[1] RUSSELL, E. J., "The Microorganic Population of the Soil," p. 14, London, 1923.

Other authorities differ widely in their estimations. On bacterial counts, results as low as 600,000 per gram and as high as 800,000,000 per gram in the same type of loamy soil have been reported. Waksman[1] explains part of the discrepancies in counts by the methods employed. By the plate method he gives the numbers of bacteria in a normal soil as between 2,000,000 and 200,000,000 per gram, and remarks, "These numbers may vary with the soil type, soil treatment, season of year, depth, content, and various environmental conditions."

Kinds of Bacteria in soil vary with conditions. When it is realized that, with the exception of a very few water forms, and still fewer pathogens, bacteria get all their food and energy

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[1] WAKSMAN, SELMAN A., "Principles of Soil Microbiology," p. 28, Baltimore, 1927.

requirements from materials which are in, or destined to become part of, the soil, it is readily understood how diversified the specific flora of any particular soil may be. Several schemes of grouping the bacteria in the soil have been used. Conn[1] groups the common soil inhabitants which will grow on artificial media as: (1) a group of very short rods, mostly immotile, which do not liquefy gelatin or, if they do, act very slowly; (2) a few short rods which possess polar flagella and liquefy gelatin quite rapidly, as Pseudomonas fluorescens; (3) a group of Actinomyces. These organisms vary greatly in physiological activities, though most of them are cellulose splitters and are therefore valuable in the decomposition of plant refuse. Some species are parasitic on plants. Many produce a yellow pigment.

One other characteristic of many of the Actinomyces deserves to be mentioned, because of their relation to the odor of soil. Many cultures of Actinomyces give off a musty odor that is very striking, and quite peculiar to this group of organisms. It is sometimes described as an earthy odor; but it would be more correct to say that soil has an Actinomyces odor, as it is undoubtedly the growth of these organisms in soil that gives it its characteristic odor. This odor is more striking in sod soil than in fallow soil, as can be proved by simple experiment; and it has been shown that Actinomyces are more abundant in sod than in fallow soil.[2]

(4) A group of large spore-forming rods like Bacillus subtilis, Bacillus megatherium, Bacillus mycoides, and Bacillus cereus. Conn[3] has shown that these organisms exist in the soil mainly as spores, so that they cannot be active. Russell states that of these groups the short non-spore formers of the Pseudomonas fluorescens type comprise about 10 per cent; the short, immotile spore formers, 40 to 75 per cent; and the large spore formers of the Bacillus subtilis type about 5 to 10 per cent of the total number of organisms found in soil. Conn infers that the Actinomyces are next in abundance to the short spore formers. Other types, of course, are present. Cocci, acid-forming rods, spirilla,

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[1] CONN, H. J., "The Microscopic Study of Bacteria and Fungi in Soil," *New York Agricultural Experiment Station, Technical Bulletin* 64, 1918.

[2] CONN and CONN, "Bacteriology," p. 170, Baltimore, 1923.

[3] CONN, "Are Spore-forming Bacteria of Any Significance in Soil under Normal Conditions?" *Journal of Bacteriology*, vol. 1, p. 187, 1916; "Soil Flora Studies," *Journal of Bacteriology*, vol. 2, p. 137, 1917.

and fæcal organisms like Escherichia coli may be present in small numbers.

Other important groups that do not grow readily on artificial media are present, as the sulphur bacteria, Thiobacteriales; nitrogen-fixing bacteria, of the genera Azotobacter and Rhizobium; nitrifying bacteria; and others.

Some agricultural bacteriologists divide the bacteria in the soil into two groups: the autotrophic bacteria and the heterotrophic bacteria—those that are capable of synthesizing carbon and those that must get their food materials from organic sources.

Again, the bacteria in the soil are divided, according to their oxygen requirements, into aërobes and anaërobes.

The morphological grouping of soil bacteria is of less importance than a grouping based on the physiological activities of the organisms, or according to the chemical changes brought about in the soil.

Waksman[1] gives the following physiological groups of soil bacteria: (1) urea decomposing bacteria, (2) denitrifying bacteria, (3) pectin-decomposing bacteria, (4) anaërobic butyric-acid bacteria, (5) anaërobic protein-decomposing bacteria, (6) anaërobic cellulose-decomposing bacteria, (7) aërobic nitrogen-fixing bacteria, (8) anaërobic nitrogen-fixing bacteria, and (9) nitrifying bacteria.

Fungi.—Next in importance to the bacteria in soil are molds and other fungi. Since 1886, when Adametz published the results of his investigation of the biochemical changes in the soil and described several species of fungi, an enormous amount of study has been devoted to the fungi in the soil. A large number of genera and species have been described, but as no entirely satisfactory specific classification has been adopted, it is hard to correlate the work of various investigators. It is sufficient to say that the common molds—Mucor, Penicillium, Aspergillus, etc.—are usually present in soil. The action of those molds, like that of the bacteria, is more or less specific. For example, Mucor splits proteins but not celluloses, while Aspergillus decomposes the celluloses readily.

The numbers are also hard to estimate. Molds are very selective in their food, and widely varying results are obtained in attempting to count molds by cultural methods. Miss

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[1] WAKSMAN, S. A., "Principles of Soil Microbiology," p. 38, Baltimore, 1927.

Jewson in an unpublished research,[1] using the same soil suspension, got the following results on various media: Coon's agar, 357 colonies; Cook's agar, 246 colonies; Czapek's agar, 215; and prune agar, 336. Even in the same medium, moreover, differences in acidity and degree of dilution make wide difference in the counts obtained.

The numbers of molds at different depths of the soil show marked differences. Takahashi[2] found 590,000 fungi per gram at a depth of 2 inches and only 160,000 at a depth of 8 inches.

Any attempt at determining the number of molds by cultural methods is, of course, open to severe criticism. If every spore grows and every little piece of detached mycelium forms a fresh "colony," it is impossible to count the number with any degree of accuracy.

The physiological activities of the molds and other fungi in the soil are quite similar to those of bacteria. Waksman[1] and Schmitz[3] claim that, in the splitting of cellulose, other fungi play a more important part than bacteria, and it would seem, in fact, as though this were at least one of the most important of the life activities of fungi in the soil.

Like the bacteria in the soil, some of the species of fungi are beneficial to plant life and some are injurious. In the decomposition of celluloses—the "woody" part of plants—fungi are probably more important than bacteria. Fungi, moreover, split some proteins more rapidly than bacteria. In this respect their benefit is a doubtful one, as, despite the fact that they destroy waste material, they use the decomposition products in their own metabolism and do not make them available for the higher plants, as do the saprogenic bacteria. Thus the fungi cannot be said to aid in nitrogen enrichment of the soil. Moreover, the fungi use such valuable salts as nitrates, which are thus lost to vegetation.

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[1] Brierly, W. B., in "The Microörganic Population of the Soil," p. 123, edited by Sir E. John Russell, Longmans, Green and Co., London.

[2] Takahashi, T., "On the Fungus Flora of the Soil," *Annals of the Phytophathic Society*, p. 1, Japan, 1919.

[3] Waksman, S. A., "The Influence of Available Carbohydrates upon Ammonia Accumulation by Microörganisms," *Journal of the American Chemical Society*, p. 39, 1917. Schmitz, H., "The Relation of Bacteria to Cellulose Fermentation Induced by Fungi with Special Reference to the Decay of Wood," *Annals of the Missouri Botanical Gardens*, vol. 6, 1919.

A symbiotic relationship between fungi and higher plants, called mycorrhiza formations, is of interest. In bogs or dense forests, there is an accumulation of partially decomposed vegetable residue known as "humus." An examination of this material often reveals a network of fungus hyphæ which penetrate the living tissues of trees. It is believed that the plant supplies the fungus with available energy material in the form of carbohydrates, while the fungus reciprocates by furnishing the tree with salts and nitrogen. Most students of the subject believe that the nitrogen furnished the plant is obtained from the fungus by digestion. Thus it would appear as though the plant were nourishing the fungus in order to consume it. The relationship between the orchid and humus fungi is an example of this mycorrhiza symbiosis. Both the fungus and the higher plant grow better when together, than when alone.

Algæ.—As algæ are chlorophyll bearing, and therefore, supposedly, dependent on the action of sunlight for their carbon synthesis, the fact that algæ may be present in the soil has not been taken seriously until recently. Moreover, we are accustomed to think of the algæ as water forms of life, or at least living upon the surface of such soil as is usually damp.

A number of workers in microbiology have shown within the past few years that many of the algæ are saprophytic and are able to obtain food from decomposing material when photosynthesis is impossible, owing to the depth of the soil. Taking 100,000 as a rough estimate of the number of algæ per gram of manured soil, it is calculated that the volume of algæ is three times as great as that of bacteria. Bristol[1] agrees with Kossowitsch that greater fixation of nitrogen is effected by mixtures of bacteria and certain gelatinous algæ than by nitrogen-fixing bacteria alone, as the algæ provide carbohydrates from which the bacteria derive essential energy. Soil algæ are sometimes important in that the loss of water from their gelatinous bodies is very slow, and, conversely, they absorb water very rapidly, thus keeping the soil moist even in dry climates.

Algæ are also of some importance in the formation of humus. Bristol[2] quotes Treub in stating that

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[1] BRISTOL, B. M., "Algae," in, "The Microörganic Population of the Soil."

[2] BRISTOL, B. M., *loc. cit.*

> . . . after the complete destruction of the island of Krakatoa by volcanic eruption in 1883, the first colonists to take possession of the island were six species of blue-green algæ. Three years after the eruption these organisms were observed to form an almost continuous gelatinous and hydroscopic layer over the surface of the cinders and stones, and by their death and decay they rapidly prepared it for the growth of seeds brought to the island by visiting birds. Hence, the new flora which soon established itself upon the island can be said to have had its origin in the algæ-flora which preceded it.

Protozoa.—Most of the protozoa in the soil belong to the three classes: Sarcodina, Infusoria, and Mastigophora. The soil protozoa may be grouped according to their food requirements into autotrophic and heterotrophic forms. The former, a comparatively small group, usually of the class Mastigophora (example: the genus, Euglena) contains a substance similar to the chlorophyll of the higher plants which enables them to synthesize carbon dioxide. The heterotrophic forms, as do all other animals, obtain their nutrition from organic substances. The food may be ingested as solid particles or only liquid food may be absorbed through the cell wall. Most of the Infusoria and Rhizopoda are able to ingest relatively large particles.

Bacteria, therefore, become the natural food supply for protozoa in the soil. In the reduction of the number of bacteria, the presence of protozoa in the soil is detrimental to soil fertility. Partial sterilization of the soil by heat or chemicals has been shown to increase fertility.

Chemical Changes.—From the preceding paragraphs it has been seen that a number of different microörganisms play a part in the physical and chemical transformations constantly going on in soil.

Since other microörganisms besides bacteria play a part in soil fertility, often duplicating the action of bacterial groups, it seems best to study the functions of soil bacteria from the chemical point of view of the processes involved. These processes have to do chiefly with the transformation of organic matter.

It must not be assumed that all organic material in the soil immediately undergoes changes due to bacterial or other microörganic activity. Possibly not more than half of it is readily decomposed. The balance is very slowly converted into available plant food and a small residue remains as a permanent soil constituent. This slowly decomposing organic matter, consisting of tannins, fats, waxes, etc., is known as "humus." It aids fertility by its capacity for absorbing and retaining water and by keeping the soil from packing.

Agriculturists are more concerned with the kinds of bacteria in soil than with numbers, and in the physiological activities of the microörganisms than in the bacteria themselves. While the changes are brought about in the soil by bacterial means, they may be interpreted to a large extent by chemical analyses. A study of the soil for crop purposes must be undertaken, therefore, from the chemical as well as from the bacteriological standpoint.

Ammonification.—In the study of bacterial enzymes the nitrogen cycle and the part bacteria play in the nitrogen economy in nature were discussed. As these essential bacteria are soil bacteria, they must be taken up in greater detail here.

In the first place, it was noted that if it were not for the saprogenic bacteria, the earth would have long ago been covered with dead organic matter. It is the saprogenic group of bacteria, with the aid of the cellulose-splitting fungi, which decomposes this matter and liberates the nitrogen from complex compounds, making it again available for use. This process of decomposition is extremely complex and consists of innumerable phases which cannot be taken up in detail. The most important one in the nitrogen cycle is ammonification, or the splitting of complex proteins into simpler and simpler compounds of nitrogen, with ammonia or an ammonium salt as the end product.

Proteins are highly complex chemical compounds of nitrogen, carbon, hydrogen, and oxygen. By the action of proteolytic enzymes they are split into various simpler compounds of the same elements, of which amino acids are examples. These amino compounds are then oxidized by bacteria according to the following equation:

$$2CH_2NH_2COOH + 3O_2 = 4CO_2 + 2H_2O + 2NH_3.$$

The ammonia liberated unites with the water, forming ammonium hydroxide, which, in turn, unites with carbonic or some stronger acid in the soil forming an ammonium salt. It is evident that the bacteria concerned in this latter reaction use the amino compounds as a source of energy, and that the carbon dioxide, water, and ammonia are liberated as by-products of combustion.

The most common bacteria concerned with the splitting of protein compounds are those of the groups of which Bacillus subtilis, Proteus vulgaris, Escherichia coli, Pseudomonas fluorescens, and various Sarcinæ are types. Up to the time (1916) of Conn's work showing that spore formers are rather inactive in the soil, it was generally believed that Bacillus mycoides and allied species were mainly responsible for ammonification.

The so-called urea bacteria are responsible for the ammonification of urea, according to the formula

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3.$$

Morphologically, these urea bacteria are rods and spheres. Some are spore forming and others non-spore bearing. The enzyme urease acts as the catalytic agent.

Nitrification.—Nitrification is a term describing the oxidation of ammonia or ammonium salts to nitrites and nitrates. The ammonia produced by the action of the organisms described above is rapidly oxidized in the soil. In fact, nitrification takes place normally at a more rapid rate than ammonification, so that at no time will any considerable amount of ammonia be present in soil.

Winogradsky[1] in 1890 in a series of papers showed that the organisms concerned in nitrification fell into two groups: first, those that oxidize ammonia to nitrous acid and its salts; and, second, those that oxidize nitrites to nitrates. These genera were named Nitrosomonas and Nitrobacter, respectively, and the reactions brought about are of the type:

$$NH_3 + 3O_2 = 2HNO_2 + 2H_2O$$

or

$$(NH_4)_2CO_3 + 3O_2 = 2HNO_2 + CO_2 + 3H_2O$$

and

$$2KNO_2 + O_2 = 2KNO_3.$$

Both of these genera belong to the family Nitrobacteriaceæ and are able to get all their food and energy requirements from simple inorganic substances. Their energy is obtained through the oxidation reaction. Although they cannot use them as sources of food, these organisms can live in the presence of complex nitrogen compounds. The processes of ammonification and nitrification can therefore go on simultaneously.

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[1] WINOGRADSKY, S., "Recherches sur les organismes de la nitrification," *Annales de l'Institut Pasteur*, vol. 4, pp. 213–231, 257–275, 760–771, 1890.

Denitrification.—Certain bacteria are able to reduce nitrates to nitrites and ammonia, and other nitrites to free nitrogen. These processes are grouped under the term denitrification.

The organisms which are able to reduce nitrates to nitrites are quite common. Escherichia coli is one example. Bacillus mycoides is more common in the soil. A group of anaërobes is also concerned with partial denitrification, and essentially with complete denitrification or the production of free nitrogen. Except in water-logged soils, however, where the lack of oxygen in the pore spaces facilitates their growth, the action of these organisms is of little importance. The following equations illustrate partial and complete denitrification:

$$2KNO_3 = 2KNO_2 + O_2.$$
$$HNO_3 + H_2O = NH_3 + 2O_2$$
$$4HNO_2 = 2H_2O + 2N_2 + 3O_2.$$

A type of anaërobic reaction is that caused by a bacterium described by Van Iterson, which, in the presence of cellulose, produces the following:

$$5C_6H_{10}O_5 + 24KNO_3 = 24KHCO_3 + 6CO_2 + 12N_2 + 13H_2O.$$

Nitrogen Fixation.—The "fixation" of atmospheric nitrogen by bacteria was first described by Marcellin Berthelot in 1885.[1]

The ancient Macedonians knew that if leguminous plants were plowed under, the ground was enriched for other plants. It was only with the appearance of Berthelot's paper, however, that an explanation of the phenomenon was forthcoming. Hellriegel and Wilfarth,[2] in studying the nitrogen nutrition of plants found that the leguminous plants were able at times to obtain nitrogen in the absence of nitrates, whereas the gramineous plants soon died in the absence of nitrates. They also showed that it was the nodules on the roots of leguminous plants that were concerned with the fixation of atmospheric nitrogen. The next step was to demonstrate that these nodules contained bacteria.

As the subject is now understood, the fixation of atmospheric nitrogen can be carried on by two kinds of bacteria: (1) those of

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[1] BERTHELOT, MARCELLIN, "Fixation directe de l'azote atmospherique libre par certains terrains argileux," *Comptes rendus*, vol. 101, pp. 775–784, 1885.

[2] HELLRIEGEL and WILFARTH, "Unterschungen über die Stickstoffnahrung der Gramineen und Leguminosen," *Zeitschrift des Veriens f. d. Ruberzucker-Industrie,* 1888.

the genus Rhizobium, of which Rhizobium radicicola is an example, and which lives symbiotically with leguminous plants; and (2) those of the genera Azotobacter and the anaërobic Clostridia, which are non-symbiotic.

In symbiotic fixation, the bacteria gain entrance into the root of a leguminous plant by way of the root hairs. Here they cause the plant to form a nodule or tubercle, in which they live and multiply. Just what the physiology of the process is, is hard to say. Lipman[1] describes it as follows:

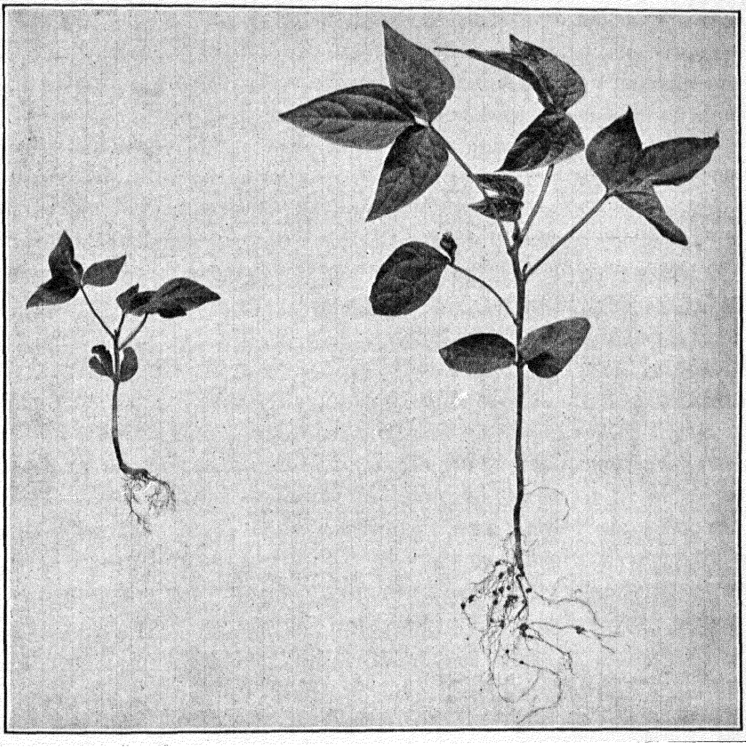

FIG. 25.—Photographic comparison of the result of soil inoculation with rhizobium. (*Courtesy, H. K. Mulford Co.*)

> It is known that the period of active nitrogen assimilation by plants coincides with the appearance of the bacteroids in the tubercules, and it is supposed that the microörganisms fashion nitrogen compounds out of atmospheric nitrogen by using the carbohydrates and organic acids

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[1] LIPMAN, in MARSHALL'S "Microbiology," p. 278, Philadelphia, 1912.

in the plant juices as a source of energy. The plants then seem to utilize the soluble nitrogen compounds that pass out of the bacterial cells.

It is thought by other bacteriologists that fixation is a reduction process with the formation of ammonia.

Even the leguminous plants seem to prefer nitrates to the nitrogen fixers as a nitrogen source. If a soil is enriched with nitrates, no tubercles will be formed. On the other hand, if phosphates, soluble sulphates, or carbohydrates are added to the soil, nodule production will be facilitated.

These nitrogen-fixing organisms show some specialization, certain species preferring certain groups of leguminous plants, and others other species. They also demonstrate a kind of "virulence" or, as Lipman calls it, "physiological efficiency." That is, a given strain may grow well but possess feeble nitrogen-fixing power. Cultivation of the strain along with a legume may rapidly increase this power.

The anaërobic bacteria which fix nitrogen are closely allied to the Clostridium amylobacter group. They will not exert this power if they can obtain nitrogen from any other source.

A large number of species of non-symbiotic aërobic bacteria are able to fix small amounts of atmospheric nitrogen. They are of little importance, however, when compared with the "nitrogen fixer par excellence," the genus Azotobacter. This organism has been estimated to "fix" 15 to 40 pounds of atmospheric nitrogen per acre per annum.

The organism goes through a rather complicated life cycle, which has been described in this book under Morphology of Bacteria. There are several species of the genus Azotobacter. Azotobacter chroöcoccum and Azotobacter agilis were first recognized, and were described and named by Beijerinck in 1901.[1]

An enormous amount of study has been given to the morphology and physiology of this interesting genus of bacteria. Lipman, Conn, Fred, and Waksman, in this country; Ashby, Hutchinson, Thorton, and Russell, in England; Christensen, in Denmark; Lohnis, in Germany; Beijerinck, in Holland; and Omelianski, in Russia are only a few of the many bacteriologists

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[1] BEIJERINCK, M. W., and A. VAN DELDEN, *Centralblatt für Bakterien, Parasitenkunde, und Infektionskrankheiten,*" abt. 2, bd. 9, p. 3, 1902.

who have published results of their researches. The genus has been found in practically every part of the world, and may be considered as almost universally present in soil.

Fermentation of Carbohydrates.—As a large part of plant tissue consists of cellulose and related carbohydrates, the decomposition of these substances must be carried out in the soil along with the rotting of nitrogenous material. As explained before, it is hard to say how these two processes differ, and where one begins and the other ends. Bacteria, algæ, and fungi are all concerned.

Cellulose $(C_6H_{10}O_5)_x$ is destroyed by at least two sets of organisms, the one aërobic and the other anaërobic. Tappeiner[1] was the first to study anaërobic fermentation. The products of anaërobic decomposition are carbon dioxide, methane, hydrogen, together with non-gaseous substances like fatty acids and starches. Clostridium butyricum and Clostridium amylobacter seem to be the principal bacteria concerned, and, as these organisms also decompose nitrogenous substances and require proteins as food, it is altogether likely that cellulose splitting is simply a by-activity of certain saprogenic bacteria.

The following equations represent a type of anaërobic fermentation of carbohydrates:

$$(C_6H_{10}O_5)_x + H_2O = C_6H_{12}O_6$$
$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4.$$

Of the aërobic fermentation of cellulose little is known. Thornton[2] describes an organism isolated by Hutchinson and Clayton, and named by them Spirochæta cytophaga, which is unable to derive energy from any carbohydrate other than cellulose.

Denitrifying bacteria and certain molds also have the power of splitting cellulose. Fats, waxes, sugars, starches, gums, and organic acids are fermented under aërobic conditions by many very common bacteria, such as Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, and by some of the "higher bacteria," yeasts, and molds. Under anaërobic conditions and in the absence of protein material, Clostridium pasteurianus will ferment carbohydrates.

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[1] TAPPEINER, *Berlin Deutsche Chemie Gesselschaft*, vol. 16, p. 1734, 1883.

[2] THORNTON, "The Microörganic Population of the Soil," edited by Sir E. John Russell, Longmans, Green and Co., London.

The gases liberated in the fermentation of cellulose and other carbohydrates may also be oxidized by various prototrophic bacteria. Sohngen[1] in 1905 showed that Methanomonas methanica was capable of obtaining energy through the oxidation of methane:

$$CH_4 + 2O_2 = CO_2 + 2H_2O.$$

Mineral Deposits.—During the ages past bacteria have been responsible for the deposition of large deposits of limestones, iron ores and sulphur. The algæ have left their traces in great silica deposits. Modern transformations of inorganic phosphorus, calcium, and sulphur compounds are largely due to microörganic life.

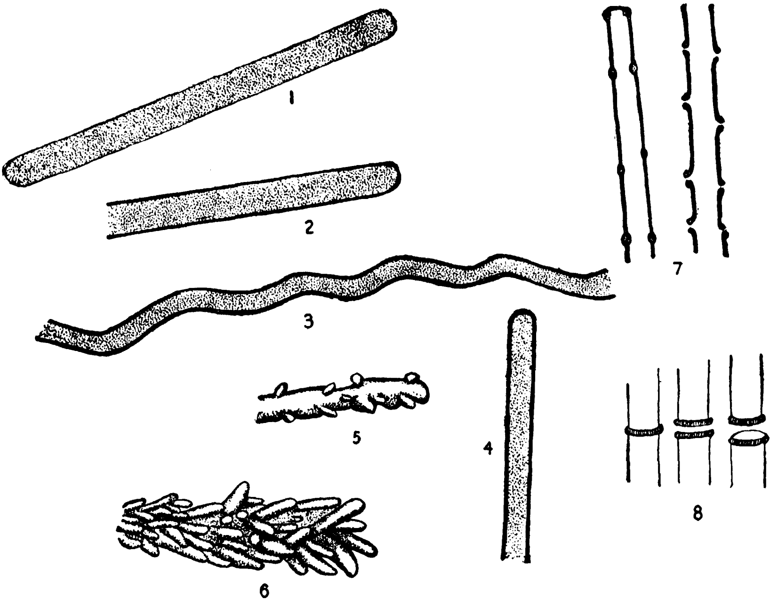

FIG. 26.—Iron bacteria, Leptothrix ochracea. (*Lutman after Ellis.*)

Iron.—Different opinions are held as to how bacteria cause the deposition of iron. Some believe that the formation of hydroxide of iron is a part of the life activity of the organism, others that because of the mucilagenous capsule of the so-called iron bacteria the iron hydroxide is mechanically accumulated, and still others

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[1] SOHNGEN, N. L., *Centralblatt für Bakterien, Parasitenkunde, und Infektionskrankheiten,* abt. 2, bd. 15, p. 513, 1905.

that the iron deposit is incidental—that the bacteria utilize the organic acid radical with which iron is in combination, and that the iron hydroxide is simply an eliminated waste product.

Harder[1] divides iron-depositing bacteria into three groups:

> . . . 1. Those that precipitate ferric hydroxide from solutions of ferrous carbonate, using the carbon dioxide set free and the available energy of the reaction for their life processes; (2) those that do not require ferrous carbonate for their vital processes but cause the deposition of ferric hydroxide when either inorganic or organic iron salts are present; and (3) those that attack iron salts of organic acids, using the organic acid radical as food and leaving ferric hydroxide, or basic ferric salts that gradually change to ferric hydroxide.

In 1865, Ehrenberg[2] suggested that living organisms may play a part in the formation of ocherous limonite deposits in bogs. Ocher is a general term applied to pulverent earths that owe their color to oxides of iron. This sort of deposit can be studied in the process of formation in a shallow pond or eddy in a stream where leaves have fallen and collected. If the silt in the bottom is disturbed, it may be of a yellow, brown, or red color. This color is due to the presence of iron hydrate formed from organic iron salts by bacterial action. When this material dries, ocher is formed.

Limestone.—The following statement by Miller[3] indicates the trend of thought regarding the role of bacteria in limestone formation:

> As a result of investigations, we have considerably modified our views concerning the origin of the various kinds of limestones. Probably most of our older geologists were taught that our limestones were all derived from the shells or skeletons of calcareous-secreting animals. Those limestones in which no recognizable fossil remains could be found were explained as due to the disintegration of the organic remains by wave action, or their obliteration by subsequent crystallization or dolomitization. Although we do find limestones of the coquina type in

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[1] HARDER, E. C., "Iron-depositing Bacteria and Their Geological Relations," Department of the Interior, U. S. Geological Survey, p.p. no. 113, Government Printing Office, 1919.

[2] EHRENBERG, C. G., "Vorlaufige Mittheilungen über das wirklige Verkommen fossiler Infusorien und ihre grosse Verbreitung," *Poggendorff's Annalen*, bd. 38, pp. 213–227, 1836.

[3] MILLER, B. L., "Origin and Utilization of the Limestones," *Proceedings of the West Virginia Academy of Science*, 1929.

almost every geologic period back to the early Paleozoic, the author has become convinced, in his studies of recent years, that bacteria and algæ have probably contributed more to the formation of our Paleozoic limestones than have the various animals that possess the ability to extract calcareous matter from the ocean waters. Seldom do we have any structures preserved that can be attributed to these low forms of plant life and consequently some persons have been inclined to believe that much of the structureless portions of limestones represents purely chemical precipitation without any connection with organisms. This view does not seem plausible because of the unusual conditions requisite to bring about sufficient concentration to result in the precipitation of the carbonates. That bacteria and algæ, by their life activities, are responsible for the deposition of carbonates in warm, shallow ocean waters at the present time has been well established, and we believe similar plant forms are largely responsible for the precipitation of the structureless portions of our limestones of previous geologic periods.

Disease Germs in the Soil.—Except in the case where a water supply is drawn from soil polluted with excreta, the soil can practically be disregarded as a means of transmission of human disease. There is no doubt that it plays an important part, however, in the infection of animals. One of the first diseases studied from the bacteriological point of view was anthrax. The organism, Bacillus anthracis, is a spore-former, and can retain its virulence for a considerable time in the soil. The mode of infection may be through abrasions in the skin or by taking the bacillus in with food. Another disease of lower animals, blackleg, is contracted in the same way.

Tetanus and gas gangrene are two diseases contracted by man in which the mode of infection is through the soil. Under ordinary circumstances these diseases are very rare, but during a war, especially in modern trench warfare, these infections are a constant menace. Both of the organisms involved are spore formers.

A few diseases in which the infective agents are not bacteria but higher organisms are contracted through the soil. Of these, the hookworm disease is the most interesting.

Burial soil has been looked upon as a possible source of infection, especially as early bacteriological analyses showed that it contains a larger number of bacteria than does virgin soil. When it is considered, however, that, except for the few organisms mentioned, disease germs are non-sporulative and are paratrophic, it can be readily seen that they cannot long survive the conditions outside of the living host.

Many plant diseases are caused by microörganisms which are capable of living in the soil. A number of fungi produce conditions known as "root rots" in which the specific fungus attacks the living plant tissue. Other plant diseases, such as the "wilts" and the "scabs," are due to plant pathogens in the soil.

CHAPTER X

BACTERIA IN WATER

Historical.—The need for pure drinking water was recognized by the earliest civilizations. Although the storage of water among the Egyptians (2000 B.C.), the Assyrians, and other ancient peoples was more for agricultural purposes than for human use, writers like Hippocrates (400 B.C.), Pliny, and others indicate that there was a general belief that impure water was to blame for disease. The famous aqueducts of Rome, one of which, the Appian, was built in 312 B.C., show how at that early period no expense was considered too great for obtaining a suitable water supply.

The true explanation of the relationship between drinking water and health did not come for two thousand years. Evidence that impure water could cause disease gradually accumulated, and, by the middle of the nineteenth century, the fact that epidemic diseases could be traced to public water supplies was widely accepted by sanitarians. Attention was now turned to the question of how disease could be disseminated through water, of what nature its infective powers were. What was the difference between a pure and an impure water? Two explanations were forthcoming. Charles Murchison, a Jamaican serving in the British army in India, attributed the disease-producing properties of water to filth.

In the same year, 1855, Max von Pettenkofer published a statement of his ground-water theory. He believed that while water had some influence in the spread of disease, it was only an indirect factor. The real contagium, he thought, came from the soil, and was liberated by the rise and fall in the level of the ground water.

Neither of these explanations was based on scientific evidence. With the rise of the chemical school of Liebig, the cause of disease in water was searched for in the chemical laboratory. In 1883, De Chaumont[1] gave the following description of good drinking water:

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[1] PARKES'S "Manual of Practical Hygiene," 6th ed., p. 22, Philadelphia, 1883.

> The general characters of good water are easily enumerated: perfect clearness; freedom from odor or taste; coolness; good aëration; and a certain degree of softness . . .

In discussing the living organisms in water, the same author evidently saw no connection between bacteria and disease, for he says:[1]

> As, however, even distilled water and the purest ice-water may contain *bacteridia*, the test cannot be used as a positive indication of good or bad water, except in connection with others, and with due regard to temperature, which has a great effect.

In this same year, Robert Koch gave to the world the first scientific explanation of the relation of water to disease. As head of the German Cholera Commission he discovered the germ of cholera and its method of transmission from one individual to another by drinking water. Two years later, John Snow, an English physician, stopped an epidemic of the disease in London by taking off the handle of the Broad Street pump.

In 1892 a cholera epidemic broke out in Hamburg, Germany, and was definitely traced to an infected water supply. The water was pumped from the river Elbe, without purification, to the city mains. The city of Altona on the opposite side of the river took its water from the same source, but the water was filtered before delivery to the mains. In Hamburg, there were more than 8,500 deaths from cholera, while Altona, except for those cases which could be traced to visits to Hamburg, was entirely free from the disease.

It is now known, in the light of our present knowledge, that many of the great "plagues" of the past were caused by water-carried bacteria. Every historic epidemic of typhoid fever, dysentery, and cholera—the causative agents of which grow in the intestinal canal of man—has been studied in the light of accumulated experience. The typhoid epidemics in Lausen, Switzerland, in 1872; in Plymouth, Pa., in 1885; in Lowell, Lawrence, and other cities in Massachusetts in 1890 have been conclusively shown to have been water-borne.

Pure Water.—Today it is known that *epidemic* diseases, like typhoid fever, cholera, and dysentery are caused by bacteria, and that water carries these diseases when, *but only when*, it contains germs of these diseases.

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[1] *Loc. cit.*, p. 66.

Water is not, however, necessarily fit for drinking purposes just because it is free from disease germs. From the sanitary standpoint, water must be free from all dangers to health. These dangers may be represented by poisonous mineral constituents, and decaying animal and vegetable matter, as well as by disease germs. In this country, unfortunately, most of the states, while attempting to require pure water, have made the requirements in terms so relative that they do but little good. For instance, a certain state board of health classified water as being either "normal" or "polluted." The difficulty is that "normal" waters may differ widely in taste, odor, color, and chemical constituents. They may, for example, contain poisonous lead salts and still meet legal requirements. On the other hand, people have become so accustomed to delight in a clear, cool, sparkling spring water, that sight is lost of the possibilities of infection through the presence of disease organisms. It must be remembered that absolutely pure water cannot be obtained outside of the chemical laboratory. Water is odorless and tasteless when pure, but, as it is a universal solvent—that is, everything is more or less soluble in it—it never exists in nature without some dissolved gaseous or mineral constituents.

Uses of Water.—Animals and plants contain a large proportion of combined water. In the human body, which consists of about 70 per cent water, it serves five distinct purposes: first, it enters into the chemical composition of the tissues; second, it is the chief ingredient of blood and lymph, thus carrying away broken-down tissue and bringing in the matter which becomes new tissue (metabolism); third, it lubricates membranes, thereby preventing friction; fourth, it is the distributor of heat in the body; and, fifth, it acts as a flush in the body drainage system.

Requirements.—The average amount of water really necessary for domestic purposes has been calculated at 17 gallons per capita per diem. Of this amount, 3 pints are used for drinking, 5 pints for cooking, and the balance, about 16 gallons, for bathing and laundry purposes, and for flushing toilets.

The actual consumption of some European cities has not been far above this amount. It is stated that Manchester, England, uses 40 gallons per capita per diem; Berlin, 18, Copenhagen, 27; Sheffield, 21; Nantes, 13; Brest, 3; Breslau, 20; Venice, 11; Brussels, 20; Vienna, 20.[1] Now compare these figures with those

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[1] *Engineering News*, vol. 41, p. 111, 1899.

of some American cities. Pittsburgh uses 250, and Buffalo 233 gallons per capita per diem. Of course, these figures do not take into account broken pipes, leaky joints, the amount used by factories, etc. The figures represent simply the total amount pumped, divided by the population. Of this amount, about 45 per cent is wasted. This, too, when it is known that there is no sanitary advantage in an excess. It is a significant fact that when the individuals in a community are required to pay for the actual amount of water consumed, which is done through the introduction of meters, the consumption falls off considerably. For instance, in two American cities which are fully metered, one, Memphis, Tenn., uses 70 gallons per capita per diem; and the other, Dallas, Tex., 56 gallons. Waste of water represents a serious problem in some cities, where it is necessary to go a long way for a supply suitable for drinking purposes.

Double Supplies.—Some European cities, Paris, for instance, have what is known as double water supplies. One tap is used for drinking and cooking and the other for washing and flushing purposes. This non-potable water is also used for street washing, fire control, and factory purposes. The advantages are in economy of filtration and in the careful supervision possible. The principal disadvantage is that, in most cities, the additional pipe lines would cost more than the purification of the entire water supply. There is a possibility, moreover, of ignorant people drinking the non-potable water and thereby defeating the very purpose of the double system. Some of the large cities in this country have a high-pressure fire supply of raw river water, but this, of course, is never available for household use.

The Sources of Drinking Water.—These may be divided into three more or less arbitrary groups: rain water, surface water, and ground water. Of course, when water falls as rain, it immediately becomes surface water—unless caught in a cistern or tank—and part of it enters the soil and becomes ground water. Ground water sooner or later comes to the surface and is again surface water.

Rain Water.—Theoretically, rain water should be the purest of all waters. Water vapor rising from the sea or from lakes gathers in the form of clouds, condenses, and falls as rain, snow, or hail. This is really a process of distillation, and the only impurities the water may gather in condensing and falling are atmospheric gases, dust particles, and air-borne bacteria. Rain water is "soft" and therefore valuable for laundry purposes. Because the source is liable to fail when most needed, and because of the difficulties of proper storage, rain water for drinking purposes is, however, not much favored by sanitarians. In the first place, its storage place is subject to contamination. The fact that it cannot be collected in large enough quantities for municipal supplies leads to the dangerous practice of having a private underground cistern too near a house or barn, where it is almost impossible to avoid pollution by seepage of sewage through the surrounding soil. Another danger arises from the necessity of having an overflow, or, at least, a cutoff, pipe. To save the expense of a double-piping system, this overflow pipe is sometimes connected with the waste pipe from the house or barn. Should there be an accident to this common pipe—and such accidents are not infrequent—sewage is likely to back up into the cistern.

While few disease bacteria are able to survive the previous drying necessary for them to get free from the air into properly stored or fresh rain water, the storing of rain water may be an indirect means of transmission of disease. Certain parasites, for example, the yellow-fever mosquito, breed by choice in the soft water in cisterns. Yellow fever, like malaria, is transmitted only through the mosquito. If the breeding place of the mosquito is abolished, the means by which the disease is transmitted is thereby destroyed.

Yellow fever was endemic in some seaport towns, such as Philadelphia and Boston, before the cistern was suppressed by law.

In cases where necessity requires a cistern as the source of drinking water, great care should be taken in the location of the cistern, in the collection of the water, and in the protection of the stored water from insects and small animals.

Surface Water.—Under this head come ponds, lakes, reservoirs, small streams, and rivers. Most cities in the United States receive their water supply from surface water. Of rivers, Rosenau[1] says:

> Streams are the natural sewers of the regions they drain, and, when used as a source of water supply, we have established a direct connection between the alimentary canals of the people living upstream with the mouths of those below.

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[1] Rosenau, "Preventive Medicine and Hygiene," 5th ed., p. 940, New York, 1927.

There are relatively few cities in this country which are not located on the banks of a river or a large stream. It is quite natural, therefore, that this large and unfailing supply of water at the very gates of the town should be looked to as the source of drinking water, yet scientific discoveries and experiences involving losses in lives and money have demonstrated that a great danger lurks in this easily obtainable supply. Three methods of procedure are possible.

First, state and federal laws may be passed forbidding the upstream cities to pollute a river which later flows by another city. If it were possible to make certain that *all* pollution entering a stream came through the sanitary sewers of a city, such a plan might be practical, but contamination from outlying communities and individual houses, storm washings, and chance pollutions by individuals while bathing, fishing or boating, make reliance on sewage purification unsafe. The procedure would not give sufficient protection.

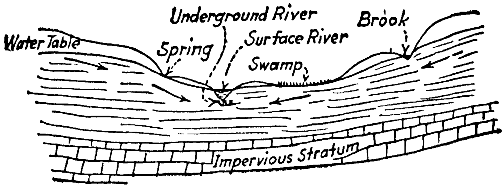

FIG. 27.—How the ground water surface or water table follows the contour of the land. (*Ehlers and Steel.*)

Second, the stream may be accepted as a polluted supply and the water may be purified by the use of chemicals and by filtration. This is the most common practice today. Millions of dollars are spent annually in purifying our municipal water supplies. Were bacterial contaminations the only objectionable substances in river water, this procedure would probably suffice. This, however, is not so. Factory wastes, packing-house debris, and other impurities do get into streams in spite of laws. Moreover, in its course, a stream dissolves chemical substances from its channels, which in themselves, or, in their oxidation products, are at least undesirable. These materials are not eliminated by bacterial-purification methods.

Third, the river may be discarded as a source and at enormous initial expense a pure supply of water may be obtained from a mountain reservoir, or from artesian wells. A few of the more prosperous or far-sighted American cities have already taken this step. Many more are considering it. What plan the future will show to be the best is uncertain. It is likely, however, that each town will have to work out the problem as an individual case.

Ponds, lakes, and artificial lakes or reservoirs are the most desirable of all large water supplies. Because of the comparative ease in watching the catchment areas, they may be kept pure rather than having to be purified.

Small cities and towns situated near mountain lakes or easily dammed mountain streams are peculiarly fortunate. The chief disadvantages of this source of supply is the liability of overgrowth of algæ and protozoa in the reservoir, which may give the water a disagreeable taste or odor. Proper treatment with an algacide or disinfectant, such as copper sulphate, will usually obviate this trouble. Another cause of disagreeable odors and tastes in impounded water is what is popularly called "working." This is an overturn of the water from bottom to top, due to convection currents.

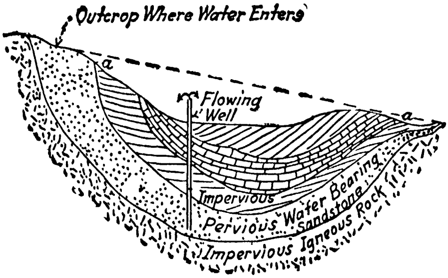

FIG. 28.—How artesian wells are formed. *aa* is the line to which the water would rise if the well casing were extended upward. (*Ehlers and Steel.*)

Large lakes are too often used as both the source of a city's water supply and the outlet of its sewerage system. Chicago has spent an enormous amount of money and has involved the government in a controversy with Canada in its solution of this problem.

Ground water is obtained from three sources: first, from shallow wells; second, from deep or artesian wells; and third, from springs. Shallow wells should be properly placed and properly watched. As the water that finds its way into a shallow well is mostly seepage from the surrounding ground surface, too much care cannot be taken to avoid contamination from house or barn drainage.

The possibilities of shallow-well pollution from a cesspool depend on several factors, of which distance, type of soil, character of underlying rock, and level of permanent ground water are the most important. Bacteria causing intestinal disease have been known to survive in moist soil for more than two months. In dry soil, these germs can only survive for a few days. In a heavily polluted soil, such as exists directly under a pit privy, the bacteria from the intestinal tract will not penetrate to a depth greater than from 10 to 12 feet, and usually not half this distance. The lateral or horizontal penetration is usually less than 3 feet. In a fairly dry, loose soil, therefore, in which the level of water is more than 10 feet below the surface of the ground, there is no great danger from seepage. There is always, of course, danger from surface drainage.

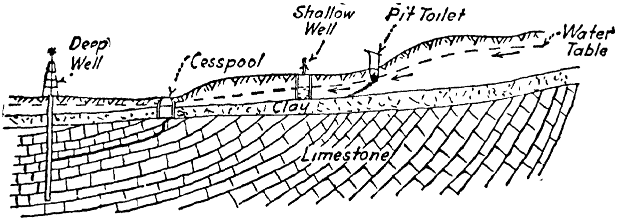

FIG. 29.—How pollution may enter wells through soil pollution and by means of fissures in limestone. (*Ehlers and Steel.*)

In a clay or other packed soil where fissures are possible, or in a soil where the level of permanent ground water rises to within a few feet of the surface, the menace is real at a considerable distance. This is true also where the soil is of little depth and is underlaid by a limestone rock in which fissures may carry pollution for a considerable distance.

In a study of experimental pollution of wells by way of ground water, a board under the chairmanship of Prof. C. W. Stiles came to the conclusion that it is possible to recover bacterial pollution at a distance of over 200 feet in the soil. A report of this study[1] contains the following interesting statement:

> Pollution sinks vertically downward until it reaches the ground-water table, which represents the water level of wells, and it spreads only in the direction of the ground-water flow. This ground water flows through the ground much in the same way that a river flows through a

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[1] *Hygienic Laboratory Bulletin* 147. abstracted in *Public Health Reports,* pp. 1581–1582, June 10, 1927.

valley. The water rises after rainfall and sinks during dry weather. As polluted water sinks, the pollution filters out into the ground; if the ground layer remains moist, the pollution may live and is carried farther when the ground water rises again; if it becomes dry, the pollution dries. Thus, these investigations have uncovered a hitherto unknown law of Nature, namely, that it is the rise and fall of the ground water, due to rain and drought, which permits the water to become purified; were it not for this fact, the underground water would contain pollution of considerable age, possibly dating back many years, and it would be difficult to find pure spring water or pure well water except under an impervious layer.

The water of artesian wells and springs is good or suspicious, depending on the nature of the material through which it flows. Water trickling through sandstone rock, for instance, is purified by filtration. On the other hand, a porous rock like limestone is readily soluble in water, and underground streams flow along carrying the organisms with which they may originally have been contaminated. If one of these streams comes to the surface as a spring, or if an artesian well taps it, it may contain the same bacteria as any surface stream.

To convince a person of this fact is one of the hardest problems a sanitarian has to face. People who can readily understand how surface water may be polluted cannot seem to understand how water drawn from a well 1,000 feet deep can be anything but pure. This applies equally to individuals on suburban estates and to city authorities.

A geologist should always be consulted before money is spent in drilling an artesian well.

Water Analyses.—The desirability of a water supply is determined by four kinds of analyses, namely, the chemical, the physical, the biological, and the bacteriological analyses.

The chemical analysis shows the suitability of the water for boiler use, domestic, laundry, and manufacturing purposes, and also whether or not it contains any harmful chemical ingredients, such as poisonous lead or zinc salts, which would make it unfit for drinking purposes.

In a "sanitary" analysis of water, the chemist is chiefly concerned with nitrogen (in the combined forms of ammonia, nitrogenous organic matter, nitrites, and nitrates); oxygen consumed (that is, the oxygen that the oxidizable compounds in water will consume when treated with an acid solution of potassium permanganate); temporary hardness (carbonates); permanent hardness (sulphates, chlorides, nitrates); residue after complete evaporation and ignition; alkalinity or acidity; iron, manganese, lead, zinc, copper, tin, and the alkaline earths.

The physical examination shows whether or not the water has any disadvantageous turbidity, color, taste, or odor, and condemns or recommends the water from the æsthetic standpoint. Turbidity readings constitute also a routine procedure in determining preliminary treatment of a water before filtration, especially "rapid" filtration, and serve as an index of subterranean conditions in an artesian supply.

The biological analysis or "microscopic examination" of water determines what and how many living forms of plants and animals other than bacteria will be taken into the human body by drinking the water.

The arbitrary difference between "microscopic organisms" and bacteria has been illustrated by the late Professor Sedgwick. Sedgwick divided all microörganisms into bacterial organisms and microscopic organisms, and drew up the following statement of differences:

Bacterial organisms require special culture methods, and are not easily examined with the microscope; are microscopic or submicroscopic in size; and are always plants.

Microscopic organisms do not require cultural methods; are easily studied with the microscope; are microscopic or larger in size, and may be plants or animals.

The chief object of the microscopic examination of water is the determination of the presence or absence of those organisms which produce objectionable tastes or odors. In certain cases the examination is also of value as an index of pollution, or as a guide to the identity of the water, *i.e.*, whether surface water or ground water.

The term "microscopic organisms" or "plankton" comprises the "higher bacteria" of the class Schizomycetes, Chlorophyceæ, Cyanophyceae, Fungi, Protozoa, Nematode worms, the smaller species of Crustaceæ, and larvæ of aquatic insects. Most of these organisms have been discussed under Microörganisms Other than Bacteria. Fragments of broken-down organic matter, fish scales, decaying vegetation, etc. are called amorphous matter and recorded as such. Clay, silt, and mineral matter in general are not included under either plankton or amorphous matter.

The procedure for the microscopic examination of water and a full discussion of the entire subject may be obtained from "Microscopy of Drinking Water," by Prof. George C. Whipple.

Whipple enumerates 188 species (110 plants and 78 animals) of microörganisms in water. Of these, only 18 are commonly found in large numbers. The most important classes are "higher bacteria," various green, blue-green and brown algæ (Diatoms) and protozoa. Of the microörganisms that have caused the most trouble in American water supplies three genera are Diatoms (Asterionella, Synedra, and Melosira), four are Cyanophyceæ (Anabæna, Clathrocystis, Caelophærium, and Aphanzomenon); and five are Protozoa (Dinobryon, Peridinium, Synura, Uroglena, and Glenodinium). The Chlorophyceæ, while commonly found in the largest numbers, do not give the water an unpleasant taste or odor and are therefore not troublesome

The bacteriological analysis of water is the most important from the standpoint of health. In the past, two kinds of analyses were included under this head. In one a so-called "total count" was made, in which the number of bacteria developing on a standard culture medium was determined. In the other, the number of organisms associated with the intestinal tract of man and animals—usually called the "B. coli" group[1]—was determined.

The significance to be attached to the results of these analyses is at present a question in the minds of bacteriologists. The mere presence of bacteria in water does not make water unsatisfactory for drinking purposes; hence, without determining the *kind* of bacteria present, a total count would seem useless. As a routine procedure in watching the efficiency of a filter, total counts are valuable.

"B. coli" determinations, as indications of pollution are, on the other hand, extremely valuable. The danger in pollution lies, not in the pollution itself but in specific disease bacteria which the pollution may contain. These dreaded disease germs are those causing intestinal diseases such as typhoid fever, cholera and dysentery. Because of the relatively few disease germs present in a contaminated water, it is impractical to look for them, for even if they were found it would only be

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[1] The term "B. coli" includes all the Gram-negative intestinal bacteria belonging to the tribe Bactereæ. Escherichia coli is only one member of this group.

after the community had been exposed to them. As they are always associated with harmless intestinal forms it is customary to approve or condemn a water on the basis of the presence or absence of any member of the "B. coli" group.

Herein lies the difficulty. No really satisfactory method has been devised for distinguishing between human intestinal organisms recently gotten into the water, on the one hand, and both old attenuated human strains and animal strains, on the other. Moreover, it is a fact that, in fæces from a typhoid-fever patient, the harmless intestinal bacteria far outnumber the typhoid germs. There comes a point in dilution therefore where the presence of "B. coli" may not indicate the presence of disease germs. Until these questions are satisfactorily answered, bacteriologists will continue to condemn water supplies which are probably no more dangerous than the ordinary hazards of life. In our present state of knowledge, no one wants to assume the responsibility of recommending a water for drinking purposes when there is the slightest possibility that it may carry dangerous disease germs.

These four kinds of analyses are by no means independent of one another. Thus, the chemical analyses may show that the water contains a large amount of albuminoid ammonia and the microscopic would show what organisms caused this condition. Or the chemical analysis might show the presence of nitrites, while the bacteriological analysis would show whether or not these nitrites were present because of sewage pollution. Again, the physical examination might show a decided taste or odor. The microscopic examination would show what organisms were causing this condition; or, if the condition was due to dissolved inorganic chemical substances, like hydrogen sulphide, chemical examination would reveal that fact. In general, the chemical analysis shows whether the water is chemically safe (contains no poisonous minerals), or whether it is usable only for industrial purposes; the bacteriological analysis shows whether or not it is bacteriologically safe (with no fæcal contamination present); the physical and microscopic analyses show whether or not it is palatable.

Bacteria in water have been divided into three groups: natural inhabitants, unobjectionable aliens, and objectionable aliens. The *natural inhabitants* are those bacteria which find all of their food and energy requirements in the salts, gases, and other substances dissolved in water. In this group are found some species of Nitrobacteriaceæ and many of the "higher bacteria," including the sulphur and iron bacteria. The *unobjectionable aliens* are the soil bacteria which are washed into water. Practically all forms of bacteria found in the soil may at times be present in water. Some saprogenic species are found so commonly in water that they might almost be considered as natural inhabitants. The *objectionable aliens* are paratrophic organisms which gain access to water from sewers or from surface seepage from contaminated soil. They are mainly intestinal forms, although any disease germ in water is an objectionable alien. As was said before, many bacteria not in themselves dangerous are considered objectionable because of their source. This group is directly indicative of danger, and the greater their numbers, the more immediate and extensive the dangerous contamination. The organisms may not themselves cause infectious diseases; they are, however, indicators of pollution, and warnings of the possible presence of intestinal forms, like Eberthella typhi and Vibrio comma, which do cause disease. These two species of bacteria have caused the death of millions of human beings through water-borne epidemics.

Rain water, even if not subjected to contamination after collection, may contain a few organisms of any of the three types, but, because the bacteria must have been dried before they got into the air, they are attenuated and are of no significance. Mycobacterium tuberculosis is a possible exception to this statement.

Surface waters may safely contain large numbers of the first and second classes and be used for drinking purposes. Their presence in large numbers shows the advisability of watching the water for objectionable aliens.

Deep-well water should not contain any of these groups, as the presence of even the first class indicates improper filtration through the soil and the probability of fissures through which pollution may find access. If present in shallow-well water, this shows surface contamination, and may mean pollution with sewage.

Natural Purification of Water.—The evaporation and condensation of rain water is one of nature's methods of purification. Slow seepage through rocks and soil is another. Early in the history of public water supplies, it was noticed that rivers receiving a large amount of sewage at one point soon distinctly improved in appearance. Chemical analyses also showed a decided improvement in the water, and the theory sprang up that "running water purifies itself." Unfortunately also, the first investigations, from the bacteriological standpoint, notably with the Seine River below Paris and the Danube River below Vienna, seemed to prove this theory. The result was that many cities took their water from a source which they knew had been polluted, through a feeling of security based on this theory. This practice has led to many avoidable epidemics of intestinal diseases. Investigations have shown conclusively that, while running water may and does become *purer*, it does not become *pure.* Of this "self-purification theory," Dr. Sedgwick says:

> The importance and far-reaching effects of this conclusion can hardly be overestimated. Relying upon it, numerous cities and towns all over the world introduced water supplies derived from sewage-polluted streams, and infinite damage was done to the public health. The theory is now abandoned, or rather accepted after so much modification that it is virtually new.[1]

Along the same line another authority, Dr. Rosenau, writes:

> It was formerly said that a stream purifies itself in 7 miles. Such a generalization is absurd. We now know that it is not the distance so much as the time and opportunity for the various factors involved to become effective. Thus, Buffalo's sewage flows to Niagara's intake, a distance of about 17 miles, in a few hours. There is little chance for self-purification to take place, and despite the high dilution, the danger from raw water is very great. Niagara's average typhoid rate for 10 years, from 1899 to 1908, was 132.9 per 100,000, the highest in the country.[2]

During the past six years, after the installation of efficient filters, the death rate has droppeed to 5 per 100,000.

That there is some purification which takes place naturally in streams, however, there can be no doubt. There are numerous explanations for this phenomenon. Oxidation, sunlight, dilution, sedimentation, and biological factors either singly or in combination with each other play a part.

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[1] SEDGWICK, W. T., "Principles of Sanitary Science and the Public Health," p. 129, New York, 1911.

[2] ROSENAU, M. J., "Preventive Medicine and Hygiene," 5th ed., p. 1007, New York, 1927.

Oxidation.—The rapid disappearance of organic matter in a rushing mountain brooklet is explained by the oxidation of these substances. With the decrease in food supply, the number of saprogenic, unobjectionable aliens diminishes. But while the water does become clear and sparkling, experience has shown that aëration does not quickly destroy objectionable aliens.

Sunlight.—Although the value of sunlight as a germicide is well known, and while it unquestionably has some effect in the purification of water, there is no doubt that its value has been considerably overestimated in this particular. Where experimental data have been collected in the laboratory, results show that even diffused light is slightly effective. Investigators in natural waters, however, have found that a few inches of intervening water materially diminish the disinfecting action of sunlight.

Dilution.—When a polluted stream receives a large influx of pure water, the number of bacteria per cubic centimeter naturally drops. It is clear that the chances of receiving one bacillus of typhoid, cholera, or dysentery are very small when the dilution is very great, and, moreover, although it is possible under certain conditions for one germ to cause disease, it is not probable, especially if the organism is attenuated, and unless the conditions are unusually favorable. This obvious lessening of the chances of transmission of disease germs is not the only advantage of dilution. Oxygen in solution in the water of a polluted stream may be used up by the oxidation of organic matter. The fresh supply of oxygen due to the dilution may be sufficient to completely oxidize the polluting material. In rapid rivers this factor is more important than in sluggish streams. Owing to these facts and to the further fact that pathogenic spore-free bacteria soon become attenuated and die in water, dilution becomes one of the chief sanitary safeguards. Although a stream may not receive any material additions by way of tributaries, yet the volume of water in a river is constantly being augmented by the influx of ground water that seeps into the drainage channels from the surrounding land, and so the extent of dilution is being gradually increased.

Sedimentation.—The most important factor, however, in the self-purification of surface water is sedimentation. This may be accomplished either by the gradual settling of the organisms themselves, or, which is by far the more effective method, by the bacteria being carried down by settling particles of insoluble matter, such as clay or silt. These particles enmesh the bacteria and mechanically carry them to the bottom of the river or lake.

Biological Factors.—Disease germs find their optimum food and temperature requirements in the human body. In the silt and muck at the bottom of a river or lake they are far removed from their natural environment and soon are attenuated or succumb through the lack of proper food. In addition to this, they are subjected to the products of proteolytic action of saprogenic bacteria which, from the nature of their paratrophism, is detrimental. Finally, they are fed upon by protozoa and other minute animals and vegetable forms.

Obviously, sedimentation and subsequent biologic antagonism take place much more effectively in a sluggish river than in a rapid stream. "Running water purifies itself." It is much nearer the truth to say that quiet water purifies itself. Sedgwick says that it may even be stated that the first requirement for the natural establishment of purity in surface waters is quiescence. But quiescence in rivers is ordinarily impossible; hence, the establishment and conservation of purity by natural means is today regarded as improbable, and no river—unless it flows from an absolutely uninhabited watershed—is to be regarded as suitable for direct use as a public water supply.

Artificial Purification of Water.—The earliest known method of purifying water, and the one that remains the best even today, was that of boiling. None of the bacteria which cause disease in man and are transmitted by water can withstand this treatment. The objections to it are its expense; that it gives the water an insipid taste—because oxygen and carbon dioxide are driven off; and that the water cannot be used until it has cooled.

A few years ago small filtering devices were often attached to faucets for household use. Of the large number of household filters on the market, only a few were of any value, and, in fact, most of them were an actual menace. While, in theory, bacteria were removed from the water passing through the filters, the rate of flow during efficient operation was so slow that the use of filters was impractical, and the frequent cleaning necessary was burdensome. As a result, in order to increase the flow, the housewife allowed the water to pass through the joints, instead of through the filter and through the accumulation of filth that collected on the outside of the filter as well. It is very fortunate that the use of these household filters has greatly diminished.

The above methods, are of course, impractical on a large scale, and the purification of water for public use must be handled in other ways.

Sedimentation.—As the chief factor in the natural purification of water is the sedimentation of suspended particles, and subsequent biological activities, it is to be expected that advantage would be taken of this fact in the purification of municipal supplies. As the suspended matter is generally sand and clay, together with a small amount of organic matter, when the currents of the water become retarded the larger particles naturally sink to the bottom, carrying with them, mechanically, large numbers of the finer particles and bacteria. In cities where this method is employed, the water is usually pumped from the river into large settling basins. The bacteria in the water are not destroyed, but simply carried to the bottom of the settling basin. Here the water and soil bacteria accumulate in large numbers; the parasitic (including, of course, the pathogenic) bacteria soon die when deprived of their host. The effluent is then drawn off, usually over a shallow weir near the surface.

Coagulation.—In case the suspended matter is too fine for ordinary settling basins to be effective, some substance is added to the water which combines with the suspended matter, forming more or less gelatinous precipitates which themselves act as coagulants, carrying down in relatively large masses the silt and occluded bacteria. Where waters are very turbid, it is more economical to allow the coarser particles to settle first and then add the coagulant. Several coagulants are in common use. Clark's process depends on the action of lime in forming insoluble calcium carbonate with calcium bicarbonate, as:

$$CaO + H_2O = Ca(OH)_2$$

$$Ca(HCO_3)_2 + Ca(OH)_2 = 2CaCO_3 + 2H_2O.$$

This process is often used to soften water. The slaked lime, or milk of lime, is first prepared in a tank, and then added to the water in a settling basin, in the proportion of about 150 pounds of CaO to each million gallons of water. The precipitate of calcium carbonate is pulverulent, however, and therefore not as effective a coagulent as the precipitate formed when alum is introduced into water.

When alum (aluminium sulphate) is introduced into water containing carbonates and bicarbonates of lime and magnesia, sulphates of lime and magnesia are formed and the aluminium salt is converted into aluminium hydroxide, a bulky gelatinous precipitate, which readily coagulates the silt:

$$Al_2(SO_4)_3 + 3CaCO_3 + 3H_2O = 3CaSO_4 + 2Al(OH)_3 + 3CO_2.$$

Some of the carbon dioxide combines with the water forming carbonic acid:

$$CO_2 + H_2O = H_2CO_3.$$

The amount of alum required, of course, depends upon the amount of fine suspended matter in the water. One or two grains per gallon is usually sufficient. The use of alum may require soda ash (Na_2CO_3) also, in order to make the water sufficiently alkaline.

The use of ferric hydrate instead of aluminium hydrate has increased greatly in the last few years. As the use of ferric sulphate is impractical on account of the expense, a number of patented processes have been proposed to form the ferric hydrate. One of these entails the use of ferrous sulphate and calcium hydroxide:

$$FeSO_4 + Ca(OH)_2 = CaSO_4 + Fe(OH)_2$$
$$4Fe(OH)_2 + 2H_2O + O_2 = 4Fe(OH)_3$$
$$Ca(HCO_3)_2 + Ca(OH)_2 = 2CaCO_3 + 2H_2O.$$

In this process, as in the alum process, sulphates of lime and magnesium are formed as well as ferric hydroxide. The lime is used to prevent the formation of ferric carbonate. It also aids in the precipitation, as in Clark's process. Alum will remove a considerable part of some kinds of color in water, but iron never removes color.

Sometimes the coagulating agent produces not only the mechanical effect of sedimentation but also acts as a germicide. These agents are not considered of much value, however, in this particular. Sedimentation with coagulants has two great advantages over plain sedimentation: first, large settling basins are unnecessary; second, the process cuts down considerably the time necessary for subsidence. In spite of the marked success attained with this means of water purification, it cannot be used as a sole means of purification where sewage pollution of the water is probable.

Filtration.—The purification of water by filtration was first used on a large scale in England. The method was introduced into this country about 1870 and was immediately adopted by a large number of municipalities.

The process has been modified considerably since first used, and today there are two distinct types of filters in use. In both, the filtering material is sand, but this fact constitutes about the only similarity between them.

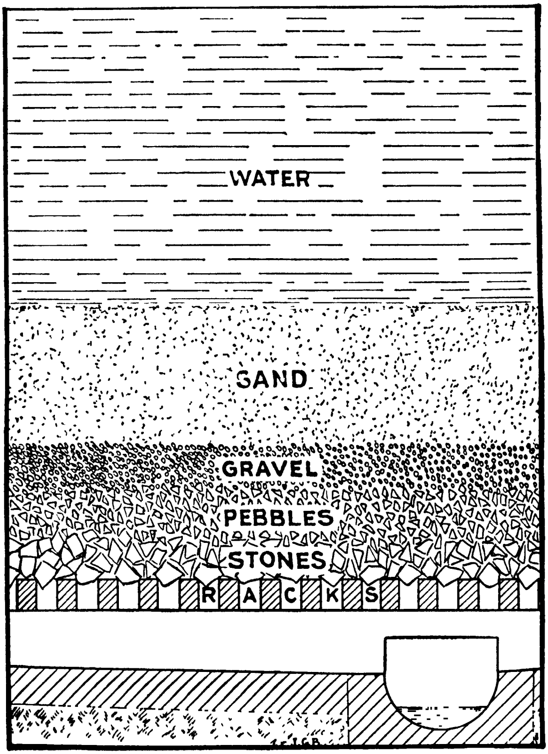

FIG. 30.—Section of a sand filter.

Slow-sand Filters.—The original filtration method depended upon the seepage of the water through layers of sand and gravel. Because it was the original method designed in England, it is sometimes spoken of as the English filter.

The filter covers about 1½ acres. The walls are made of concrete or brick or clay and are sloped or stepped toward the bottom in order to prevent the formation of channels between the sand and the walls. At the bottom of the bed is a system of drain pipes, or usually perforated tile, leading to an underdrain or collector. The bottom layer of gravel is about 9 to 12 inches thick. Above this is a layer of small gravel of about the same thickness. Above this is a layer of still finer gravel, and then two layers of sand, the top one of the finest grains. This top layer is usually about 3 feet thick. Some authorities claim that the total thickness of the bed should be between 42 and 48 inches, as more than this does not add to the efficiency of the filter.

The depth of the water over the filter is usually held between 3½ and 4 feet. The rate of flow is from 4 to 5 vertical inches an hour. In this country the latter rate is usually accepted, which gives about 3,000,000 gallons per acre per day.

No preliminary treatment of the water is necessary in slow-sand filtration.

It is a curious fact that this type of filter works best when apparently the dirtiest, due to the formation of a zoöglœal mass on the surface of the sand (see p. 89), known as the schmutzdecke (dirt cover). The schmutzdecke catches the bacteria in the water and thus aids the filter.

In the course of time, depending upon the amount of silt, etc. in the water, the filter must be cleaned. Several methods are used, varying from simply scraping off the schmutzdecke and a few inches of the top layer of sand to the use of rather elaborate cleaning machines.

Even in cases where no definite schmutzdecke forms on the surface of the sand, it is easily demonstrated that chemical changes take place in the substances in the water. There is a definite oxidation of organic material, and the diminution of bacteria cannot be accounted for simply by mechanical removal. It is likely that the same set of factors are at play in the slow-sand filter as are found in a sedimentation tank.

The efficiency of a properly operated slow-sand filter is approximately 99 per cent—that is, only about 1 per cent of the bacteria originally in the water pass through the filter.

The objections to the slow-sand filters are chiefly of an economic nature. For a large city, a great area of land is necessary, and, especially in the temperate zone, the filter bed must be enclosed and roofed in order to prevent freezing during winter and the growth of algæ at other seasons.

Rapid-sand Filters.—The rapid-sand filter is a typical American development. The filter consists of one or several iron, steel, wood, or concrete units, containing sand. The water instead of being allowed to seep through the sand, as in the English process, is forced through the filters by gravity at the rate of 100,000,000 to 150,000,000 gallons per acre per day. Thus, an acre of surface of American or mechanical filters will filter about forty times as much water as the same surface area of English filters.

In this process, a substance, such as alum or iron, which will precipitate the flocculent material in the water, and at the same time form a gelatinous hydrate with the water itself is always used. The aluminium hydroxide entangles the suspended matter and acts as an artificial schmutzdecke. There are different methods for adding the alum. One of these is to have a small automatic measuring and mixing apparatus attached to the filter.

The filters are cleaned by reversing the flow and at the same time agitating the sand and gravel by mechanical arms and rakes. Recently, compressed air, admitted from beneath, has been used to great advantage.

The action of rapid-sand filters is purely mechanical; no time is allowed for any biologic action.

Chemical Purification.—The use of chemicals as coagulants must not be confused with that treatment known as "chemical purification." In the former case the chemical is simply used to precipitate the dissolved and suspended matter. The water, from the bacteriological standpoint, is purified simply by mechanical means. In true chemical purification, the chemical is added to destroy living organisms by poisoning them.

The most common chemicals used for this purpose are hypochlorite of lime, chlorine, copper sulphate, and ozone. While not truly a chemical treatment, the use of ultra-violet light may be mentioned, as it has definite germicidal action on bacteria.

The *ozone* method depends on the production of nascent oxygen, which has a pronounced germicidal action, particularly on pathogenic bacteria. Experimental results indicate that the efficiency of ozone is greater than that of any other germicide. Absolute sterilization by this process cannot, however, be obtained, and up to the present time it has proved impractical in the purification of large water supplies, because of the cost.

At Koenigsberg, East Prussia, the water is first coagulated with alum, and then ozonized. The resulting water is said to be "sparkling, and nearly sterile."

Ozonization systems are not used for the purification of any public water supply in America. In Europe, the practice has had a limited use. Powell[1] summarizes the extent of the use of the method of purification in Europe:

> In 1919, there were between 60 and 70 municipalities in Europe employing ozone for the sterilization of public water supplies. The majority of these plants now in use are comparatively small, although some large units have been constructed and operated. The St. Maur system at Paris, with a daily capacity of 24,000,000 gallons, and the Petrograd plant in Russia, treating about 13,000,000 gallons of water daily, are notable installations.

These two supplies would approximate that needed to supply the towns of Bridgeport, Conn., and Dallas, Tex., respectively.

As an argument in favor of the use of ozone, the following may be quoted:

> The ozonizers of the Baltimore County Electric Company have been so modified that efficient results have been obtained. Organic colors, either in solution or in colloidal suspension, and odors have been eliminated; bacteria have been satisfactorily removed without the addition of objectionable chemicals and without leaving any detrimental after effects from the treatment. The cost of operation is less than $4 per million gallons. The process should be found efficient and economical, provided due consideration is given to the adaptability of the method for each particular supply.[2]

The efficiency of *copper sulphate* is due to the fact that it acts as a mineral poison to bacteria, and especially to algæ (a group of microscopic organisms which, by their taste and odor, cause so much trouble in reservoirs). The germicidal action of the copper sulphate is probably that of a crystalloid, permeating the bacterial cell wall and thereby producing the toxic effects. In the presence of the bicarbonates of calcium and magnesium the following reaction takes place:

$$CuSO_4 + Ca(HCO_3)_2 = CaSO_4 + Cu(OH)_2 + 2CO_2.$$

The chemical change, while it destroys the toxic power of the salt, really aids in the ultimate purification of the water, since

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[1] "Water Works Practice," Manual of the American Water Works Association, p. 266, 1926.

[2] POWELL, S. T., *Journal of the New England Water Works Association*, vol. 29, pp. 87–93, 1915.

the hydroxide of copper formed acts as a coagulant, precipitating the suspended organic matter. This salt is rarely used, except in large reservoirs.

Chlorinated Lime.—The chemical treatment that has had the most marked success, is the one proposed by Traube, in 1893. This consisted in the introduction of a very small amount (about 0.1 to 0.4 parts per million of available chlorine) of chlorinated lime, commonly called "hypochlorite of lime" or "bleaching powder." Usually in the treatment of raw water, 0.3 to 0.4 parts per million are used, while in filtered water, 0.12 to 0.2 parts per million of available chlorine are added, depending on other conditions. Chlorinated lime consists of a mixture of calcium chloride ($CaCl_2$), calcium hydroxide ($Ca(OH)_2$), and calcium hypochlorite ($CaOCl_2$). It owes its germicidal action to the formation of hypochlorous acid, free chlorine, and nascent oxygen when it dissolves in water.

$$2CaOCl_2 + H_2O = Ca(ClO)_2 + CaCl_2 + H_2O$$
$$Ca(ClO)_2 + CO_2 + H_2O = CaCO_3 + 2HClO$$
$$HClO = HCl + O \text{ (nascent)}$$
$$HClO + HCl = H_2O + Cl_2$$
$$Cl_2 + H_2O = 2HCl + O \text{ (nascent)}$$

or

$$CaOCl_2 + CO_2 = CaCO_3 + Cl_2$$
$$Cl_2 + H_2O = 2HCl + O \text{ (nascent).}$$

It will be seen from the above that three distinct types of reactions take place when chlorine is put into water. First, there is a direct oxidation of organic and inorganic substances in the water; second, a direct chlorination of the organic matter (this probably accounts to some extent for the objectionable odors and tastes in water thus treated); third, and most important, is the bactericidal action of the chlorine itself and of the nascent oxygen liberated from the water.

The chlorinated lime is generally introduced into the water in the form of a concentrated solution of the salt in water, through a mixing and feeding apparatus. At the present time, this is the most practical method of purifying water that does not contain a large amount of suspended matter. It is often used as a sole means of purification, and is quite generally used in conjunction with other methods of purification.

Liquid Chlorine.—Chlorination by means of liquid chlorine is rapidly replacing the use of bleaching powder in this country. About 80 per cent of all the water-purification plants in America make use of liquid chlorine. The chlorine is stored in cylinders and allowed to escape as a gas into the water to be treated:

$$Cl_2 + H_2O = 2HCl + O \text{ (nascent)}.$$

This is usually effected by the use of a perforated carborundum disc. Sometimes the chlorine is dissolved in a small amount of water before being injected into the main supply. In comparing the practical use of liquid chlorine with bleaching powder, it is claimed that liquid chlorine application is more compact, requires less storage, produces no sludge, reduces danger of taste and corrosion, is more effective, is more uniform, is more easily regulated, and is handled with a low labor cost.[1]

A comparison of all available data would indicate that, under the most intelligent and careful supervision, chemical treatment will, in actual practice, remove 85 to 95 per cent of the suspended organic matter, and 10 to 15 per cent of that in solution, or 80 to 93 per cent of the total suspended matter and 50 to 60 per cent of all organic matter. Moreover, it will destroy all disease bacteria.

Electrolysis.—The purification of water by electrolysis is carried on to some extent in large office buildings. Like the use of ozone, its cost makes it impractical on a municipal scale. Chevrier and Salles[2] have recently described an apparatus in which ordinary light current (110 to 120 volts) can be employed. The cathode is a metal cylinder. Through the axis of the cylinder passes a platinum wire, which acts as the anode. According to the authors, the sterilizing action is due to the formation of ozone and of free chlorine. The latter is liberated from the small amount of chlorides contained in the water.

In deciding upon the type of purification process to be used by any community, several factors must be taken into consideration. The nature of the impurities other than bacteria is important. Chlorine will not remove organic matter and will often produce unpleasant tastes and odors due to partial oxidation of these substances. On the other hand, a clear supply with little or no

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[1] HALE, F. E., *Engineering Contracting*, vol. 43, p. 173.

[2] CHEVRIER, D., and M. SALLES, "Sterilization of Potable Waters by Electrolysis," *Comptes rendus*, vol. 185, pp. 230–231, 1927.

impurities other than bacteria does not lend itself well to efficient filtration, and chemical purification is best. In more temperate climates, where land values are not of great relative importance, slow-sand filtration will be more desirable than rapid filters. Often it is cheaper and better to drill artesian wells or impound a mountain stream than to attempt to purify a more accessible water supply.

CHAPTER XI

BACTERIA IN SEWAGE

Sewage[1] may be defined as the waste water of a human community, or as a municipal water supply after it has served its purpose.

The water-carriage sewerage system, which is the chief interest here, dates back a little over a century. Sewage contains water that has been used for washing floors, clothes, dishes, etc., animal and human urine and fæces, vegetable decomposition, waste from factories, and, in fact, anything and everything that is the waste of city life and is small enough to enter the sewer. Some of this matter is in solution in the water which forms the greater part of the sewage, some in colloidal suspension, and some simply carried along in small or large masses.

The American Public Health Association and the American Society of Civil Engineers have adopted the following comprehensive definition of sewage:[2]

> *Sewage.*—(1) Wash water and water-carried animal, culinary, and, in some cases, industrial wastes. (2) Liquid wastes containing human excreta, and other matter, flowing in or from a house-drainage system or sewer. Excreta include fæces, urine, secretions from the skin, expectoration, etc. (3) Liquid wastes from dwellings and institutions, stables, and business buildings. It may also contain liquid wastes from industries. (4) A combination of (*a*) the liquid wastes conducted away from residences, business buildings, and institutions, and (*b*) from industrial establishments with (*c*) such ground, surface or storm water as may be admitted to or find its way into the sewers. (5) The ordinary liquid contents of a sewer containing organic wastes, which may or may not include street wash.

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[1] Sewage is the liquid contents of sewers. Sewerage is a term applied to the pipes, mains, etc., which physically make up the disposal system.

[2] "Definition of Terms used in Sewerage and Sewage Disposal Practice," American Society of Civil Engineers, Manuals of Engineering Practice, no. 2, New York, 1928.

It will be noted that the above descriptions of sewage do not take into account the disposal of human wastes in rural communities. It is not because the problem of getting rid of human excreta and kitchen wastes on the farm or in the camp is of little importance that the subject is neglected here—more than half of our population live in homes not provided with sewers. But, in these individual farmhouses or small community homes, the problem is chiefly one of sanitation, not of disposal. The bacteriologist, after emphasizing the fundamental danger of permitting any seepage from cesspool or latrine to the well or cistern, finds here no interest in the problem of sewage disposal such as he does in the larger city sewerage systems.

Volume of Sewage.—The volume of sewage in a community sewerage system will average somewhat more than the amount of water consumed in the city's water supply. This is due to a small extent to the added human-waste products, vegetable matter, etc., that form a part of the sewage, and to a greater degree, especially in small towns, to added water, chiefly from private wells, cisterns, etc. Rain and snow and street washings are usually excluded from sanitary sewers. Sewage volume is considerably increased in some cases by manufacturing plants having their own water supply and using public sewers. While great differences exist, the average content of city sewers will approximate slightly over 100 gallons per capita daily.

Composition of Sewage.—When the greatly varied kinds of material which get into a sewerage system from time to time are considered, it can readily be seen how chemical analyses would differ. Sewage is, moreover, undergoing chemical changes due to bacterial action from the time it enters the sewer. If it were possible to analyze a definite lot of sewage at one point in the sewer main and later analyze the same definite volume at another point, it would be found that the two analyses would not agree.

The following table[1] gives the estimated chemical contents of an average sample of sewage, in parts per million, and in grams per capita, assuming a per capita volume of 100 gallons per diem.

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[1] Rosenau, M. J., "Preventive Medicine and Hygiene," 5th ed., p. 1099, New York, 1927.

Estimated Constituents of Average Sewage

(*After Fuller*)

		Grams per capita daily[1]	Parts per million[2]
Oxygen consumed	Two minutes' boiling	15.0	39.6
	Five minutes' boiling	22.0	58.0
Nitrogen as	Free ammonia	7.0	18.5
	Albuminoid ammonia	2.5	6.6
	Organic	8.0	21.1
	Total	15.0	39.6
Chlorine		19.0	50.2
Fats		19.0	50.2
Dissolved matter	Total	136.0	359.0
	Mineral	99.0	261.0
	Organic and volatile	37.0	98.0
Suspended matter	Total	66.0	246.0
	Mineral	58.0	140.0
	Organic and volatile	40.0	106.0
Total solids	Total	229.0	605.0
	Mineral	152.0	402.0
	Organic and volatile	77.0	203.0

Bacteria, 322,000,000,000 per capita daily.

[1] These figures also indicate parts per million if the per capita volume of sewage is 264 gallons per day.

[2] Assuming a per capita volume of 100 gallons per day.

Number of Bacteria in Sewage.—The composition of sewage makes it an excellent culture medium for bacteria. The total number of bacteria in sewage varies greatly. Investigators have reported from 500,000 to 12,000,000 per cubic centimeter. The variations in numbers are due to many factors. The age of the sewage, the kind of water, the proportion of organic matter present, etc. influence the number of bacteria found in sewage from different communities. The same factors cause changes in the number present in the same sewage at different times, and in samples collected simultaneously at different points of the sewerage system.

The Kinds of Bacteria in Sewage.—The bacteria present in sewage vary even more greatly in kind than in numbers. Any germ which finds its natural habitat in the human or animal body —except a few obligate parasitic organisms; any bacterium which is a natural habitat of water; or any soil form may be found from time to time in sewage. To enumerate the species of bacteria found in sewage therefore would mean to name practically every known germ.

In different methods of sewage treatment in which bacteria present in the sewage itself are used to break up the solid portion, the processes are divided into two broad groups, one in which air is freely admitted, and the other where oxygen is excluded. On this basis, the bacteria in sewage may be divided into two groups, the aërobes and the anaërobes. The list of the *aërobes* is a long one. All of the common species of the surface soil, of water, of the air, and of the human and animal intestinal tract are present at times. The more common species are Bacillus subtilis, Proteus vulgaris, Bacillus mesentericus, Aërobacter aërogenes, and Escherichia coli. In numbers the predominating aërobic bacteria are soil bacteria, entering by way of the washtub and basin, the common intestinal bacteria of man (the "B.coli" group), and common water and air forms. Among the common anaërobic species are Spirillum regula, Clostridium welchii, Clostridium amylobacter, and Clostridium butyricum.

Microörganisms in Sewage.—Besides bacteria, many other forms of life are found in sewage. Of these the chief interest is centered on a comparatively few forms of algæ, molds, and protozoa. Of the latter, the Mastigophora (mostly of the order Flagellata) are the most important, both in numbers and in physiological activity. The Infusoria (ciliates) and the Sarcodina (Rhizopoda) are also present, often in large numbers. An objectionable condition in biolytic treatment processes known as "foaming" is caused by protozoa. The number of protozoa in sewage depends largely upon the availability of oxygen and the amount of organic acid present. Objectionable numbers can be prevented from developing by adding sufficient lime to the sewage to produce an alkaline reaction.

Algæ and molds have somewhat the same rôles in sewage as they have in the soil. They are mainly concerned with cellulose decomposition, although some forms are capable of splitting proteins. They are also of considerable importance in the formation of "sewage humus," a black humus-like sludge that is a valuable fertilizer.

Disease Germs in Sewage.—As human excreta form a part of sewage, the organisms found in fæces are included in the bacterial flora. In fact, the sewer has been defined as the direct continuation of the intestine. Where intestinal disease is present in the community, the sewage must contain disease germs.

Typhoid fever, Asiatic cholera, and dysentery are the great epidemic diseases, the causative agents of which are liable to be present in sewage and in water polluted by sewage. These are not the only ones, however. Infantile diarrhea, summer cholera, and other gastro-intestinal complaints are caused by bacteria from sewage. Tuberculosis, jaundice, and septic sore throat may also be transmitted through sewage.

It must again be emphasized that none of these diseases is ever contracted from drinking the sewage itself. No matter how unpleasant the thought, drinking sewage which has been boiled for 15 minutes is a perfectly safe procedure. It is only when the specific typhoid germ or cholera germ or dysentery germ gets into sewage from the alimentary tract of a person harboring it, that the sewage can cause typhoid fever or cholera or dysentery. In other words, the sewage is only a temporary carrier or medium for the transfer of these germs from one person to another.

Enzymes in Sewage.—As is the case with the bacteria in the soil, the bacteria in sewage, through the action of their enzymes, cause various chemical changes to take place. Certain methods of sewage disposal take advantage of this fact. Because of the many and various kinds of material which enter sewers, and their still more varied bacterial content, the expressions "kinds of bacteria found in sewage" must be understood to mean functional groups rather than any specific classes.

These changes are oxidative, reducing, proteolytic, amylolytic, and steatolytic, in accordance with the five groups of enzymes produced by bacteria. The sewage, therefore, soon contains many decomposition products formed by the action of bacteria on the original material.

The soil organisms present are the most important in their action on the material in the sewage. Various genera of the family Nitrobacteriaceæ oxidize ammonia to nitrites, nitrites to nitrates, sulphur to sulphites and sulphates, etc. As fresh sewage contains a large amount of dissolved oxygen, the aërobic action of bacteria starts immediately, and unless artificial methods are employed, this action soon ceases on the depletion of oxygen.

The anaërobic bacteria are usually saprogenic, producing putrefactive changes in the protein material. Just what these changes are, chemically, it is hard to state. The result, however, is that proteins are liquefied or rendered soluble in the water, so that the large clumps of fæcal and other nitrogenous matter are destroyed.

The changes occurring during putrefaction always involve the formation of simpler substances out of the more complex. Proteins are changed to albuminoses, to peptones, amino acids, amines, ammonia, and free nitrogen. Other anaërobes are concerned with the reduction of nitrates (denitrifying bacteria) and sulphates. Still others are associated with the saponification or hydrolysis of fats. These are the glycerogenic bacteria and their action is due to steatolytic or lipolytic enzymes. Others are concerned with denitrifying changes, such as the hydrolization of urea and ureates.

Finally, a large group of anaërobic zymogenic bacteria ferment cellulose. In a previous chapter, the difference was shown between fermentation induced by zymogenic bacteria and putrefaction caused by saprogenic bacteria. In the chemical changes taking place in sewage, however, it is almost impossible to draw a clear line of division between the two processes. Many saprogenic bacteria are also zymogenic, in that they attack cellulose as well as proteins; and zymogenic bacteria may be proteolytic. In nature, moreover, cellulose is often closely bound up with nitrogenous matter, and it is almost impossible to say where putrefaction ends and fermentation begins. Of the anäerobes mentioned, Spirillum regula, Clostridium amylobacter, and Clostridium butyricum seem to be most intimately connected with cellulose splitting.

The biochemical activity of bacteria and other forms of life in sewage depends, of course, upon the enzymes which they produce. The particular type of enzymes active in the decomposition of sewage depends in turn, upon the predominance of specific bacterial forms. In general, it may be stated that the enzymes active in decomposition of organic matter and in oxidation and reduction in the soil find their counterpart in sewage. Diastase, rennet, trypsin, and lipase are the most active in the conversion and splitting of carbohydrates, proteins, and fats.

The parts played by the various groups of bacteria and other microörganisms are described above. Just what organisms are concerned, however, and under what conditions of symbiosis, and environmental relationships, it is impossible to state. Certainly, the changes produced are not always exactly the same. At times one set of organisms predominates and again another entirely different set. In two sewage-treatment plants, the action will be entirely different, although, apparently, all factors are alike. In fact, the microbiology of sewage can be spoken of only in general terms.

Sewer Gas.—In these processes of sewage decomposition, certain gases, as hydrogen, hydrogen sulphide, carbon dioxide, methane, and ammonia, are formed as by-products. The term "sewer gas" is generally used to describe a mixture of these gases.

Much discussion has taken place regarding sewer gas. For years it was believed that the "noxious odors" from sewers were the cause of disease. Professor Sedgwick said:[1]

> It is commonly believed that much sickness is directly caused by the emanations of gases from sewers, drains, cesspools, or other receptacles for sewage and similar foul or decomposing substances. This belief even goes so far popularly, and sometimes professionally, as to serve as the all-sufficient explanation for the occurrence of certain specific diseases, such as typhoid fever, dysentery, diphtheria, and scarlet fever.
>
> Closely examined, the belief in the efficiency of sewer gas as the cause, not only of general, but also of specific, disease appears to rest upon the idea that in some way or other poisonous gases, after having been formed in sewers, cesspools, and the like, by active decomposition of the foul substances therein, escape into the air, and, being inhaled, either by virtue of their chemical character or by means of microörganisms, for which they are a vehicle, produce insidious general poisoning or specific disease. It is very seldom, however, that the sewer-gas theory of disease is thus explicitly and clearly defined. More often it takes the form of the simple statement or belief that typhoid fever, dysentery, diphtheria, or malaria are directly produced by broken drains; and it is this form chiefly of the theory or belief which requires to be corrected.

The facts with regard to sewer gas, and the part which it plays in the causing of disease, appear at present to be as follows, quoting Professor Sedgwick:

> In the first place, there is reason to believe that the dangers of sewer gas have been very much exaggerated. There is no doubt, of course, that sewage is a decomposing liquid, and that it may, and often does, contain the germs of specific disease. But, on the other hand, the facts that workmen frequently spend much of their time in sewers, with

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[1] Sedgwick, W. T., "Principles of Sanitary Science and the Public Health," p. 347, New York, 1911.

impunity, or work upon or about sewage in sewage-purification works or on sewage farms, seem to show that experience does not confirm the idea that the gases emanating from sewage are always or necessarily dangerous. Furthermore, careful chemical and bacteriological examinations of the air of sewers have shown not only that dangerous gases cannot ordinarily be detected in such air, but even that sewer gas is singularly free from microörganisms. A little reflection will show that these results might have been expected, for decomposition of sewage in the sewers is seldom very advanced or extensive, while the air of sewers, being very quiet, ought to contain few bacteria.

Reasons for Sewage Disposal.—The enormous financial outlay of American cities for the purpose of sewage disposal are for two purposes: first, to get rid of wastes in an inoffensive manner; and, second, to get rid of these wastes in a way that will not endanger health. The chief concern, from the public-health viewpoint is that pathogenic or disease germs may be present in sewage. For practical purposes, these disease germs must be considered as actually present. While complete sterilization is not practical, the sewage must be so treated that the dangers to public health are eliminated. This includes the destruction of disease organisms, not only in the liquid portion of the sewage, but those which may be imbedded in clumps of solid matter.

The two fundamental requirements, therefore, of any disposal system are to get rid of the solid matter which may hold pathogenic bacteria, and the purification of the liquid sewage. Closely linked, then, to these necessary requirements is the aesthetic desire of getting rid of the putrescible organic matter in the sewage.

Natural Purification of Sewage.—The "natural purification" of sewage has been discussed under the natural purification of water. Dilution, oxidation, sedimentation, and biological factors have the essential rôles. That these "natural" means are not sufficient is self-apparent. Some "artificial" means of handling sewage must, therefore, be used so that it is not a menace to the health of the community, or to other communities.

Artificial Purification of Sewage.—The "artificial" methods of sewage disposal may be divided into three broad groups, which are, roughly, (*a*) mechanical methods, (*b*) biological methods, and (*c*) chemical methods. The type of method selected by any particular community depends upon local considerations. Sometimes, because of economic or natural factors, one type represents decided advantages. In other cases, another type is more suited to peculiar conditions. Often a combination of two or of all three types is used.

Mechanical Disposal.—The term "mechanical disposal" is here used to describe methods of getting rid of sewage without the aid of either bacterial or chemical action. This may be either a single method of bulk disposal or may be divided into three processes: the separation of liquids and solid sewage, the disposal of the liquid, and the disposal of the solid matter.

The earliest and simplest method of getting rid of municipal wastes was to empty the sewage into rivers or the ocean. The former method is a decided menace to public health, and laws are being rapidly passed to abolish this practice. Emptying the sewage into salt water, however, is not dangerous, except where shellfish are gathered from sewage-polluted water. Where the pipes are far enough from shore and are equipped with backwater gates and valves to prevent tidal flow-back, this method of disposal need not create a nuisance.

Broad irrigation, or "sewage farming," over land used for farming purposes, has been tried in this country, but with little success. The sewage is run over the ground through ditches. The theory was that the farm produce raised would pay for the sewage disposal. Unfortunately, sewage possesses little fertilizing power, and only in a very few cases has irrigation by this method been found practical. The chief value of the sewage is in the water itself. Unless the ground is sandy and porous, the method may create a positive nuisance. Several European cities, among which are the capitals, Berlin and Paris, are still using this method with apparently satisfactory results. The size of the beds necessary depends upon the porosity of the soil. The rate of application is usually from 5,000 to 15,000 gallons per acre per diem.

Subsurface Irrigation.—In some small communities, where the underlying ground is sandy, subsurface irrigation is employed. The sewage is discharged through numerous small pipes laid in rows 15 inches to 2 feet under the surface of the ground. The rate of application is about twice that of broad irrigation.

Sedimentation.—If sewage is allowed to flow slowly through a tank or basin, most of the suspended matter will settle out. Tanks constructed for the purpose are built to take advantage of all factors to produce the greatest amount of settling. The intake usually consists of a weir, or of a number of openings below the surface, to avoid a direct current across the tank. The process may be continuous, the flow being constantly maintained at a velocity of about 2 feet per minute, and the sludge drawn off at regular intervals; or it may be intermittent. In the latter case, the sewage is allowed to flow rather rapidly into the tank until it is filled. The flow is then stopped and the contents allowed to settle for ½ to 6 hours. The liquid is then drained off and the process repeated. Sedimentation is not in itself a method of sewage disposal, as it simply separates the solids from the liquid portion. Both portions must receive subsequent treatment.

In some cases sedimentation is forced by alum or some other coagulant. This process is known as "precipitation," and will be taken up under the discussion of chemical methods of sewage disposal.

Biological Disposal.—By far the most successful methods of sewage disposal, both in economy and in desirability from the safety and aesthetic points of view are the so-called "treatment" plants. In these methods, advantage is taken of the bacteria normally present in sewage, and conditions are made most favorable for their enzymic action in destroying the organic material. The primary advantage of these methods over the mechanical methods is the elimination of the bulk of the solids and the resultant ease in purification and disposal of both the solids not acted upon, and the liquid sewage.

The different processes coming under this head are rarely used alone. Usually, two or three follow each other, and a combination of methods is employed.

Preliminary Methods.—As sewage may contain anything small enough to enter the sewers, it may readily be seen that substances which cannot be acted upon by bacteria are always present. In order to get these bodies out of the sewage the following preliminary methods are used.

Screening.—Screens or strainers, when used as a part of a sewage-disposal system, are usually placed directly at the outlet of the outfall sewer. They may consist of metal rods (bar screens or racks), screens of about ½-inch mesh, or may be quite elaborate in the form of a revolving cylinder of wire mesh (band screens). When bar screens are used, they are usually set at an angle of 30 degrees, and they slope in the direction of the flow of sewage. Cleaning apparatuses are necessary, and a further means of disposal of the material collected in the screens is essential. Incinerators are usually employed.

Grit Chambers.—At times the sewage is allowed to flow through a detention compartment somewhat larger than the sewer. This is known as a "grit" compartment. The object of the grit chamber is to check the velocity of the sewage in order to permit gravel and other heavy, solid matter, which would not burn in the incinerator, to be deposited. In most treatment plants, both screens and grit chambers are used, the screen being placed so that the sewage passes through it just before entering the grit chamber.

Treatment Methods.—With the exception of the electrolytic process, all the modern treatment methods of sewage disposal make use of the bacteria present for the digestion of the sewage solids. In some of the processes, anaërobic bacteria are the principal agents, in others, both aërobic and anaërobic bacteria and in still other, the aërobic organisms alone are used. In each case, the structure of the plant is such that optimum conditions are prepared for the type of bacterial growth desired.

Septic Tanks.—The fact that sludge will be dissolved by bacteriological means if left standing is taken advantage of in a method of sewage disposal known as the "septic-tank process." The method was first proposed by Donald Cameron, in England.

The essential difference between sedimentation tanks and septic tanks is the length of time the sludge is allowed to stand in the basin, with the resultant greater bacterial action in the latter. Another difference is that, owing to the bacterial action in the septic tank, gases are formed, which, on rising to the surface, carry up solid material which forms a scum on the top of the liquid. This scum excludes the air from the tank and thereby produces a favorable condition for the development of the saprogenic and zymogenic anaërobes. It also, on account of its foamy nature, serves as a protection of the sludge from temperature changes.

These organisms putrefy the sludge in the tank to a great extent, and produce gases and soluble split products, thereby diminishing the cost of disposal as compared with that when dealing with the large amount of sludge in the sedimentation method.

The term "septic" means putrefactive, and indicates the kinds of bacteria chiefly invloved. The changes in the organic matter in the sewage are, in general, the same as occur in the destruction of organic material in the soil. Ammonification and the destruction of cellulose are the principal processes. Denitrification by reducing bacteria also takes place.

The tank is constructed of concrete, or of brick or stone, lined with cement. It should be water tight and not allow any seepage. Commercial septic tanks made of cast iron or cement are obtainable for small installations.

The rate of application of the sewage is between 200,000 and 400,000 gallons per acre per diem. The tank capacity should be between 15 and 30 gallons per capita per diem, allowing an 8-hour overturn.

Among the factors which aid or retard the efficiency of the septic or digestion tank are reaction and temperature. Organic acids, produced as split products, retard digestion, and lime is added to neutralize these acids. The most favorable growth occurs in an alkaline medium (pH 7.8). When the temperature falls below 50°F., digestion stops. In modern plants, sewer gas is burned to furnish heat in winter. Digestion in the septic tank is never complete, and the accumulated sludge, as well as the overlying scum, has to be removed periodically. In the installation of a new tank, some of the material from a well-operating tank is added as a culture or "starter."

Imhoff Tanks.—As the beneficial effects of the scum on the septic tank are largely outweighed by the necessity of its ultimate disposal, various modifications of septic tanks have been proposed. In the Imhoff, or two-story tank, the sewage enters through a V-shaped trough, the sides of which do not quite meet at the bottom. The solid material settles down into the tank through this opening. The sides of the trough prevent gas bubbles from carrying masses of the sludge back into the effluent, and prevent a scum from forming. Gas-outlet vents are provided, and also a means of sludge removal from the bottom of the tank. The solid material is acted upon by the anaërobic bacteria exactly as in the one-story septic tank. The organic nitrogen and albuminoid ammonia are greatly diminished in amount. There is also a marked reduction in the amount of cellulose, indicating that the zymogenic bacteria, like Clostridium amylobacter, play an important part in the anaërobic change. Septic tanks are usually constructed in batteries to take care of 100,000 to 300,000 gallons a day. At Columbus, Ohio, is a large tank having a capacity of about 20,000,000 gallons a day. The retention period is shorter than that of the septic tank, being usually from 2 to 6 hours.

As is the case with the septic tank, not all of the sludge is liquefied by this process, and the removal of the undigested sludge at regular intervals is necessary. Usually, a small amount of the old sludge is left in the tank after cleaning, as a "starter" for the new sewage. The use of the septic tank and the Imhoff tank should never be considered as complete processes. Further treatment of the effluent is necessary, and the undigested sludge must be disposed of.

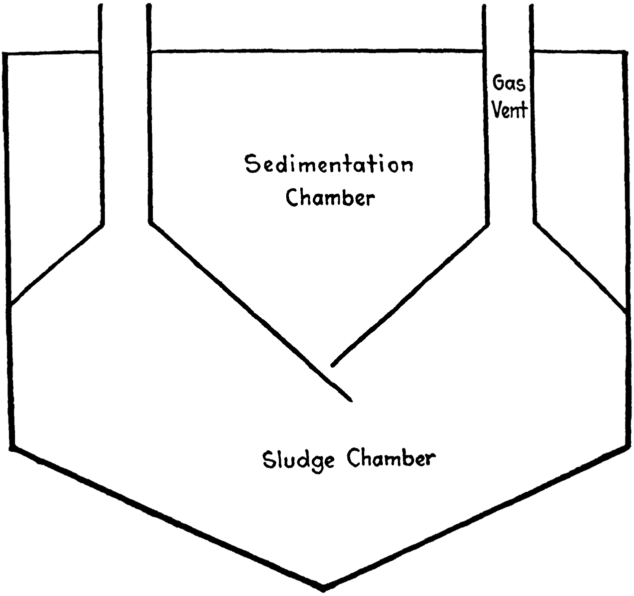

FIG. 31.—Imhoff tank. Diagrammatic sketch.

Contact Beds.—Since the biological changes are mainly anaërobic, the effluent from a septic tank often has a more disagreeable odor than the raw sewage. Contact beds have been designed to prevent the development of this feature by allowing the intermittent action of saprogenic and oxidizing bacteria. These beds are water-tight basins filled with slag, clinkers, broken crockery, sharp stones, coke, or slate. The bed is filled with sewage and allowed to stand for a time to permit the action of the anaërobes. The sewage is then drained off and the material clinging to the slag allowed to stand exposed to the air. During this exposure, the oxidizing bacteria have an opportunity to act on the material and quickly change it into inoffensive salts, like carbonates and nitrates. As a rule, about 2 hours is allowed for filling the bed, 2 hours for anaërobic action, 2 hours for emptying, and 2 hours for aërobic action, making a complete cycle in 8 hours. Contact beds are generally used in series of two or three, the action in the final bed being practically aërobic. The rate of application is 300,000 and 800,000 gallons per acre per diem. It has been noticed that the effluent from the contact beds contains many nitrogen-fixing bacteria of the genus Azotobacter. This would make the sludge very valuable as a fertilizer.

Sand beds for intermittent treatment are very popular in New England and other places where the raw material is to be found in ample quantities locally. These beds are constructed of sand, in three layers. The top layer, of 14 to 18 inches in depth, is of fine sand about 0.01 inch in diameter. This layer actually filters out a majority of the bacteria. It also regulates the flow and ensures a minute subdivision of the water and the subsequent thorough oxidation in the lower layers. The top layer rests on a bed of coarse sand about 3 feet thick which, in turn, covers a layer of medium-sized gravel of about the same thickness as the top layer. The total depth of the bed is about 6 feet. Underdrains are provided for the collection of the fluid passing through the bed. The sewage is run over the surface of the bed through distributor pipes. It rapidly soaks into the bed, which is then allowed to stand for from one to several days before another lot of sewage is run on. The efficacy of this type of bed depends partially on the action of oxidizing bacteria in the coarse sand and gravel beds, and partially on a mechanical filtration in the top layer. This top layer must be scraped at regular intervals to permit air to get into the interior of the bed.

Trickling Filters.—The trickling filter is in no sense a filter, as the term is generally used. It is constructed of broken stone, slag, coke or even of small branches of trees, or broken lath. The beds are between 6 and 7 feet in depth. This process is a purely oxidative one, and must follow some preliminary process, such as screening or septic action. Except for the anaërobic action, it is the same as that of the contact bed. No attempt is made to "filter" or strain out bacteria by this method, as in the filtration of water.

The essential conditions are very slow motion of very thin films of liquid over the surface of the particles (gravel, slag, stone, etc.) that have spaces between them sufficient to allow air to be in contact with the films of liquid. With these conditions it is essential that certain bacteria be present to aid in the process of nitrification.[1]

The sewage is usually applied to the filter by means of sprinkler nozzles. About two or three million gallons of sewage per acre per day can be handled in this way. The process removes almost 100 per cent of the organic matter. The sludge obtained from sewage after passing through a trickling filter is sometimes called "humus sludge," as it resembles humus in appearance. This method is also very efficient from the bacteriological standpoint.

Serious objections have been raised against this process on the ground that it gives off disagreeable odors and is a breeding place for flies in summer. However, 1,000 feet is considered an ample distance to prevent a nuisance from trickling filters. For instance, various beds at Phillipsburg and Madison, N. J., and Philadelphia, etc. are less than 100 feet from houses and have not caused any complaint. Trickling filters and contact beds are sometimes given the general name of coarse-grained filters, to distinguish them from the sand beds.

Activated Sludge.—In this method of treatment, raw sewage is run through aërating tanks, where it comes in intimate contact with air and biologically active sludge previously produced by the same process. Air is pumped through the sewage by an air compressor, through perforated pipes near the bottom of the tank, or through perforated plates built into the bottom of the tanks. The air serves a double purpose. First, it aids the development of oxidizing bacteria, and, second, it thoroughly distributes, by means of the currents it sets up, the aërobic (oxidizing, nitrifying, and nitrogen-fixing) organisms from the activated sludge "starter" into the fresh sewage entering the tank. A modification of this method, known as bio-aëration consists of mechanically agitating the sewage with paddle wheels. After thorough aëration, the sewage is run into sedimentation tanks, and the sludge collected. Experiment shows that it requires from 1.5 to 2.0 cubic feet of air to treat a gallon of sewage containing 325 parts of suspended matter per million gallons in a tank carrying 10 feet

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[1] Folwell, A. P., "Sewerage," 8th ed., p. 367, New York, 1918.

of liquid sewage, when the sewage has a temperature of 50°F. and upward.[1]

By this process the oxidizable matter in the sewage is almost completely oxidized; the dried sludge contains between 10 and 15 per cent nitrates. The sludge is practically odorless, and contains, in addition to the usual flora of nitrifying and other oxidizing bacteria, great numbers of nitrogen-fixing organisms. It makes, therefore, a valuable fertilizer. The term "activated sludge" is derived from its bacterial content.

Various other special types of sewage disposal have been proposed and are in use to a limited extent. Among these are "wave filters," or rapid intermittent filters for rapid oxidation and cleaning, upward filtration or cultivation filters to combine oxidation and liquefaction in the same filter.

It will be seen that all of the treatment processes above described are biologic, that is, the digestion of the sludge or transformation of the organic matter into non-putrescible substances depends upon the action of anaërobic and aërobic bacteria. While pathogenic bacteria may survive these treatments, it is altogether unlikely that they do to any extent, or, if they do, that they retain their virulence. Pathogenic bacteria are paratrophic and live best at 37°C. In a septic tank, contact bed, or filter bed, they are in competition for food with an enormous number of other bacteria which are under their most favorable environmental conditions. It may readily be seen, therefore, how the pathogenic bacteria are "overgrown" by other organisms, with a resultant attenuation and finally death.

Chemical Disposal.—The disposal of sewage by chemical processes is no longer practiced to any great extent. New methods may be suggested in the future, however, which will take advantage of the enormous value of these wastes and prepare them for use either as a cheap fuel or as a source of fats and fertilizer material.

In the process of sedimentation, a coagulant, such as alum, lime, sulphate of iron, or animal charcoal, is sometimes used to precipitate the solid matter. The general method is known as the A.B.C. process (alum, blood, clay). The coagulant is usually added to the sewage and mixed with it just before it enters the sedimentation basin. About 100 pounds of alum are required for 1,000,000 gallons of sewage. Some further method of disposal of

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[1] HATTON, T. CHALKLEY, *Engineering News Record*, vol. 75, pp. 1–16, 1917.

the sludge and purification of the liquid is necessary after precipitation.

Electrolysis.—The electrolytic treatment of sewage is the only modern digestion method in which bacteria do not play a part. Purification by electric current for a time found wide application. The first process for the electrical treatment of sewage was designed by Webster in London, England, in 1889. In the Webster process, the sewage was allowed to flow through iron troughs made of sections insulated from each other, and through which an electric current was passed. The oxygen liberated from the water had a germicidal effect on the sewage, but also oxidized the iron electrode, forming oxides of iron, which, in turn, coagulated the sludge. This action, therefore, was mainly one of chemical precipitation.

In another electrolysis process, salt was added to the raw sewage. In this case, electrolysis liberated chlorine from the brine, which has its chemical effect on the sewage.

In the Landreth direct oxidation process, lime is added to the sewage before it passes through the electrolysis chambers. Steel plates are used as alternating anodes and cathodes. The lime serves a double purpose. It aids in sedimentation and also neutralizes the sewage and acids formed during electrolysis, thus permitting but little chemical action on the electrodes. The sole action, then, of the electricity is to form nascent oxygen from the water, which acts as a germicide. Screening is essential as a preliminary course, and the solid particles, removed by the screen, are burned.

The main advantages of electrolytic purification over most of the others is that rapid oxidation takes place, with the elimination of all noxious and disagreeable odors.

> Bacteriological study of this process shows that it: . . . brings about a reduction of over 92 per cent in the total bacterial count, while the lime (precipitation) treatment alone effects a reduction of 82 per cent, and electrical treatment produces a reduction of only 38 per cent. The reduction in Escherichia coli amounts to 99.95 per cent with the electrolytic lime treatment, and to 92.3 and 81.8 per cent, respectively, with the lime treatment and the electrical treatment.[1]

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[1] Creighton, H. J. M., and B. Franklin, "Electrical Treatment of Sewage: The Landreth Direct Oxidation Process," *Journal of the Franklin Institute*, vol. 188, pp. 2, 157–187, 1919.

Relative Germicidal Efficiency of Methods.—The following table prepared by Whipple[1] shows the bacteriacidal efficiency of different sewage processes. It must be borne in mind that bacterial reduction is not the sole purpose of a disposal method. In fact, during the process, *specific* bacterial growth is the factor aimed at. The resultant material, however, should not be putrescible and the bacterial content thereby lowered.

Process	Percentage Removal of Bacteria
Coarse screens	0 to 5
Fine screens	10 to 20
Grit chambers	10 to 25
Sedimentation	25 to 75
Septic sedimentation	25 to 75
Chemical precipitation	45 to 80
Contact beds	80 to 90
Trickling filters	90 to 95
Activated sludge process	90 to 98
Intermittent sand filters	95 to 98
Broad irrigation	97 to 99
Disinfection of raw or settled sewage	90 to 95
Disinfection of filter effluents	98 to 99

Surface area necessary for different sewage treatment processes.

Process	Rate of Application
Broad irrigation	5,000 to 15,000 gal./acre/diem
Subsurface irrigation	10,000 to 30,000
Sedimentation	500,000 to 1,000,000
Septic tanks	200,000 to 400,000
Contact beds	300,000 to 800,000
Sand beds	100,000 to 150,000
Trickling filters	2,000,000 to 3,000,000

Sludge Disposal.—In the various methods of sewage disposal mention has been made of the fact that sludge, or the accumulation of moist solid organic matter, is deposited and requires additional methods of disposal.

In the sedimentation basins the sludge comprises practically all the solid matter of the raw sewage; in the septic and Imhoff tanks, contact beds, aëration beds, and after electrolytic action, the sludge is less in volume and has been modified by the treatment. In any case some means of disposal is necessary.

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[1] Rosenau, M. J., "Preventive Medicine and Hygiene," revised by G. M Fair 5th ed., p. 1113, New York, 1927.

That sludge contains valuable material is an established fact. After certain methods of sewage treatment it contains organisms and substances which are useful in soil fertility. All sludge contains ammonia and fats. But it also contains fatty acids combined with alkaline earths, grease, or other substances which make it unfit for direct use as fertilizers; and always water in such quantities that it cannot be easily handled unless dried.

Several methods of treatment of sludge are in use and in some cases the cost of such treatment is balanced by the sale of the products reclaimed.

Treatment with sulphuric acid (acidulated sludge), in order to recover the grease, has been partially successful. The destructive distillation of the sludge with the recovery of oils and ammoniacal liquors has been tried. Filtering presses, or centrifuges for drying and "caking" the sludge, boiling with a solvent, like petroleum naphtha for simultaneous dehydration and degreasing, and numerous methods for recovery of material have been, and are, in use. Where a cheap method of discarding the sludge is practicable, however, such as dumping it into the ocean, or filling natural basins where it will not create a nuisance, such procedures are still considered the best.

Disinfection.—This is usually the means of final purification of the liquid sewage following any one or any combination of the above processes. This process consists of the destruction of the bacteria by chemical means. Chlorine and bleaching powder have been found to be the most satisfactory disinfecting agents, although copper sulphate at one time was quite extensively employed. The methods used are quite the same as those discussed under Water Purification. The action depends upon the chemical reaction of chlorine with water, forming atomic or nascent oxygen, which kills the bacteria. Four and one-half to six parts per million are required.

It was stated that the methods of sewage disposal described above were generally used in combinations. The following example will be of interest to engineering students. This description of the Baltimore, Md., sewage works is given by Theodore C. Schaetzle.[1]

[1] SCHAETZLE, T. C., "Nine Years' Operation of the Baltimore Sewage Works," *Engineering News-Record*, vol. 87, pp. 2, 50, 1921.

The existing plant consists of preliminary vertical bar screens, spaced 1 inch apart; a meter house with five 42 by 21 inch venturi meters; three preliminary plain sedimentation tanks, known as hydrolytic tanks; twenty-eight radial-flow Imhoff tanks; nineteen separate sludge digestion tanks, and 7.8 acres of sludge-drying beds; four cylindrical revolving screens, trickling filter house control; thirty acres of trickling filters; four small pump houses each equipped with an electrically driven centrifugal pump; a hydroelectric power house generating power from available head in the treated sewage; and a final effluent conduit discharging into Black River.

The planning of a municipal sewage disposal plant is distinctly an engineering problem. The choice of a combination of processes will depend on the local conditions, area required, and the economy of local materials of construction.

CHAPTER XII

BACTERIA IN THE AIR

Composition of Air.—Air is a fairly uniform mixture of gases consisting of approximately 80 per cent nitrogen and 20 per cent oxygen. There is also present about 0.04 per cent carbon dioxide and slight traces of other gases. Water, either in the form of vapor or mist, is always present in varying amounts. In almost any sample of air there will be found some suspended matter consisting of dust of various kinds, pollen of plants, and bacteria. This suspended matter in air is by no means uniform in kind or amount, as is the gaseous content.

Dust.—In the vicinity of large industrial plants the air contains a great deal of dust, the nature of which depends upon the nature of the operations. Near steel and iron works, the dust in the air will consist in part of minute metallic particles which are abrasive in nature. Near industrial plants the dust may consist of particles of carbon. Chemical plants send off into the air fumes which are sometimes gaseous and sometimes made of small particles of solid matter which may be highly corrosive. Dust, therefore, does not mean the cloud thrown by a passing automobile on a dirt road, but a complex and varying mixture of minute solid particles in the air.

Suspended matter tends to sink to the earth, so that the air becomes purer as we ascend from the ground. Dust may be entirely absent immediately after a rain and over the ocean at some distance from land.

Water is suspended in the air in the form of vapor, and the amount so suspended determines the humidity of the air. When air contains all the water it can carry in the form of vapor, it is said to be saturated, and any excess will be carried in the form of minute globules, which become visible in the form of fog or cloud. The capacity of air to hold water in suspension increases with the rise in temperature.

Sunlight, by heating the air and thereby lowering its relative humidity, dispels fog and causes clouds to disappear.

A constant supply of oxygen is necessary to human life, and, therefore, fresh air, or outside air, is essential to the body. In the process of breathing, the air undergoes marked changes. Expired air is saturated with water vapor, and the carbon dioxide content raised and the oxygen content lowered.

Bad Air.—Bad or vitiated air has a decided physiological effect, the symptoms of which are dullness, nausea, and headache. Experiments have shown that these effects are not due to the increase in carbon dioxide, the decrease in oxygen or to any "body emanation." The reasons why air becomes bad are threefold. The most important of these is improper temperature. Almost invariably this is too high. Next to temperature comes improper humidity. As temperature rises, the relative humidity is lowered. The third reason lies in lack of movement in the air.

The following resolutions were adopted by the American Public Health Association at their annual meeting in 1925:

> *Whereas,* Hundreds of thousands of dollars are wasted every year on this continent in the installation and operation of systems of school ventilation which are not only not beneficial but are positively harmful to the health of school children; and
>
> *Whereas,* In the light of current knowledge, the supply of as large an air volume in schoolrooms as 30 cubic feet per minute per capita is necessarily accompanied by dangerous overheating of the schoolroom in order to avoid resulting drafts; and
>
> *Whereas,* The use of ozone and other chemicals for treating schoolroom air has little or no scientific justification and little or no practical value; therefore be it
>
> *Resolved,* That the system of ventilating schoolrooms by fresh, untreated, outdoor air, admitted at the windows with gravity exhaust ducts for removing vitiated air from near the ceiling, is the most generally satisfactory method of school ventilation; and be it
>
> *Resolved,* That we recommend that State laws and city regulations interfering with such scientific and economical methods of school ventilation should be repealed in the interest of the public health.

Air Bacteria.—In discussing microörganisms in the air (bacteria borne through the air by winged creatures will not be treated in this discussion), it must be understood that the air is not a natural environment for bacterial life. There are no "air bacteria." In other words, the air does not contain the necessary food for the growth and multiplication of bacteria—not even to the extent that water contains food for its natural bacterial inhabitants, which can take their food from the very simplest of chemical compounds. Yet bacteria are usually present in the air, and they are of considerable economic importance.

Historical.—The idea of the air being responsible for disease was accepted long before the nature of disease was realized. Hippocrates described a pestilential state of the air as occasioned by southerly winds or a warm, humid, clouded atmosphere. Galen thought that a putrid air gave rise to pestilential diseases. The contagium theory of transmissible disease was based upon this notion. During the Rennaissance, the contagium theory became modified to the extent that it was believed that infection might take place in ways other than by breathing foul air, as seen in the following passage regarding the manner in which syphilis is contracted.[1]

> When the Venereal Disease first made its appearance in Europe, it was not known that the infection was propagated by coition, the diseased either cautiously concealing the manner how they were infected, in order to hide their wickedness, or it may be not suspecting that so severe an illness could possibly be contracted by copulation solely, as it seemed so unusual a way of conveying contagion. For which reason the physicians who lived at that time in general believed that this new disease *was epidemical like other pestilential Distempers,* and owing to an external and common cause, which some of them thought to be a malignant influence of the Stars or bad aspect of the Planets, and others *an unwholesome disposition of the air,* brought on by rains and inundations.

By the beginning of the nineteenth century the idea of a tangible "substance" in the air rather than the air itself was taking hold. Noah Webster,[2] in 1799, gave us a fair idea of the beliefs of the time.

> From the date of the earliest historical records, the opinions of men have been divided on the subject of the causes and origin of pestilential diseases. All enquiries of the philosopher and the physician have hitherto been baffled, and investigations, often repeated, have ended without leading to satisfactory conclusions.
>
> In the history of opinions on this mysterious subject, there is a remarkable distinction between the ancients and moderns (1800).

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[1] AUSTRUC, JOHN, "A Treatise of the Veneral Disease," p. 136, translated from the Latin by William Barrowby, London, 1737. Italics, the author's.

[2] WEBSTER, NOAH, "A Brief History of Epidemic and Pestilential Diseases," p. 9, Hartford, 1799. Italics, the author's.

The ancients derived most of their knowledge and science from personal observations, as they had very few books and little aid from the improvements of their predecessors. The philosophers of antiquity, attentive to changes in the seasons and to the revolutions of the heavenly bodies, attempted *to trace pestilential diseases to extraordinary vicissitudes in the weather*, and to the aspects of the planets. Modern philosophers and physicians, on the other hand, unable to account for pestilence on the principle of extraordinary seasons, and disdaining to admit the influence of the planets to be the cause, have referred to *invisible animalculæ* and to infection concealed in bales of goods or old clothes, transported from Egypt or Constantinople, and *let loose, at certain periods*, to scourge mankind and desolate the earth.

In the year 1859, while Louis Pasteur was pursuing his "studies on fermentation which are of great interest, connected as they are with the impenetrable mystery of Life and Death,"[1] occurred the "historic stench" in London. During the summer of this year the sewage-polluted Thames gave forth such foul odors that its notoriety traveled around the world. Dr. Sedgwick[2] uses a description of this phenomenon to illustrate certain popular beliefs as to the causes of disease.

For months together, the topic almost monopolized the public prints. Day after day, week after week, the *Times* teemed with letters filled with complaint, prophetic of calamity, or suggesting remedies . . . But more significant still of the magnitude of the nuisance was the fact that five millions (of pounds) of money were cheerfully voted by a heavily taxed community to provide the means of its abatement. With the popular views as to the connection between epidemic disease and putrescent gases, this state of things naturally gave rise to the worst forebodings.

Members of Parliament and noble lords, dabblers in sanitary science, vied with professional sanitarians in predicting pestilence . . . Meanwhile, the hot weather passed away; the returns of sickness and mortality were made up, and, strange to relate, the result showed, not only a death rate below the average, but, as the leading peculiarity of the season, a remarkable diminution in the prevalence of fever, diarrhea, and other forms of disease commonly ascribed to putrid emanations.

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[1] VALLERY-RADOT, "The Life of Pasteur," Pasteur's letter to Chappuis, p. 87, New York, 1923.

[2] SEDGWICK, W. T., "Principles of Sanitary Science and the Public Health," p. 354, New York, 1911, quoted from William Budd, "Typhoid Fever, Its Nature, Mode of Spreading and Prevention," pp. 148–151, London, 1873.

The refutation of the spontaneous-generation theory by Schwann, Pasteur, and Tyndall was based on the fact that bacteria, molds, and other microscopic forms are usually present in the air. These observations led the way to a new idea of "contagium," and directly to our modern bacterial view of disease transmission. Today we believe that, while vitiated air, sewer gas, abrasive dust, etc. may have physiological ill effects in irritation of the mucous membranes of the throat and bronchial passages, and thus prepare the respiratory tract for infection, the air itself cannot cause disease, and only rarely carries the bacteria which do cause specific diseases.

Conveyers of Bacteria in the Air.—Ever since bacteria were found in the air, investigators have tried to explain their presence. The idea that bacteria floated through the air on "rafts" of dust was the first explanation, and one that is generally believed today by the uninformed.

Raft Theory.—The first attempt to account for the presence of bacteria in the air was known as the "raft theory." According to this, bacteria were floating in the air on "rafts" of dust. In the early researches it was noticed that the number of bacteria in the air seemed to bear a relationship to the amount of dust present. This fact can easily be demonstrated by exposing petri dishes to the air in a room where the air has been quiescent, and later when considerable dust has been stirred up. On the other hand, the number of bacteria present in the air is not always proportionate to the amount of dust present. In a very severe dust storm in a desert region there will not be found the number of organisms that are present in the air above a dusty country road.

Free Floating.—When it is considered that the weight of a bacterium is less than a billionth of a gram it will be realized that the minute size of bacteria will in itself account for their presence in the air in the free state and will render unnecessary the assumption that they need "rafts." The fact is that the same conditions (drying and wind) account for the presence of both dust and bacteria in the air, provided they are present on the underlying surface.

In discussing this point, David Ellis[1] says:

> The number of bacteria, however, does not bear a relation to the amount of dust in the atmosphere. Thus, the air in the workroom of

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[1] Ellis, David, "Outlines of Bacteriology," p. 77, New York, 1909.

a certain skin curer was found to be densely impregnated with dust particles from the skins, but there was scarcely a microbe present; while, on the other hand, the atmosphere of the polishing room of a hat firm was found to be remarkably free from dust, yet several kinds of bacteria were isolated.

Water Droplets.—Bacteria may also be present in the air in minute droplets of water blown from the underlying surface in the form of spray, or they may be blown from the human mouth during coughing, sneezing, and rapid and forceful talking. This latter method of dissemination is responsible to a considerable extent for the transmission of certain diseases.

That air contains, as a rule, very few bacteria blown from watery surfaces has been shown in the discussion of "sewer gas." Sewer gas is practically free from bacteria, although the conditions seem to be favorable for their presence in large quantities, since they are in the sewage in enormous numbers, and the gases bubbling from the surface of the sewage could account for their getting into the air. Winslow,[1] in a bacteriological examination of the air of various plumbing systems in Boston, found sewage bacteria only four times in 200 liters of air. In all of these cases there was mechanical spraying of the sewage at the point where the samples were collected.

It must be remembered that these "droplets" are much heavier than air, or even than dust particles, and they very rapidly fall to the ground. The "droplets," therefore, while a source of bacteria in the air, are present only for a short time, and can travel only a very short distance.

Number of Bacteria in the Air.—The factors which influence the number of bacteria in the air are of three kinds; first, those which affect the relative numbers of the bacteria that get into the air; second, those which control the length of time bacteria are suspended in the air; and, third, the viability of organisms. The most important of the first group of factors are: (1) the bacterial contents of the surface acted upon; (2) the dryness of the surface; and (3) the velocity of the air current.

1. *The Earth Surface.*—The nature of the earth surface is the most noteworthy of the factors which influence the relative numbers of bacteria which get into the air. Bacteria must

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[1] Winslow, Report to Sanitation Committee, National Association of Master Plumbers, *Abstracts American Journal of Public Hygiene*, vol. 5, p. 640, Boston, 1909.

exist on the underlying surface in order to get into the air, and, when the nature of the underlying surface is not favorable to bacterial growth, the natural conclusion is that few bacteria are found in the air. The study of the nature of the surface and its bacterial content may be divided into: (*a*) the kind of soil; (*b*) the nature of vegetation; and (*c*) the community.

a. Kinds of Soil.—In general, a rich, fertile soil contains many more microörganisms than a sandy or clay surface; a tract of cultivated farm land is richer in bacterial growth than is a desert region. If the same rate of air currents or wind velocity is present above the farm land and above the desert, the air above the cultivated region will contain more bacteria than that above the desert. In fact, the air of a desert region is practically free from bacteria.

b. Vegetation.—Where the soil is covered with vegetation, as in a grassy field, fewer bacteria can get into the air than where the earth is bare. This is simply a matter of exposure, as when the earth is bare the top surface and the bacteria can, under proper conditions, be blown into the air, while vegetation holds the earth together and protects it from the wind.

c. Community.—In any place where human activity is present, a large number of bacteria are likely to get into the air. Thus, on city streets, in classrooms, in certain kinds of work shops, etc., where the surface contains large numbers of bacteria due to contamination and is being constantly agitated, dust and bacteria are stirred up and get into the air.

2. *Dryness of the Surface.*—Next to the presence of microörganisms on the surface, the condition of the surface as regards moisture is of greatest importance. Dust cannot be blown from a moist surface. Neither can bacteria be blown from a wet surface, except during mechanical agitation, such as bubbling or spraying. Molds, with their reproductive bodies at the tip of aërial hyphæ, liberate their spores into the air even where the surface itself is moist. Moreover, molds bear spores that are relatively small and possess a surface that is not easily moistened. According to Buchanan:[1]

> These spores are resistant to desiccation and light, and remain viable for considerable time even under unfavorable conditions. Furthermore, the fruiting bodies of many, though not all, molds show a distinct nega-

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[1] BUCHANAN, R. E., "Microbiology of the Air," in Marshall's "Microbiology," p. 185, Phiadelphia, 1912.

tive hydrotropism, *i.e.*, the mycelium remains in contact with the moist substratum while the threads which bear the spores rise at right angles to it. These latter are so sensitive that they can detect slight differences in the moisture content of the air and grow in the direction which bring the spores into the driest situations. A slight current of air will detach the spores from these structures and carry them long distances. Bacteria and yeasts lack the specific adaptations for wind distribution found in molds. The material upon which they have been growing must be dried and pulverized before they can be blown out. Many species produce spores or other resistant cells, and physiologically are as well adapted for air distribution as are the molds.

3. *Velocity of Wind.*—The third factor which plays an important part in getting bacteria into the air is the velocity of the air current or wind. Bacteria can only get into the atmosphere by being lifted into it by the wind. Other factors being equal, the greater the wind velocity, the greater the number of bacteria present in the air. Bacteria can *never* leave a moist surface, unless the wind velocity is sufficient to raise the water in the form of spray.

Length of Time Bacteria Stay in the Air.—Passing now to the factors which control the length of time bacteria are in the air, the following are found: (1) the velocity of the air current; (2) the size of the particles upon which they may be, if they are so attached; (3) the relative humidity of the air; (4) the proximity of moist surface with which they may come in contact; and (5) altitude.

1. *Velocity of Wind.*—Of these controlling factors, the wind plays the most important part. According to Buchanan,[1] "An air current having a velocity of only a few centimeters per second will sustain many organisms indefinitely."

Muir and Richie,[2] quoting indirectly from Flugge, state that he (Flugge)

> . . . in dealing with this subject in an experimental inquiry distinguishes between large particles of dust which require an air current moving at the rate of 1 centimeter per second to keep them suspended, and finer dust particles which can be kept in suspension by currents moving at from 1 to 4 millimeters per second. In the former case, when the particles settle, they cannot be displaced by currents of air

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[1] Buchanan, R. E., "Household Bacteriology," p. 465, New York, 1913.

[2] Muir, Robert, and James Ritchie, "Manual of Bacteriology," 5th ed., p.150, New York, 1911.

except when they are moving at least 5 meters per second, but the brushing, shaking, or beating of objects, may, of course, distribute them. In case of the finer dust, the particles will remain long suspended, and, when they have settled, can more easily be displaced.

2. *Size of Particles.*—Being heavier than air, bacteria will soon settle out of quiet air. When the bacteria are in dust particles or droplets of water, the rate of sedimentation will be even greater and increase directly with the weight of the conveyor.

3. *Humidity.*—Dry air usually contains more bacteria than moist air, because a humid atmosphere soon means a damp surface, and the bacteria which were in the air are caught and held at the surface. Even more important is the fact that bacteria become the nidi of drops of condensation and fall with their surrounding water.

4. *Moist Surface.*—Over a snow-capped mountain, at sea, and after the ground has been wet by rain, there are few, if any, bacteria in the air. In the first place, rain or snow will wash the atmosphere free of bacteria, and, once caught, they cannot depart from a wet surface. There are, therefore, more bacteria in the air in summer than in winter when the ground is covered with snow, or when drying of the soil is uncommon. The fact that bacteria cannot leave a wet surface accounts for the absence of organisms in sewer air. It is also the reason why gently expired breath is sterile. The air coming from the lungs passes through the moist passages of the upper respiratory tract and mouth. While there are many bacteria in the human mouth, they cannot leave the saliva in which they are held.

5. *Altitude.*—As bacteria settle out of air, the rate of subsidence is greater at high altitudes than at low, due to the rare atmosphere. Even at relatively low altitudes, such as at the tops of buildings, fewer bacteria will be found than near the surface of the ground. In "The Life of Louis Pasteur," Rene Vallery-Radot describes Pasteur's attempt to show that air alone would not produce fermentation, but that bacteria in the air were necessary. To prove his point, Pasteur opened a number of flasks of sterilized yeast water to the air of the Alps where he suspected few bacteria were to be found.

> The next morning, twenty flasks, which have remained celebrated in the world of scientific investigators, were brought to the Mer de Glace. Pasteur gathered the air with infinite precautions; he used to enjoy

relating these details to those people who call everything easy. After tracing with a steel point a line on the glass, careful lest dusts should become a cause of error, he began by heating the neck and fine point of the bulb in the flame of a little spirit lamp. Then raising the vessel above his head, he broke the point with steel nippers, the long ends of which he had also heated in order to burn the dusts which might be on their surface and which would have been driven into the vessel by the quick inrush of the air. Of these twenty flasks, closed again immediately, the contents of only one was altered.[1]

Viability of Organisms.—One other factor is of extreme importance in determining the number of bacteria in the air—the viability of the organisms themselves. In speaking of bacteria in the air, live bacteria are meant. Drying and sunlight have a pronounced germicidal effect on bacteria, so that even those organisms which are in the air in droplets of water, due to the splashing of waves, agitation from bubbling, or expulsion from the human mouth in coughing, are soon freed from their surrounding water by evaporation and are subjected to the harmful effects of desiccation and sunlight. Between 90 and 100 per cent of all vegetative forms are killed within 24 hours in this way.

The following table[2] gives figures from various authors, showing the bacterial content of air.

Locality	Number of bacteria per cubic meter	Observer
Outdoor air, Boston	100 to 150	Sedgwick and Tucker
Open air	100 to 150	Fischer
Open field	250	Uffelman
Seacoast	100	Uffelman
Mountain altitude, 200 meters	0	Pasteur
Mont Blanc	4 to 11	Ellis
Spitzbergen (Artic region)	0	Levin
Middle of Paris	4,000	Ellis
Paris street	3,500	Fischer
Tailor's room in White Chapel	17,000	Ellis
Boat workshop	25,000	Ellis

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[1] Translation by Mrs. R. L. Devonshire, p. 98, New York, 1923.

[2] BUCHANAN, R. E., in Marshall's "Microbiology," p. 189, Philadelphia, 1912.

Kinds of Organisms in the Air.—The microörganisms in the air may be divided into four groups: the fungi (yeasts and molds), spore-formers of the genus Bacillus, Sarcinæ, and pathogenic bacteria.

Yeasts are not usually present in large numbers, though they can often be isolated from the air.

Molds on the other hand, because of the peculiar manner in which the parent liberates its spores, and because of the moisture-resistant surface of these spores, are found very frequently in large numbers. Penicillium is the mold most commonly found, with Mucor and Aspergillus less common.

Bacilli (aërobic spore-forming rods) from the soil are found quite frequently in air. Bacillus subtilis is the most common species of bacterium found in nature. It is a hardy saprophyte and is quite as common as molds in the atmosphere. Its common name, the hay bacillus, indicates its natural habitat on vegetation and its ability (in the spore stage) to withstand drying. In fact, a plate exposed almost anywhere, indoors or out, will soon show the presence of this ubiquitous organism.

Sarcinæ (together with Micrococci) comprise another group of saprophytes commonly found in air. Many species of chromogenic spheres may be isolated.

Pathogenic Bacteria in the Air.—The organisms described above are all saprophytic. In studying the pathogenic organisms in the air, certain special facts must be noted. In the first place, pathogenic bacteria get into the air directly and indirectly from persons suffering from disease.

The indirect method may be spoken of first because it is relatively of little importance, occurring where the organisms have been eliminated from a diseased person and, after drying, have been blown or swept into the air. Inasmuch as drying kills, or at least attenuates, all pathogenic bacteria, the disease organisms getting into the air by this method are negligible. Mycobacterium tuberculosis may be, to some extent, an exception to this rule, as this organism can retain its virulence through considerable desiccation.

Anthrax, a disease of animals, is sometimes contracted by humans working with hides or animal hair. For this reason it is called "wool sorter's disease." The spores of this germ have been found in the air of wool-sorting rooms, and no doubt some cases of the disease may have been contracted through aërial infection.

It is more likely that the actual handling of wool from anthrax-infected sheep has been the usual mode of infection.

Pathogenic bacteria may get into the air directly from a person suffering with disease through sneezing, coughing, or talking. This has been mentioned previously as the "droplet" method.

Long before the discovery of bacteria, the air was considered a means of transmitting disease. Up to within the last hundred years the infective agent of transmissible disease was supposed to be a gas carried in the air. The gas was supposed to be given off by persons suffering with disease, either in the breath, or by body emanations; or it could be given off by certain decaying materials in the ground. The idea that night air contained more of these gases than air during the day was an outgrowth of this belief.

When specific bacteria were demonstrated as the cause of disease, the first idea was that these pathogens were carried about in the air. Later, it was realized that air-borne transmission is confined to droplet infection, as it is apparent that the disease organisms cannot retain their virulence after drying. Flugge[1] pointed out that during coughing, sneezing, and energetic talking, tiny droplets of saliva were thrown out of the mouth, and that these droplets may remain in the air for several hours. In 1909, Winslow and Robinson[2] made a survey of the literature and a rather exhaustive study of the possibilities of infection by air-borne bacteria, and came to the following conclusions:

> Most of the particles in the mouth spray are rather coarse and settle out rapidly. In fact, the spray is like rain falling through the air rather than mist suspended in it. In estimating aërial infection, it is the bacteria suspended in the air which are important, because these only could be inhaled with the inspired air. The true aërial infection is therefore relatively small by comparison with the distribution of the heavier particles of spray. These conclusions are in harmony with the conviction now generally gaining ground that aërial infection of any sort is a minor factor in the spread of zymotic disease; and in regard to tuberculosis they accord well with the opinion that ingestion rather than inhalation is the principal channel of infection even in the case of bacilli originally discharged through the air in the form of mouth spray.

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[1] Flugge, *Zeitschrift für Hygiene und Infektionskrankheiten,* vol. 25, p. 179, Leipzig, 1897.

[2] Winslow and Robinson, "Investigation of the Extent of the Bacterial Pollution of the Atmosphere by Mouth Spray," *Journal of Infectious Diseases,* vol. 7, pp. 17–37, Chicago, 1910.

Chapin[1] shows that epidemiological investigations prove that there is little evidence in favor of aërial transmission of disease in any form. He discards the theory as a working hypothesis and says:

> It will be a great relief to most persons to be freed from the specter of infected air, a specter which has pursued the race from the time of Hippocrates, and we may rest assured that if people can, as a consequence, be better taught to practice strict personal cleanliness, they will be led to do that which will more than anything else prevent aërial infection also, if that should in the end be proved to be of more importance than now appears.

Economic Importance of Bacteria in the Air.—Aside from the disease germs which may be present in the air of a room, due to coughing and sneezing, there are many bacteria which get into the air that cause economic losses. These are the saprogenic and zymogenic bacteria which cause decay and fermentation. In any industry in which food or food products are handled, these germs in the air become of enormous importance. In dairies, canning plants, sugar refineries, etc., care is taken to get the air as germ free as possible. In the large biological laboratories, where vaccines and antitoxins are prepared, the most elaborate precautions are taken against air contaminations.

Air Bacteria in the Laboratory.—The importance of excluding bacteria in the air from bacteriological cultures is soon appreciated by every student of bacteriology. In the physiological and biochemical study of an organism, a single bacterium entering from the air may lead to a totally false observation. Even the expert technician realizes the difficulty of carrying pure cultures through frequent transplantings.

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[1] CHAPIN, "Sources and Modes of Infection," New York, 1910.

CHAPTER XIII

BACTERIA IN FOODS

Food Defined.—The human body, like that of every other living thing, is made up of a few chemical elements in the form of protoplasm. Through the process of metabolism these cell components are undergoing constant change—wearing out and replacement. The substances taken into the body to supply the elements eliminated through waste are classed as foods.

In a narrower sense, physiologists are right in stating that food is not what is eaten what but is digested. But from the bacteriological point of view, anything taken into the alimentary system—with the exception of water—may be considered as food.

In this discussion, foods are not of especial concern from the physiological viewpoint, nor is the term "food" limited to its rôles in the human body. It might be said, for convenience, that foods are divided roughly into three classes: carbohydrates, proteins, and fats; and after digestion have the functions of supplying energy (heat or motion), of replacement in anabolism, and of regulating or functional control (vitamins, etc., in the endocrine glands). The sole interest here, however, lies in the action of microörganisms on these foods, and the kind of organisms present. These substances, which are classed as foods, are organic, of either animal or vegetable origin.

Classes of Food Bacteria.—The study of bacteria in foods may be divided into three phases: first, the rôle which microörganisms play in the preparation of foods, such as molds in cheese, yeasts in bread, and bacteria in butter making and in the preparation of vinegar, sauerkraut, etc.; second, how microörganisms cause the spoilage of food products, either in fresh foods like the Lactobacilli in milk, or in canned goods, like the saprogenic anaërobes; third, the part which food products play in the transmission of disease germs like Eberthella typhi, Mycobacterium tuberculosis, Corynebacterium diphtheriæ, etc.

It has already been pointed out that different groups of bacteria use different kinds of substances for their food and energy requirements. In order to utilize material as pabulum, bacteria secrete enzymes which convert the material into substances which can be used in their cell metabolism, just as human beings, through the enzymes (pepsin, trypsin, pancreatin, etc.) in the digestive tract, convert food into forms which can be utilized by the body cells. The changes which are produced by bacteria have been defined, in general, as oxidation, reduction, fermentation, decay, and fat splitting, the process depending on the type of enzyme produced and the nature of the material acted upon. All of these chemical changes have been discussed previously. Two of them, oxidation and reduction, are usually confined to simple inorganic compounds and are of little or no importance in this discussion; the other three, however, are of considerable interest. It must be borne in mind that, in the subject under discussion, human foods are acted upon by bacteria as their foods. In this action, the environmental factors which bear a relation to bacterial life must be borne in mind. The relation of bacteria to moisture, to temperature, and to chemicals has special application.

Bacteria in Food Production.—The discovery that bacteria and other microörganisms play a part in food production has been entirely empirical. Soured-milk foods, like matzoön, cheese, and butter, as well as other foods, like sauerkraut, wines, vinegar, etc. were used as articles of diet long before such things as bacteria were known to exist.

Alcohol, Wines, and Beer.—It was in connection with the manufacture of alcohol that Louis Pasteur made his remarkable discovery of the cause of fermentation, a discovery which soon led to the formation of a new science—bacteriology.

Although wine and beer making had been practiced since the dawn of history; although alcohol, the "active principle" of these intoxicating beverages, had long been known; although, for years, alcohol had been used as a solvent and had been manufactured by an industrial process, no one prior to the year 1856 had understood its chemical formation.

In the summer of 1856, about two years after his election as professor of chemistry at Lille, Pasteur was asked by a pupil, M. Bigo, to extend help to Bigo, Senior, who was experiencing difficulty in the manufacture of alcohol from beet sugar. The immediate results of Pasteur's investigation justified the request. To quote M. Bigo's own words:[1]

> Pasteur had noticed through the microscope that the globules were round when fermentation was healthy, that they lengthened when alteration began, and were quite long when fermentation became lactic. This very simple method allowed us to watch the process and to avoid the failures in fermentation which we used so often to meet with.

In the manufacture of alcohol and of foods containing alcohol, as wines, beers, etc., the principle is the fermentation of sugars by means of a yeast. Although the methods of manufacture are different according to the product to be obtained, all alcoholic fermentation processes depend upon the following chemical reaction:

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$

The actual yield of alcohol in accordance with this reaction does not equal the theoretical yield. Other products are formed, of which glycerine is usually the greatest in amount, averaging about 3 to 5 per cent. Organic acids, as succinic, lactic, and acetic, are also formed in small quantities.

The yeasts used in making beers and ales are usually cultivated strains, while, in wine making, the yeasts which normally collect on the skins of the grapes or other fruit used are allowed to cause the fermentation. The latter are known as "wild yeasts."

Bread.—The same reaction is used in making bread as is in the manufacture of wines. In this case, however, the carbon dioxide is the desirable product of the reaction. This gas bubbles through the dough, causing innumerable small holes, which make the bread light and porous. Before it was known that a yeast was the cause of this phenomenon, bread was made by saving a little of each previous lot of dough and using it as a starter for a subsequent batch. This starter was known as "leaven," and leavened bread was distinguished from "unleavened bread," in which the dough was not fermented.

The housewife makes use of a similar process, not microörganic, when she makes biscuits, using sour milk and baking soda. The lactic acid in the milk reacts with the sodium bicarbonate, forming sodium lactate, and liberating carbon dioxide. The carbon dioxide causes the biscuit dough to "rise."

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[1] VALLERY-RADOT, "The Life of Pasteur," p. 79, translated by Mrs. R. L. Devonshire, New York, 1923.

Milk Products, Sour Milk.—The earliest known milk products formed by bacterial means were foods prepared by spontaneous souring. Buttermilk and clabber are two which are used extensively in this country. Matzoön, yohourt, kumiss, kefir, and others have been used by peoples in Eastern countries for ages.

Sour milk, unless contaminated with pathogenic organisms, is wholesome and nutritious. It is probably more easily digested than sweet milk, and in certain afflictions, such as autointoxication, it has a beneficial effect. Organisms like Lactobacillus bulgaricus, the "bacillus of long life," Lactobacillus acidophilus, and others which cause the souring of milk can replace, to some extent, the gas-forming organisms in the human intestines and thus diminish the production of indol, skatol, and other poisonous products.

Butter.—Butter making is essentially a bacteriological process. The cream is separated from milk either by gravity or by mechanical separators. The cream is then "ripened" or soured and the butter churned from the sour cream. On the farm this "ripening" is usually accomplished by allowing the cream to sour spontaneously through the agency of the lactic-acid bacteria in the cream. In large dairies, however, the cream collected from various sources is mixed and pasteurized and then ripened by means of a "starter," or pure culture of a particular species of bacterium. The churning of butter is a purely mechanical means of demulsifying the fat, the minute globules of the fat cohering to form larger and larger globules until a solid mass is obtained.

The ripening of cream in butter making imparts the desired taste and aroma. While spontaneous ripening is usually employed by farmers in making small lots of butter, the chances of getting a poor flavor in a large creamery batch are great, so that the "starters" employed are carefully guarded by competent bacteriologists.

Cream normally contains many more bacteria than milk, due to the inverse sedimentation carried out by the gradual rising of the fat globules. So, while whole milk contains 50,000 to 200,000 bacteria per cubic centimeter, cream will normally contain 200,000,000 to a 1,000,000,000 bacteria per cubic centimeter. Cream ready for churning usually contains about 500,000,000 bacteria per cubic centimeter. Because of their ability to live in the acid of sour cream, the bacteria of the genus Lactobacillus are usually dominant. Other organisms do persist, however, and at times cause considerable financial loss, due to the taste imparted to the butter. Common spore formers in the air, like Bacillus subtilis, produce a metallic taste in butter.[1] Other organisms impart unpleasant tastes and odors which may cause the product to be known as insipid, bitter, tainted, tallowy, strong, or putrid. These abnormal odors and tastes are called by the trade "butter faults." Old or rancid butter owes its characteristic taste to the changes produced by Clostridium butyricum.

Cheese.—Cheese has been used as a food ever since the very earliest times. The literature of all peoples shows that it was, and is, a universal food. In America, alone, there are over 300 cheese factories, and, in addition, much cheese is made independently by farmers. About 150 kinds of cheese are made, although only a few are of great importance commercially; about nine-tenths of the cheese consumed in this country is of the ordinary Cheddar type.

Cheese may be classified according to the kind of milk used, the process of making, and the seasoning or ripening. From the bacteriological standpoint, the chief concern is with the ripened cheese. Cheeses which are eaten fresh, such as Philadelphia cream cheese, cottage cheese (smierkaese), and American Neufchatel may be disregarded.

In general, fresh milk is curdled by the addition of rennet, a substance obtained from the stomach of calves. This precipitates the casein from the whey, the process taking from 10 to 15 minutes at about 30°C. The curd is then separated from the whey with the least loss of fat, and pressed into the desirable form. It is then ready for the ripening. In this process, the casein is peptonized and changed into forms that are digestible, and the characteristic flavors are produced.

There are five broad groups of microörganisms which may be present in cheese during the various changes in its manufacture: (1) the lactic-acid group of bacteria of the genus Lactobacillus. Lactobacillus casei (varieties α and β) are predominant, and, together with the other members of this group form about 95 per cent of the total number of organisms present during the early stages of the ripening; (2) the casein-digesting bacteria, which

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[1] RUEHLE, G. L. A., *Michigan Agricultural Experiment Station Quarterly Bulletin*, vol. 3, pp. 103–104, 1921.

assist the ferments normally present in liquefying the curd and otherwise altering it; (3) a group of gas formers like the genus Propionibacterium (Orla-Jensen), organisms which are responsible for the "eyes" in Swiss or Emmental cheese; (4) certain molds like Penicillium roqueforti, etc., which impart a taste and aroma; (5) a heterogeneous group of bacteria and molds which are incidental contaminations from the milk, air, or utensils employed in making the cheese. To the latter class belong the organisms which cause "disease" of cheese and impart an unpleasant taste or odor. These abnormal ripenings give rise to what are called spotted, inflated, tainted cheese, etc., and unless carefully controlled, may cause enormous economic losses.

Conn divides soft cheese into three classes according to the relation of bacteria and molds: (1) those in which bacteria alone are concerned in the ripening; (2) those in which molds contribute to the ripening, growing on the surface and extending into the interior (Brie, Camembert, etc.); and (3) cheeses in which molds play an essential rôle and grow throughout the entire mass.

Sauerkraut is prepared from cabbage by bacterial action. The vegetable is mixed with salt and thoroughly bruised, the salt dissolving in the extracted juice to form a brine. The whole is packed tightly in a crock, and a perforated cover smaller than the diameter of the crock and heavily weighted is pressed down on the solid matter.

Fermentation is then allowed to take place at room temperature. The vegetable sugars are converted into organic acids, principally lactic, by the bacteria normally present. After fermentation is complete, the fermented juices are drawn off and replaced by brine.

Pickles.—In the pickle industry, a group of lactic-acid bacteria are the essential organisms. These bacteria are always found in vegetables which grow near the ground and increase rapidly in the juices of these vegetables, producing lactic acid. In *Farmers' Bulletin* 1159, Le Fevre[1] gives directions for the home production of pickles. A strong brine (1 pound of salt to 10 pounds of cucumbers) is necessary to withdraw the juice of the cucumber and to check spoilage organisms. The fermentation continues for 10 to 30 days, while the total time of curing may

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[1] See also, LE FEVRE, EDWIN, "The Bacteriology of Pickle Fermentation" *The Canner*, vol. 53, pp. 39–40, 1921.

be 1 to 2 months. The best temperature is 30°C. As the brine fermentation is anaërobic, these bacteria are facultative anaërobes. Yeasts are also present in pickle brine, but must be considered as contaminating organisms.

Food Spoilage.—Disregarding for the moment the illnesses caused by the consumption of spoiled food products, the subject of food spoilage may now be considered solely from the economic viewpoint. In the large canning and packing industries, the decomposition of foodstuffs is a serious financial problem, and its proper control is the very heart of the industry. The losses due to food spoilage in the individual home are far greater in their aggregate than in the large plants, but it has become customary to accept these daily small losses as part of home economics.

Milk.—Milk is the secretion of the mammary gland. In America milk is usually thought of as cow's milk, and when a dairy is spoken of, a distributing point for cow's milk is meant. In some countries, horse's milk and in others, goat's milk, are commonly consumed. The composition of milk is very complex, as may be understood from the following scheme:

- Milk... 100
  - Water . 87.9
  - Solids . 12.1
    - Fat 3.6
    - Solids not fat 8.5
      - Nitrogen compounds . 3.3
        - Casein 2.5
        - Albumin 0.8
      - Lactose 4.5
      - Ash . .. 0.7
      - 8.5
    - 12.1
  - 100.0
  - Gases
    - Carbon dioxide
    - Nitrogen
    - Oxygen

Under fats, there are nine or ten different chemical compounds; under nitrogen compounds, at least five; under ash, in varying amounts, about ten salts, usually in the form of oxides and acid salts. Besides these, there are a large number of ferments or enzymes, such as galactase (proteolytic) and lipase (steatolytic). All milk, moreover, contains a number of blood cells and broken-down body cells, and a great many other substances, such as volatile oils, which may be introduced if the cows graze on garlic, onions, etc.

For the first year of a human life, milk is the only food; it is the principal food of the sick; and it forms a large proportion of the food of all people at all times. Milk contains all the food principles necessary for human existence. The casein and albumin represent the proteins, and the cream, the fats;

the lactose, or milk sugar, represents the carbohydrates—all of these being in combination with salts and water. About 16 per cent of the food of the people of the United States consists of milk and milk products.

The study of milk and dairy practice is of especial interest to the bacteriologist and the chemist, since milk is the principal standard article of human diet obtained from animal sources which is consumed in its raw state. As it is so readily decomposed, it is the most difficult of all foodstuffs to obtain, handle, and deliver in a clean, fresh, and satisfactory condition.

The government divides milk into three classes: (1) certified milk, or milk obtained under the special supervision of a medical milk commission. Certified milk should not be more than 24 hours old when delivered. (2) inspected milk. This term is limited to clean, fresh milk from cows tested by veterinarians. (3) market milk. All milk that does not come under either of the above heads and all milk that is of unknown origin is known as market milk and should be pasteurized before use.

Because milk is almost a perfect food, it contains, as a rule, a large number of bacteria. In fact, sometimes more bacteria are found in milk than in sewage. Mere numbers, however, within certain limits, are of no great importance. Excessive numbers indicate that the milk is dirty, old, or has been kept in a warm place. Certified milk should not contain over 10,000 bacteria per cubic centimeter, inspected milk not over 100,000, and market milk not over 500,000. New York has placed the limit at 1,000,000 per cubic centimeter. Even this standard is not rigidly enforced. Boston has a standard of 500,000; Rochester, 100,000.

Most of these organisms belong to the "aciduric" group, including the lactose-fermenting cocci, various species of the genus Lactobacillus, and the so-called "bacillus lactis acidi" group (members of the genus Escherichia). These are the organisms that cause souring and thereby the greatest losses to the dairy industry. Souring of milk is the fermentation of the milk sugar, lactose, with the production of lactic acid. This acid causes the coagulation of the milk casein with the resultant "clot," or "curd."

If sour milk is allowed to stand for a time, a liquefaction of the clot may take place, and a rancid, unpleasant odor replaces the rather agreeable sour odor. This is due to the putrefactive changes in the casein or milk proteins by saprogenic organisms like Bacillus subtilis.

Other microörganisms, as certain species of yeasts and molds, are present in milk, and often cause spoilage, especially in cream and ice cream. Oidium lactis is a mold which is found normally in sour milk. Torula amara, a yeast, causes a bitter taste in milk.

In addition to these common milk spoilers, there are a number of species of bacteria which occasionally cause losses to milk dealers. Bitter milk is sometimes caused by a micrococcus and sometimes by a rod form. Blue milk is due to Bacillus syncyaneus (*Bacillus cyanogenus* Flugge). "Bloody milk" is usually due, not to blood, but to various bacteria, such as Serratia marcescens, and species of the genus Sarcina. Ropy or slimy milk is due to a number of different species of which Achromobacter viscosum and Micrococcus freudenreichii are the chief offenders.

Pasteurization.—The process known as "pasteurization" consists of heating milk to a temperature at which all pathogenic germs are destroyed. No attempt is made to sterilize the milk, and in fact, as much care is taken not to overheat the milk as is taken to heat it sufficiently. Overheating affects the vitamines, especially vitamin C, and changes the physical and chemical properties of the milk. There are two well-established methods of pasteurization. In one, the milk is held in a vat at a temperature of 142 to 145°F. for 20 to 30 minutes, with constant circulation of the milk to ensure a uniform temperature. The milk is then rapidly cooled by running it over cold brine pipes. In the other method, known as the flash method, the milk is heated momentarily over a steam coil to a temperature of 160 to 185°F., and rapidly cooled.

Eggs are also animal food sold to the consumer in the "fresh" state. Examination of large numbers of eggs has shown that bacteria are always present inside the shell. Rosenau[1] states that "bacteria doubtless gain entrance to the egg while in the oviduct." It is also likely that, in eggs not strictly fresh, entrance is gained through the shell by bacteria. There is, therefore, no particular type of bacteria common in eggs. Yet because of the highly nitrogenous substance acted upon, the bacteria developing in eggs and causing spoilage are chiefly saprogens.

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[1] Rosenau, W. J., "Preventive Medicine and Hygiene," 5th ed., p. 770, New York, 1927.

The odor of rotten eggs is due to hydrogen sulphide liberated in the protein decomposition.

Other Fresh Foods.—Most other articles of food, as well as milk and eggs, are subject to spoilage by bacteria. Fish and other sea-food, vegetables, fruits, and meat, until comparatively recent years, have had to be consumed soon after collection to avoid losses due to decay. Today there are a number of great industries which deliver foods that have been preserved from the action of bacteria.

Sugar.—In the manufacture of sugar, bacteria, yeasts, and molds are often responsible for enormous losses. The raw juices of sugar cane and beets form a favorable culture medium for these organisms, and, unless proper cleanliness is observed and rapidity in handling is carried out, large amounts of the sugar will be fermented. The yield will not only be diminished thereby but the subsequent crystallization will be more difficult, on account of the formation of organic acids.

Preservation of Foods.—By the term "preservation" of food is meant protecting the food from the action of bacteria. In some cases, like the pasteurization of milk, the process is mainly for the destruction of any disease germs which may be present. In most cases, however, preservation is for the purpose of keeping out saprogenic germs which cause the rotting or putrefaction of the food.

Canning.—Canning and preserving are two processes for the preservation of food which depend upon heat for killing the bacteria present in the food and upon the exclusion of subsequent contaminations. In preserving reference is usually to a food with a high sugar content. The fact that food would not spoil if heated and then sealed from the air was first discovered by a Frenchman, Nicholas Appert, early in the nineteenth century. In general, the process of canning is the same for all canned goods, although details, such as the temperature of sterilization, length of sterilization, and preliminary preparation, vary with the different foods. The food, usually cooked, is placed in the can and the cover closed. A small hole is left in the cover to allow steam to circulate in the can when heated. The can, with its contents, is now placed in an autoclave and sterilized by steam under pressure. Upon removal from the autoclave, while the can is still hot, the small opening is sealed with a drop of lead. Upon cooling, a partial vacuum is created in the can, due to the condensation of steam. Usually the sealed cans are subjected to a second heating. This heating is known as "processing." Sometimes, because of incomplete sterilization, the presence of resistant organisms, or defective cans, bacteria remain in the cans after they are on the market, and cause spoilage. This spoilage is not to be confused with rare cases where food poisoning results from the consumption of the food. It is generally noticeable before the cans reach the consumer, either in the hands of the distributor, the wholesaler, or the retailer. While the product thus spoiled may not be dangerous, it is unwholesome and not marketable.

Cold Storage.—Cold storage or refrigeration of foods takes advantage of the fact that saprogenic bacteria cannot grow and multiply at low temperatures. Unlike heat, cold does not necessarily kill bacteria. On the other hand, low temperatures do not readily affect the taste of foods thus preserved and it is possible to deliver to the consumer foods in their original state.

The temperature of refrigeration depends upon the kind of food. Some foods, meat and fish for example, are kept in temperatures below freezing; others, including eggs, fruit, and vegetables, are best kept at a temperature slightly above the freezing point. One essential requirement of refrigeration is dryness.

Other Preserving Methods.—Canning and refrigeration are the two principal methods of food preservation used on a large commercial scale. Other methods of destroying bacterial life or of inhibiting their action in foods are used in special cases. Drying of meats and vegetables is quite frequent, sometimes on a large scale. Desiccation inhibits bacterial growth because bacteria can only absorb food in a moist state. Examples of desiccated foods are dried or chipped beef, prunes, and apricots.

Pickling or salting is employed in the preservation of fish—mackerel, for example; meats, as salt pork; and a few vegetable foods like ripe olives. This process depends upon the dehydrating effect of concentrated salt solution on bacteria, and to some extent upon the destruction of bacterial life by the dissociated chlorine ion of the salt.

Chemical preservatives, such as salicylic acid, boric acid, formaldehyde, sodium fluoride, etc. were formerly used to "keep" foods. Such procedures, while not necessarily harmful to consumers, are forbidden by the government through the Pure Food and Drugs Act, on the ground that these methods allow foods already partially decayed or dirty, to be kept in a salable condition.

Foods in the Transmission of Disease.—The dissemination of disease through foods is one of the chief concerns of sanitarians.

Foods may cause diseases which are not due to bacteria. Many plants and animals are poisonous in themselves, and severe illnesses are caused by eating mushrooms, or certain kinds of fish.

Moreover, specific diseases may be transferred from one person to another through the indirect medium of food. Thus, flies traveling from the fæces of a typhoid patient to food may infect a healthy person.

Again some people "react" to particular proteins with severe symptoms. This condition is commonly called food "idiosyncrasy." Scientifically, it is known as atopy, allergy, anaphylaxis, or hypersensitiveness. Clams and other sea-foods, strawberries, buckwheat, and egg albumen are some of the foods which cause this condition in certain individuals.

Specific bacterial diseases are frequently spread in epidemic forms through particular foods.

Milk.—Of the pathogenic bacteria found in milk, those most frequently accused of causing epidemics are the organisms of tuberculosis, typhoid fever, diphtheria, scarlet fever, sore throat, and summer complaints of infants and adults. It is known that the virus of foot-and-mouth disease, which is ultramicroscopic, is almost constantly found in the milk of the affected animals. On the other hand, tubercle bacilli do not pass the mammary gland unless there is tuberculosis of the udder. Typhoid fever, which takes the first place in the rank of milk-borne epidemics, diphtheria, scarlet fever, and other diseases are transmitted through milk which has been contaminated by human sources.

Although the dangers of milk as a vehicle for the dissemination of typhoid fever and other disease are quite generally understood by sanitarians, some investigators think that they are not so great as is generally supposed. In a study of statistics, collected in Massachusetts, of cases and deaths due to typhoid fever, diphtheria, scarlet fever, and septic sore throat, correlated with the number of deaths and cases for which milk was responsible, it was observed by Kelley that milk is not an important channel of infection in this group of diseases.

> The transmission of diphtheria through milk is negligible. Scarlet fever is transmitted through milk more frequently than is diphtheria, but has a small percentage significance. Typhoid fever was attributed to milk in 5 per cent of the cases reported. Septic sore throat is of milk transmission—forty-eight out of forty-nine deaths were due to milk.[1]

While there is no doubt that the danger of transmission of disease is real, there have been a number of reports on both sides of the question of the relationship of milk supplies to typhoid fever.

Although there are other ways of attacking the problem, perhaps the best of all opportunities for estimating the influence of milk supplies on a municipal typhoid rate is found in cities where an abrupt change is made in the efficiency of control by ordinances requiring universal pasteurization.

As a rule, a milk-borne epidemic of any disease is easily differentiated from a water-borne epidemic by the way the primary cases follow the dairyman's route, and also by the large proportion of severe primary cases to the total number of cases. This, of course, is due to the fact that a very short time elapses between the infection of the milk and its consumption; and also to the fact that bacteria do not become so readily attenuated in this favorable medium as they do in water.

One recent epidemic of typhoid fever, definitely traced to milk, was that at Montreal, Canada. Nearly 5,000 cases with more than 450 deaths occurred in that city during the spring and early summer of 1927. The epidemic was traced to a single dairy.[2]

During the past few years a number of cases of undulant fever have been reported in America. The organism causing this disease is either the same as, or very closely related to the germ responsible for contagious abortion in cattle. The possible rôle of milk in the dissemination of undulant fever is of considerable interest.

Ice Cream.—As a manufactured product, ice cream is liable to dangerous contamination from the milk and cream used, from utensils, and especially from the direct introduction of pathogenic

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[1] Kelley, E. R., "The Quantitative Relationship of Milk-borne Infection in the Transmission of Human Communicable Diseases," *Journal American Medical Association*, vol. 67, pp. 1997–1999, 1916.

[2] Report of the U. S. Public Health Service on the Montreal Typhoid Fever Situation, *U. S. Public Health Report* 42, p. 1893, 1927.

bacteria by employees. Fabian[1] has gathered records of 35 minor epidemics of typhoid fever, scarlet fever, and diphtheria which were traced to ice cream. Although the effect of continued freezing upon certain pathogens like Eberthella typhi is still an open question, the possibilities of the spread of various diseases through this food should not be overlooked.

Oysters.—Epidemics of typhoid fever and gastroenteritis have been caused by eating of oysters which have been "fattened" in sewage-infected water. The U. S. Public Health Service has made a thorough study of the situation and has advised the individual states in whose water shellfish are grown that effective sanitary controls must be exercised over the industry. A certification plan has been instituted whereby only those concerns which meet with sanitary requirements will be permitted to sell shellfish. Other foods, like watercress, etc., have also been blamed as sources of infection.

Food Poisoning.—There are three forms of food poisoning caused by bacteria. The first is grouped under the general term "ptomaine poisoning." In this case, certain saprogenic bacteria break down food substances into poisonous products. These poisons are formed before the food is eaten and the poisons, not bacteria, cause the disease. True ptomaine poisoning is very rare. The second type of food poisoning is often miscalled ptomaine poisoning. This is caused by bacteria of the genus Salmonella, of which Salmonella enteritides is the most common. Salmonella schottmulleri (B. para-typhosus B), Salmonella aertrycke, Salmonella suipestifer, and other species have been identified in this connection. In this type of disease, an actual infection takes place and the bacteria liberate their poisonous substances in the intestinal tract of the sufferer. The organisms grow well at temperatures between 6 and 38°C., and are dangerous in that they do not give the infected food offensive odors or tastes. The symptoms of this type of poisoning are like those of true ptomaine poisoning of the gastrointestinal type—nausea with vomiting and purging. Nearly all cases of food poisoning are of this type. The death rate is low, about 2 per cent of the morbidity rate.

The third form of food poisoning is due to Clostridium botulinum, a spore-forming anaërobe which liberates a very poisonous exo-

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[1] Fabian, F. W., "Ice Cream as a Cause of Epidemics," (Bibliography) *American Journal of Public Health*, vol. 16, pp. 873–879, 1926.

toxin. This type of disease differs from ptomaine poisoning in that the poison is secreted by the bacteria themselves and not made out of the infected food. It differs from the second type in that the bacteria produce their poison outside the body and therefore do not cause an infection. The mortality due to botulism runs as high as 70 per cent, the poison attacking the central nervous system. As a rule, botulism has been caused by eating canned goods where anaërobic conditions are favorable for the growth of the organism. Fortunately, in this case, external signs, such as swellings of the cans and a pronounced odor and taste, give warnings. Thom, in an article in the *American Food Journal*[1] gives definite rules for the prevention of botulism. The main points for the housewife are:

> . . . The ends of the cans should be flat or incurved, never bulged; there should be no sign of leakage, corrosion, or blackening of the inside of the can; suction should be inward with no outrush of gas; and there should not be any abnormal appearance of the products themselves.

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[1] THOM, CHARLES, "Food Poisoning and Its Prevention," *American Food Journal*, vols. 15, 16, pp. 33–36, New York, 1922.

CHAPTER XIV

THE PATHOGENIC BACTERIA

Disease may be defined as an abnormal condition of life, or as an irregular functioning of life processes. The word "disease" is the converse of the word "health." Any deviation from health is disease. The original meaning of the term was even broader than this. Any temporary bodily discomfort caused by external conditions—such as heat or cold—would be *dis-ease.*

In the folklore of every primitive people there are found explanations of the reasons for man's sufferings. And without exception a god is blamed, usually represented as acting through the agency of a woman, like Pandora or Eve. With the dawn of history the art of medicine is found to be well established, and coming down through the centuries, with the humoral theory, the alchemic theory, the contagium theory and the bacterial theory replacing one another in the minds of physicians, there has gradually been worked out a doctrine of cause and effect, which represents modern medicine.

That bacteria may be associated with disease was first suggested by Robert Boyle (1627–1691). Pasteur, Koch, and Lister, however, in the latter part of the nineteenth century, definitely proved that these tiny plants can cause disease. By the clever and thorough experimental work of Pasteur and his contemporaries, pathogenic bacteriology was established as a true science. It is now known that what are called infectious diseases are phenomena caused by the products formed, in the carrying out of their life processes, by bacteria and other microörganisms in the body of a host.

As a result of the early work of these men, the interest of the entire scientific world was aroused and soon adherents to the new "germ theory" were found enthusiastically claiming that *all* diseases were due to bacteria.

Today it is definitely known that certain diseases are caused by bacteria, and that others are not. In some, like cancer and diabetes, it is not certain whether the cause is bacterial or not.

For purposes of distinction, the non-bacterial diseases—like rickets, scurvy, gout, jaundice, apoplexy, etc., which are due to disturbances in nutrition and metabolism, or to injury to a particular organ as a result of such disturbances—are called "constitutional" diseases. The constitutional diseases are never transmitted from one person to another, although a tendency to suffer from them may be passed on from parent to offspring.

Pathogenic Bacteria.—Disease bacteria are those germs which cause ailments when introduced into the bodies of higher plants and animals.

Relation to Other Bacteria.—From the point of view of food requirements, bacteria are divided into three groups—the prototrophic organisms, which obtain their food from inorganic materials; the saprophytic or metatrophic organisms, which obtain their food from dead organic matter; and the paratrophic organisms, which must have living organic matter from which to get all or part of their food requirements.

As bacteria are a group of organisms at the very foot of the scale of life, it is necessary to conceive of their presence on the earth long before such highly complex organisms as man or trees appeared. So far as is known today, however, only a few bacteria are capable of carrying out their life processes without organic food. It must be assumed, therefore, that the present-day bacteria differ essentially from their early ancestors in food requirements; by *adaptations* they have gradually accustomed themselves to foods far different from those upon which the first bacteria thrived. As would be expected, moreover, in the organic evolution of species, the habitual consumption of certain food materials led to the development of specific food necessities. Some bacteria have come to need highly specialized foods—as, for example, in the case of the species Erwinia atroseptica, which grows in the stems of the potato plant; the species Clostridium chauvei, which develops in cattle but not in man; and Eberthella typhi, which attacks man but not the lower animals.

The paratrophic bacteria have been defined as those bacteria which are parasites, invading the tissues of other plants or animals which act as hosts. The relationship between the parasite and its host may be beneficial to both, as in Rhizobium radicicola and certain leguminous plants. This nitrogen-fixing organism aids the plant in its assimilation of nitrogen, while it receives from the plant certain food and energy material. This particular form of parasitism, in which the organisms are mutually benefited, is called "symbiosis." Cases have been studied in which bacteria infecting ants have been transmitted through many generations of the animal through the egg. While at first harmful, these organisms later lost all their harmful effects, and now may be beneficial to their hosts, through enzymes which they secrete.[1] Symbiosis is not confined to the paratrophic bacteria, as this condition exists between many species of saprogenic bacteria and also between different species of the prototrophic bacteria.

On the other hand, the relationship between the parasite and its host may be one of constant warfare, the one or the other necessarily being destroyėd. Thus, when a bacterium like Corynebacterium diphtheriæ invades the body of a human being, it not only lives at the expense of the host, but it produces a substance which is extremely poisonous to the host.

Paratrophic organisms of this kind are known as "pathogenic bacteria." The poisonous substances produced by bacteria are protein in nature. *Why* bacteria produce these poisons is not clear. The poisonous substances may be associated with their process of metabolism, but it is more likely that the poisonous nature of the excretion is purely incidental or accidental, as is that of the toadstool or ivy. The fact that the most strictly parasitic bacteria are not the ones which cause the most dangerous infections bears out this idea. Corynebacterium diphtheriæ and Clostridium tetani produce poisons which, if not counteracted by medical means, are ordinarily fatal to man. Yet these organisms can be readily grown on artificial culture media. On the other hand, the organism of cowpox cannot live outside the animal body, yet it produces a relatively mild disease.

Classification of Pathogenic Bacteria.—Bail[2] classifies the pathogenic bacteria strictly on their ability to invade living tissue.

1. *Pure Saprophytes.*—Organisms, like the botulinus germ, which do not invade living tissue. Clostridium tetani and Corynebacterium diphtheriæ also belong to this class.

2. *Pure Parasites.*—Bacteria like the anthrax bacillus, which, when introduced into the body, rapidly spread throughout the

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[1] Glaser, R. W., "Biological Studies on Intracellular Bacteria," *Biological Bulletin*, vol. 39, pp. 132–145, 1920.

[2] Bail, "Das Problem der Bakterien Infektion," Leipzig, 1911, quoted by H. Zinsser, in "Infection and Resistance," p. 10, New York, 1923.

body. It will be noticed that a pure parasite according to Bail's classification may be a saprophyte in the ordinary sense. The anthrax germ grows well on laboratory media and would therefor be considered saprophytic. Bail, however, looks at it from the point of view of the body invaded, and since this organism multiplies readily in the human body, he considers it a pure parasite.

3. *Half Parasites.*—Most pathogenic bacteria belong to this group. They are the bacteria which need a particular path of entrance into the body, must be introduced in large numbers in order to get a foothold, or must have some other special advantage not needed by the pure parasites.

Pathogenicity.—It will be seen from the above that the term "pathogenic" is merely relative. An organism which is pathogenic to plants may or may not be harmful to man. No organism is known which is uniformly pathogenic to all animals. On the other hand, common saprophytic bacteria of the soil may produce pathological changes in the body under certain conditions.

The infection and the subsequent harmful effects give rise in the host to a symptom complex which is known as disease. Thus, from the very nature (parasitism) of bacterial disease, infection can only take place when the parasite gains entrance to the body of its host and there finds conditions favorable to its growth.

Virulence.—One factor which must be taken into account in the conception of pathogenesis is the virulence, or disease-producing ability of different strains of the same pathogenic species. A strain of Eberthella typhi, the organism which causes typhoid fever, may become entirely unable to cause disease if cultivated for a sufficient length of time on artificial media. Diplococcus pneumoniæ, when artificially cultivated, may become so weak in its infective ability that billions of the bacteria injected into a white mouse will not be harmful. Yet this same strain, if repeatedly passed through mice by a series of inoculations and reinoculations from one animal to another, may become so highly virulent that a single organism injected into a mouse can multiply and produce a fatal septicemia.

To illustrate by a specific example: A culture of Streptococcus mitior (viridans) was isolated at post-mortem from a fatal case of endocarditis. The organism was grown in bouillon and an 18-hour culture killed a rabbit within 24 hours when $1/10$ cubic centimeter was injected. Here was a highly virulent organism.

The culture was transplanted from bouillon to bouillon several times at monthly intervals, and at the end of 6 months was tested for virulence. Now the same strain which killed in 1/10 cubic centimeter of the bouillon culture, did not even produce the slightest symptoms when 10 cubic centimeters of the culture were injected into a rabbit; 20 cubic centimeters of the culture did kill a rabbit in 4 days, however, and the organism was isolated from its blood. By repeated animal passage, that is, injecting sufficient of the culture into a rabbit to produce fatal results and then isolating the organism from the heart blood, growing in bouillon, and again injecting into a rabbit, and repeating the process several times, the virulence of this strain of Streptococcus was raised so that 1/10 cubic centimeter would again kill a laboratory animal.

It would seem from this that the degree of virulence of a strain of pathogenic bacteria depends simply on how well the bacteria have accustomed themselves to the defensive actions of the host when they passed from one animal to another. Bail and his co-workers explain this variation in virulence by the comparative ability of bacteria to secrete certain non-toxic substances which enable them to counteract the offensive warfare of the body. These substances are called "aggressins." Bail assumes that aggressins are produced by bacteria only when in the body of their host and not in test-tube cultures. This would explain the fact that virulence increases with animal passage. Rosenow has extracted substances from pneumococcus cultures which, when mixed with an avirulent strain of the pneumococcus, enables it to cause disease. These substances, which are probably the same as Bail's aggressins, he calls "virulins.' Other workers attack Bail's theory and believe that the so-called "aggressins" are simply poisons, produced by the bacteria, which harm the host and thus enable the germs to multiply unhindered. However the subject is looked at, the aggressins must not be confused with the specific poisonous products of pathogenic bacteria—the toxins which give rise to the different symptom complexes which are called disease.

It is apparent, therefore, that there are two factors in virulence: one is simply a defensive action against the protective substances of the host; the other is an offensive secretion.

Infection.—The entrance of pathogenic bacteria into the body of their host is known as *infection.* The term "infection" is rather loosely used. Some able writers have used the word as synonymous with the term "bacterial disease," and speak of a person suffering with a "Streptococcic infection" or an "infected tooth," etc. Properly speaking, the word should be limited to the actual invasion of pathogenic microörganisms. This does not necessarily mean, however, that all infections come from outside the body. A person may harbor pathogenic germs in the body for a long time without harm, and only when the body resistance is lowered or an accident happens does infection take place.

The manner of entrance of the pathogenic bacteria into their host is varied, but is, as a rule, the same for any one species. There is, however, one general rule for infection; namely, that the infecting agent must pass directly or indirectly "from an orifice of the infector to an orifice of the infectee." The orifice may be the mouth, the nose, the anus, the external sexual organs, or an abrasion of the skin or mucuous membrane.

External Existence.—The manner of entrance of a particular organism depends to a great extent upon the ability of the organism to live outside its host. Thus, gonorrhea is rarely transmitted except by direct contact of the infector with the infectee, because the causative agent, Neisseria gonorrhϾ, can only exist for a very short time outside of the human body. The same may be said for cerebrospinal meningitis and syphilis. On the other hand, anthrax may be contracted years after the organism causing the disease has left its former host. This is also true to a lesser extent of typhoid fever, tuberculosis, bubonic plague, cholera, and many other diseases.

Selectivity of Tissue.—Another factor which influences infection is the manner in which the disease germs have adapted themselves to particular tissues of their hosts. The typhoid germ grows well in the intestinal tract of man, from whence it enters the blood stream. Yet, if typhoid germs were introduced into a wound, they would, at worst, cause a local lesion. On the other hand, the organisms of tetanus and gas gangrene, which are the constant dread of army surgeons, may lead a harmless existence in the alimentary canal. There is no danger of the transmission of tetanus even in the closest contact with a person suffering with that disease. On the other hand, there is extreme danger of infection in coming into the same room with a person sick with diphtheria. The gonococcus may be placed with impunity on the skin but causes a severe disease if introduced into the urethra or on the conjunctiva.

Ports of Entrance.—The ports of entrance of bacteria into the bodies of their hosts depend, as explained above, to a large extent upon the ability of bacteria to live outside the body, but to an even greater extent upon tissue selectivity. The port of entrance of any particular organism is fairly constant. In the case of gonorrhea, two different diseases may be caused by the same germ, depending upon the port of entry. Gonococcal urethritis, or common gonorrhea of the male, is caused by the germs getting into the urethra. Ophthalmia neonatorum, a disease of the eyes of newborn infants, is caused by the same germ. Pasteurella pestis may cause bubonic plague or pneumonic plague (the black death), depending upon whether the port of entrance is through the skin by the bite of the rat flea, or into the respiratory tract through the coughing of a sufferer. In general, the ports of entrance are the respiratory tract, the digestive tract, the urino-genital tract, and the abraded or injured skin and mucous membrane.

The chief pathogenic bacteria entering through the nose and mouth are those causing diphtheria, septic sore throat, cerebrospinal meningitis, tuberculosis, measles, scarlet fever, whooping cough, mumps, influenza, and pneumonia.

The bacteria which set up disease in the digestive tract are those causing typhoid fever, cholera, and dysentery. These organisms are usually transmitted from the intestinal discharges of the infector to the alimentary canal of the infectee through water or food.

The venereal diseases are the most common infections of the urino-genital tract. Gonorrhea, chancroid, and syphilis are almost always transmitted by direct personal contact.

The pathogenic bacteria entering the body through the skin may be roughly divided into two classes—those carried by insects and transmitted by their bite, as bubonic plague, spotted fever, and yellow fever; and those entering through abrasions of the skin, as tetanus, anthrax, and gas gangrene. Then, there are certain diseases in which there is considerable doubt as to the port of entry of the causative agents. Among these are smallpox and infantile paralysis.

Sources of Infection.—By sources of infection is understood the medium by which disease germs are transferred from a person suffering with the disease to the infected individual. The old idea of miasmic dissemination through "bad" air gave place to the more modern but almost equally false idea of pathogenic bacteria floating in the air. One of the greatest steps forward that sanitarians have made is in giving up the dogma of air transmission of disease and thus obtaining mental freedom in looking for the *real* sources of infection. The following list of methods through which disease germs are transferred is given in the order of the ports of entrance cited above.

Droplet Infection.—While disease germs are not floating around in the outdoor air, they may be present in the air of a room in which a sufferer is sneezing or coughing.

Although the quietly exhaled breath is sterile, loud talking, sneezing, or coughing throws from the mouth a spray of small particles of water. In a diseased person, these drops may be heavily contaminated with the disease germs which may be in the mouth. Since they come directly from the person infected, these organisms are likely to be highly virulent, and a very few bacteria therefore may set up a fresh infection on the mucous membranes, or through the indirect medium of fingers, food, or sickroom objects. While it is not likely that the infected droplets can be carried through the air for any great distance, they have been clearly shown to have traveled more than 20 feet—the length of an ordinary room.

Indirect Contact.—One of the exploded theories of disease transmission was that of "fomites," or inanimate objects. Books, bed linen, clothing, etc. of a person ill or dead of a disease were supposed to be particularly dangerous. As in the case of aërial transmission it has been learned that the only way inanimate objects carry disease is by the almost simultaneous use by infector and infectee. There is no doubt that the common drinking cup may spread respiratory diseases, that towels may cause skin infections, or that food may transfer typhoid fever, yet these inanimate objects cannot support bacterial life for any considerable length of time.

Water.—From the earliest times water has been believed to be the means of transmission of disease, but it is only in recent years that the true relation of water to disease has been satisfactorily studied. Typhoid fever, cholera, and bacillary dysentery are the only diseases which are commonly spread by water. Because of sanitary measures in water filtration and sewage treatment, these diseases have been practically eliminated in this country. More than a quarter of a million people still die annually in India of cholera alone. As the organisms of these diseases cannot multiply nor long retain their virulence in water, it follows that the rôle water plays in the spread of these diseases is that of a medium in which infection is transmitted through indirect contact.

Milk has been shown, in the same way, to be a medium for the spread of diphtheria, typhoid, and scarlet fever. This usually results from drinking milk infected by persons suffering with the disease. As this food is usually consumed within 48 hours, the germs do not all die off before being introduced into the body of the infectee. In the case of typhoid fever, the practice of washing milk cans with sewage-contaminated water is another source of the spread of the disease. The ways in which other infected foods may cause disease have been taken up in a previous chapter.

Direct Contact.—Direct contact is practically the sole source of infection in the venereal diseases. It is also probably an extremely important means of transmission of a number of other diseases. For example, influenza is spoken of as a "hand to mouth" disease, the idea being that the hands of the infected individual are the means of picking up the disease germs and introducing them into the mouth. From here they get into the throat, where infection takes place.

Insects.—Insects disseminate disease in different ways. Microörganisms may be externally carried by insects on their legs or mouth parts and transmitted mechanically into wounds made by the carrier. Or they may in the same way infect food; flies have been responsible for transmitting typhoid fever in this way. The microörganisms may be taken into the alimentary tract of the insect through biting an infected person, and passed along unchanged to an infectee. The organism causing trench fever apparently is transmitted not solely through the bite of the louse, but by contamination of the bite with the fæces of the louse.[1] Bubonic plague is transmitted from the rat to man through the bite of the rat flea in about the same way. An insect may act as the "alternate host" of the organism, and the disease may be spread by the insect bite. Malaria is an example of a disease

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[1] Bachholtz, M., "Trench Fever: a Summary from the Literature," *Public Health Report*, vol. 34, pp. 677, 1919.

transmitted by this method. Finally, insects may be indirectly responsible for disease in the subsequent external infection of an insect wound.

Carriers.—One of the most insidious sources of infection is the person who without knowing it harbors disease germs. Such a person is called a "carrier." A carrier may have recovered from the disease and, long after symptoms disappear, harbor in his body virulent pathogenic bacteria. On the other hand, he may have an infection but because of a slight immunity show no evidence of the disease. The length of time that a person remains a carrier varies. During a diphtheria or meningitis epidemic a great many carriers may be discovered who soon become normal. In the case of a person convalescing from typhoid, the carrier state may exist for months or years.

Heredity.—The hereditary transmission of disease has long been a subject of spirited discussion among scientists. "Heredity" in its limited biological sense, refers to the transmission of definite characters from the sperm of the father and the ovum of the mother to the body cells of the offspring. Weismann[1] has shown that the germ cells (sperm or ovum) are distinct from the body cells (somatic). Thus, the child inherits from the parent germ cell, not from the parent body. Body characters which the parent has acquired during life, whether they be the loss of an arm or the acquisition of a bacterial disease, cannot be inherited by the child.

The germ cell, if not destroyed by a microörganism, could simply carry it as a foreign body. This foreign body could not be a part of the protoplasm of the cell, and therefore could not play a part in inheritance. There is no proof, moreover, that the sperm or egg is ever infected. The fact, then, that children may be born with a bacterial disease like smallpox, measles, or syphilis raises the question, "If these diseases are not inherited from the parent, how are they contracted?" There are two or three answers, any one of which may be true in any one case. Thus, though a child cannot inherit tuberculosis in the biological sense, it can inherit a tendency to the disease in the form of "weak" lungs, which are unable to withstand the invasion of the germs of tuberculosis when they gain entrance.

Bacterial disease, moreover, may be transmitted through the placenta, the organ transmitting nourishment to the embryo

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[1] WEISMANN, "Essays upon Heredity," 1889.

in the body of the mother during the fetal period. This is known as congenital infection. Syphilis, because of the great penetrability of the causative agent, is frequently transmitted in this way. On the other hand, tuberculosis is rarely congenitally transmitted.

Again, disease may be contracted by a child at the time of birth. Thus, even though it inherits no tendency to a disease and is not infected in utero, it may contract the disease during its passage through the vagina of the mother while it is being born. Ophthalmia neonatorum, or inflammation of the eyes of the new-born, is, in practically every case, due to a gonococcal infection, from the mother in this way.

Although bacterial diseases are not heritable, the tendency to constitutional disease is. The reason for this is that anatomical variations or abnormalities are not infrequently due to variations in the germ plasm and are therefore passed on from parent to offspring.

The knowledge of the source of infection and the way pathogenic bacteria enter the body is important to the bacteriologist and physician in determining the prophylactic measures necessary to avoid the spread of the disease. During the winter of 1917–1918 the detection of meningococcus "carriers" and their isolation was to a great measure responsible for the success in combating the epidemic of cerebrospinal meningitis which swept the army cantonments. To a lesser extent, the same may be said of the discovery of anthrax germs in shaving brushes.

As soon as Reed and his co-workers proved that yellow fever was transmitted by the mosquito, it was possible to take measures which eliminated this dreadful disease from Cuba and the canal zone.

Malaria in the southern states affects 3,000,000 people and causes an estimated economic loss of $1,000,000,000 yearly. In the control of malaria, mosquito eradication, especially at the breeding grounds of the pest, is the most effective means. The elimination of mosquito breeding grounds is primarily an engineering problem, and innovations and inventions in the mechanical problems of drainage will undoubtedly be of utmost economic importance in the future.

Until the source of infection of any particular disease is known, eradication is almost impossible. Sanitarians do not know how to protect the infectee from the infector. During the winter of

1917, there was a brief epidemic of infantile paralysis. No apparent reason could be found for infection. Contact, droplet infection, and insects were blamed, but no proof could be established connecting any of these sources with the disease. The epidemic fortunately stopped. But should there be another outbreak of the disease, physicians will still be ignorant of the measures necessary to combat it.

Incubation Period.—Even after virulent disease germs get into the body, they may not set up the symptoms called disease. There are other factors involved, such as the natural defenses of the body, which may prevent the bacteria from getting a start. These factors will be discussed later under the subject of Immunity.

If the disease organisms find conditions in the body of their host suitable for their growth, they multiply and ultimately set up a disease. Between the time when the bacteria enter the body and the time when the typical symptoms of the disease appear, there is a period during which the host is apparently perfectly well. It will be easily realized, when the manner of action of pathogenic bacteria in producing disease is understood, that, before any symptoms can appear, the comparatively few organisms which get into the body must multiply and must reach enormous numbers before the secretion of their toxic products can be noted from the major symptoms. The time that elapses is known as the "incubation period." The incubation period varies with different diseases and even in the same disease. In typhoid fever the incubation period is usually 2 weeks, although the fever may be apparent in 1 week; or the period may extend to 3 weeks.

The incubation periods of some of the more common bacterial diseases are:

Influenza	1 to 2 days	Meningitis	2 to 10 days
Cholera	1 to 5 days	Gonorrhea	3 to 5 days
Pneumonia	2 to 3 days	Smallpox	8 to 16 days
Diphtheria	2 to 5 days	Rabies	2 to 6 weeks
Dysentery	2 to 7 days	Syphilis	3 to 6 weeks
Scarlet fever	2 to 6 days	Typhoid fever	1 to 3 weeks

These periods may be shorter or longer, depending upon the virulence of the infecting organism, the amount of the infecting organism, and the individual susceptibility of the person infected.

How Bacteria Cause Disease.—The behavior of pathogenic bacteria after they have gained entrance to the body varies according to the nature of the organism. They may set up an inflammation at the site of entry, as in Clostridium tetani, or near the site of entrance, where the conditions are more favorable, as in Corynebacterium diphtheriæ in the throat, Neisseria gonorrhœæ in the urethra, or Eberthella typhi in the intestines. Or they may get into the blood stream and there set up a general infection of the blood known as bacteremia. Having gotten into the blood stream, however, the bacteria usually locate in some part of the body where conditions for their growth are most favorable and there set up a focus of infection. Mycobacterium tuberculosis usually settles in the lungs, although other organs, such as the bones, kidneys, etc., often become foci for this species of bacteria.

Except in rare cases where they may clog up capillaries, the mere growth and multiplication of bacteria inside the body do but little harm. Disease germs have, however, the property of producing, as a result of their metabolism, certain chemical substances which act as poisons to the body tissues. These poisons or toxins are of two kinds, the toxins excreted by the bacteria—called true toxins or exotoxins—and the toxins which remain in the bacterial cell until the cells are destroyed by some agency in the body. Corynebacterium diphtheriæ and Clostridium tetani produce exotoxins, Eberthella typhi, Diplococcus pneumoniæ, etc. produce endotoxins. These toxins may act at the site of infection, killing the surrounding body cells, as in boils, or the toxin may get into the blood stream and affect organs far removed from the focus of infection. Corynebacterium diphtheriæ, for example, sets up an infection in the throat. Here it develops and remains localized. It gives off a toxin, however, which gets into the blood stream and harms various organs of the body, notably the heart. In the same way, Clostridium tetani, while remaining in the tissue at the site of infection, produces a toxin which destroys the nerve cells in far-distant parts of the body.

Some pathologists believe that disease germs produce enzymes which cause certain specific chemical changes to take place in the body proteins, and that it is the products of these changes which cause the symptoms of the disease.

It is apparent that what are recognized as the symptoms of disease—fever, diarrhœa, headache, etc.—are simply the signs of a battle going on between the body and the disease germs. Aggressins, toxins, and enzymes are the offensive weapons of the bacteria. The defensive and offensive measures of the body will be taken up in the chapter on Immunity.

Types of Infection.—Bacterial diseases are grouped in various ways. They may be specific or non-specific, simple or mixed, primary or secondary.

Specific infections are those which show a definite symptom complex and are always caused by the same species of organisms. Thus diphtheria is always caused by Corynebacterium diphtheriæ; typhoid fever, by Eberthella typhi; tuberculosis, by Mycobacterium tuberculosis; tetanus, by Clostridium tetani; etc. On the other hand, the disease known as pneumonia, while usually caused by Diplococcus pneumoniæ, may be caused by Streptococci, Hemophilus influenzæ, and other organisms. Tonsilitis may be caused by Streptococci, Staphylococci, the Freidlander bacillus, etc. Boils, carbuncles, abscesses, and other pus formations may be caused by various species of the genera Staphylococcus and Micrococcus, Pseudomonas aëruginosa or Escherichia coli. The latter diseases are *non-specific.*

Simple infections are due to single species of organisms in pure culture. In other words, only one type of bacterium is involved. This is usually the case in specific infections, and it may be the case in non-specific infections. On the other hand, in addition to the specific organism causing the disease, other species may be present and add to the severity of the disease. Or, in non-specific infections, two or more species may at the same time be equally responsible for the diseased condition. These are called *mixed infections.* The presence of Staphylococci or Streptococci in tuberculosis is an example of mixed infection. Gonorrhea, caused primarily by Neisseria gonorrhœæ, rarely remains a simple infection, that is, an infection in which a single species of bacterium is present, but is usually a mixed infection in which Staphylococci, Streptococci, Escherichi coli, and bacteria of the "diphtheroid" group may singly, or all together, play an important pathological rôle. In fact, the gonococcus may disappear entirely from the infection and the disease continue and become chronic, due to the complicating organism or organisms.

Primary infections are specific or non-specific simple infections, as typhoid fever, whooping cough, etc. Sometimes, however, this primary infection is complicated by a *secondary infection*, which, by chance, or as a result of the weakened condition of the body, gains entrance. Thus, typhoid fever is often complicated by tuberculosis or pneumonia. Deaths from syphilis, while common, are few in proportion to the deaths which are caused by other diseases gaining ready access to the syphilitic, whose vitality is lowered by the primary infection.

Focal Infections.—Localized inflammations, such as abscesses of the teeth, kidneys or ears, sinusitis, tonsilitis, appendicitis, cystitis, etc. are usually caused by the pyogenic cocci, although other pathogenic bacteria may occasionally be responsible. These localized inflammations are called focal infections. The systematic effects of these infections are due to the liberation into the blood stream of toxic substances or enzymes of the bacteria themselves and of products of tissue destruction. At times the bacteria causing the local infection get into the blood stream, in which case they may set up new infections at other parts of the body. Generally, these new sites are localized, forming so-called secondary foci. Sometimes a general septicemia develops from a focal infection.

The treatment of focal infections is not agreed upon by the medical profession. One group believes that, whenever possible, the diseased organ should be removed by surgical means. The other group, holding that every organ of the body has a definite purpose, believes in cure by proper treatment and rely on surgery as the last resort. Kopeloff[1] in speaking of focal infections of the tonsils says:

> The tonsils, because of their crypts, harbor many bacteria. Despite the fact that they are more or less easily infected, the tonsils are effective weapons of defense against the invasion of bacteria into the blood stream. For it is in the tonsil that many disease-producing microrganisms are caught in a mesh, die, or are annihilated by the white blood cells, or scavengers. Consequently, the haste to remove tonsils on the slightest provocation is to be deplored. The same may be said for adenoids, which are similar in structure to tonsils. Merely because tonsils and adenoids are frequently infected does not mean that they cannot be restored to health and should therefore be removed. It is never too late to replace caution and conservatism, if they are ineffective, by radical procedures.

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[1] Kopeloff, N., "Why Infections?" p. 172, New York, 1927.

Koch's Laws.—In order to avoid confusion and to place the relationship of any species of bacteria to a particular disease on a scientific basis, bacteriologists have adopted a set of rules which must be satisfied before any organism is accepted as the specific cause of a disease. These conditions are known as "Koch's postulates,"[1] or "Koch's laws":

1. The specific organism must be present in every case of a particular disease.
2. It must be isolated from the diseased individual and cultivated in pure culture.
3. The pure culture when inoculated into a susceptible animal must produce the same disease.
4. The specific organism must be reobtained in pure culture from the animal in which the experimental disease has been produced.

Transmissible Diseases Not Caused by Bacteria.—While diseases caused by microörganisms other than bacteria have not been specifically discussed, it must be understood that these other organisms behave, in general, like bacteria. The more common protozoan diseases of man are malaria, sleeping sickness, and amœbic dysentery. There are several mold diseases like "barber's itch," "mat itch," etc. A few rather rare yeast diseases are known.

Diseases of Animals.—In speaking of the pathogenic organisms, those forms are usually referred to which cause disease in man. Many of these same organisms also cause disease in the lower animals. On the other hand, there are some diseases of man which the lower animals do not contract, as, for instance, gonorrhea, smallpox, meningitis, and infantile paralysis; and there are some diseases of animals from which human beings do not ordinarily suffer, as glanders and hog cholera.

Diseases of Plants.—There are other pathogenic microörganisms which do not attack either man or animals but produce specific diseases in members of the vegetable kingdom. The science of phytopathology, which treats of plant diseases, is as important economically as the study of animal diseases.

The terms applied to plant diseases are not, as a rule, high-sounding Latin words, as are the names of human and animal

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[1] Robert Koch stated his "postulates" in a paper on the tubercule bacillus in 1882. These conditions had previously been expounded by both Jacob Henle (1840) and Edwin Klebs (1877).

diseases, but what they lack in scientific terminology they make up in practical applicability among the men who are, and should be, interested—the farmers. Thus, the terms, "wilt," "rot," "scab," "smut," "gall," "blight," and "spot" are used, which, while they are couched in popular terms, refer to definite symptom complexes and are, therefore, scientific.

Parasitism among higher plants is common. The members of the sandalwood family have root branches which are supplied with suckers enabling them to attach themselves to the roots of other plants and thus obtain food through the efforts of the host. The common mistletoe is another example. This plant obtains practically all its food from its host. It does not kill its host, but causes dwarfing.

Most of the diseases of plants are caused by molds and other fungi, although not a few are bacterial, and, of course, many are due to animal parasites.

Stevens[1] divides the causative agents in plant diseases into three classes, according to the manner of life in their host: the parasite living in the sap or in cavities or parts devoid of living protoplasm; the parasite for the major part of its life drawing its nutriment from host cells that are still living; and the parasite living within host cells which recently have been killed or partially destroyed by it.

The following short list will give an idea of the names of plant diseases and the type of organisms causing them:

Bitter rot of apple—fungus
Powdery mildew of apple—fungus
Rust spot in potatoes—bacterium
Tobacco wildfire—fungus
Tobacco wilt—bacterium
Damping off, seedlings—fungus
Curly top of sugar beet—bacterium
Apple blotch—fungus
Leaf blight in pear and apple trees—bacterium
Soft rot in melons, cucumbers, turnips, etc.—bacterium
Black knot of cherry and plum—fungus
Chestnut blight—fungus
Corn smut—fungus
Wheat rust—fungus
White pine blister rust—fungus.

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[1] STEVENS, F. L., "Problems of Plant Pathology," *Botanical Gazette*, vol. 63–64, pp. 297–306, Chicago, 1917.

CHAPTER XV

IMMUNITY

The ability of an animal to resist the microörganisms of disease is called "immunity." The term "immunity" is taken from the Latin and literally means, "freedom from service," or as it would be popularly expressed, "exemption."

Immunity is the opposite term to "susceptibility." Both must be considered as relative terms, and never absolute. The following example of this is of historic interest. Pasteur, in one of the earliest experiments to show the relationship between bacteria and disease, found that ordinarily chickens would not contract anthrax, but that if a chicken were placed in water, in order to lower its temperature after inoculation, it would readily contract the disease. It is known that the American Indian is more susceptible to tuberculosis than are people of the white race, yet not all Indians "take" tuberculosis, and large numbers of the Caucasians die of this disease annually.

Historical.—The ancient Greeks realized that persons who had recovered from the "plague" could associate with those suffering from that disease and not contract it again. Following this idea, the Turks and other Eastern peoples purposely contracted smallpox while they were perfectly well, in order that they might not contract the disease during the great epidemics, or when they were not physically fit. This was accomplished by transferring some of the material from a pustule of a smallpox patient to the scarified skin of a healthy person. This practice was introduced into England in the early part of the eighteenth century by Lady Mary Montague, who had had her young son inoculated in Turkey. The practice of variolation, as it was called, immediately spread through Western Europe and even to the American colonies.

In the last quarter of the eighteenth century

> . . . came one of the greatest triumphs in the history of medicine—the successful introduction of preventive inoculation by Edward Jenner.[1]

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[1] Garrison. F. H., "History of Medicine, "3d ed., p. 386, Philadelphia, 1924.

Jenner, an English country doctor, established the fact that vaccination with cowpox virus protects against smallpox.

For more than three-quarters of a century, smallpox was the only disease against which artificial immunization was attempted. With the establishment of the science of bacteriology, experimental evidence of protection soon followed. In 1880, Pasteur showed that chickens inoculated with an old (attenuated) culture of the organism causing chicken cholera would not contract this disease when later exposed to infection, or even when inoculated with virulent bacteria.

During the next decade the science of immunology took its place in human knowledge.

Early Attempts to Explain Immunity.—Pasteur's observations were soon confirmed by others, and protection against anthrax, Asiatic cholera, and diphtheria was established in animals by inoculation with attenuated or dead cultures or even the products of bacterial growth. As was quite natural, attempts were immediately made to explain this phenomenon. Three of the outstanding explanations, though since discarded, indicate the trend of thought.

The Exhaustion Theory.—According to the exhaustion theory of Pasteur, pathogenic germs, during the course of a disease, used up the supply of certain substances in the body of their host which were necessary for the growth of the particular species. Theoretically, this idea is untenable as it seems unlikely that any body substance could be exhausted by foreign means and not be replaced by the body. As soon as it became known that dead bacteria, or the products of bacterial growth, could confer immunity as well as the living germs, this theory was discarded.

The Retention Theory.—The retention theory, as expounded by Chauveau and others, was as easily disproved. This theory held that certain products of the specific organisms concerned in a disease were retained in the host after recovery, and that these products were harmful or "noxious" to the species. The rapidity with which the body frees itself of foreign substances alone contradicts this theory.

The Acquired Tolerance Theory.—This theory was based on the known fact that, if an individual takes a poison like arsenic or morphine in gradually increasing doses, he can acquire a tolerance to the drug and take it in quantities that would ordinarily prove fatal. The believers in the theory applied this fact to disease germs and claimed that the body of a person immune to a disease had acquired a tolerance to the bacteria and their toxins. As a statement of results of what happens in the body of a susceptible animal, this theory is all right. The fault is that it does not attempt to explain the mechanism of immunity. Moreover, it does not lend itself to "test-tube" proof. Diphtheria toxin can be neutralized in a test tube by the serum of a person who has recovered from the disease. Morphine cannot be rendered harmless by mixing with it the blood serum of a "dope fiend."

Modern Views of Immunity.—Today the world is still in the dark as to the actual mechanism of immunity. It is known that there are several factors which play a part in immunity and it is likely that a combination of these factors, any one of which may be greater or less at various periods in the human life, gives the real explanation.

Some of these factors are non-specific, that is, the protection is not against any particular disease germ and may be held by a person who has never had a disease. Others are truly specific—the protection is only against specific diseases.

Tissue Resistance.—The fact that living tissue resists invasion has already been stated. This impenetrability of living substance confers a degree of immunity against all of the pathogens in Bail's group of saprophytes, and to a great extent against the large numbers of disease germs which he calls "half parasites."

Outer Defenses.—Moreover, there are certain "outer defenses" of the body which resist invasion by pathogenic bacteria.

The skin normally has present on it, at times, virulent pathogenic bacteria, yet these organisms do not penetrate the unbroken surface. If they do, through abrasion, successfully invade the subcutaneous tissue, they may set up a local infection, but except in rare cases, the organisms are resisted by the rapid production of new connective tissue which effectually blocks off the site of infection.

The exposed mucous membranes of the body, such as those of the mouth, throat, and genitals, offer a protection by the mucous which washes them. The mouth, for example, contains a large variety of bacteria, often of pathogenic species, yet an infection of the mouth is seldom heard of. The tonsils, on the other hand, are rather permeable, but under normal conditions of health offer a fair resistance.

The cilia of the upper respiratory tract constantly tend to catch any pathogens that may be taken in by the breather, and by their upward motion deposit the germs on the moist surfaces of the mouth and nose, where they are held impotent, or are ejected.

The eyes are constantly irrigated by tears, which wash off any bacteria that might lodge there.

In the stomach, bacteria come in contact with digestive juices which are at least antiseptic. The intestines also contain antiseptic enzymes and bile. The entire alimentary canal, moreover, is covered with mucous membrance which, as has been seen, is a natural barrier to pathogenic bacteria.

It is apparent, however, that these defenses are not always sufficient and that syphilis and gonorrhea may be contracted through the apparently unbroken mucous membrane of the genito-urinary tract, typhoid fever through the intestinal lining, etc. Moreover, the presence of these outer defenses does not explain why an individual recovered from a disease rarely contracts the same disease again.

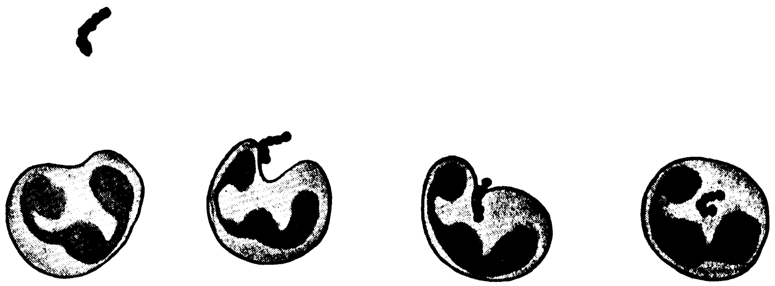

FIG. 32.—Phagocytosis. The leukocytes in the blood have the power of engulfing and destroying bacteria, thus aiding the body in combating disease. The picture shows the gradual ingestion of a chain of streptococci by a white blood corpuscle. (*Bull and Thomas, J. B. Lippincott Company.*)

Phagocytosis.—In 1882, Metchnikoff observed that certain of the white corpuscles in the blood (polymorphonuclear leucocytes), which he called phagocytes, had the power of ingesting and destroying disease germs which had passed the outer line of defense. The phagocytes are amœba-like blood cells varying in size from 5 to 15μ in diameter. When a local infection occurs, the phagocytes immediately gather at the site of invasion. Here they ingest the bacteria. Some of the phagocytes are in turn destroyed by the poisons of the bacteria. This mass of phagocytes and bacteria, together with a certain amount of serous exudate, is known as pus.

The phagocytes of all animals have the power of destroying bacteria to a certain extent, but the phagocytes of a person who has recently recovered from a particular bacterial disease show a markedly increased activity toward the organisms responsible for that disease. That the phagocytes themselves do not become more efficient in destroying bacteria in an immune individual but that there is something in the blood serum of immunized animals which assists the phagocytes in destroying bacteria may be demonstrated in the test tube by two experiments. First, if the blood from an immunized person be centrifuged and the phagocytes washed clear of all serum, they will not ingest bacteria; but if the serum be again added, their original power is regained. Second, if the serum from an immunized individual be added to the washed phagocytes of a normal individual, it will be found that these "normal" phagocytes attain the same power of ingesting bacteria that the phagocytes in the immune blood possessed. This substance in the blood of immune individuals, which prepares the bacteria for ingestion by the phagocytes, is called opsonin (Greek, "I prepare food for").

The "opsonic index" is the ratio of the average number of bacteria absorbed by the phagocytes in a test specimen of blood to the number of bacteria absorbed by the phagocytes in a specimen of normal blood. Thus, if the average number of bacteria absorbed by phagocytes in a "test" blood were 3, and the average number of bacteria absorbed by the phagocytes in a normal blood were 1.5, the opsonic index would be 2.0. If the figure for normal blood and test blood were reversed, the opsonic index would be 0.5.

Antibodies.—The examination of the blood of individuals ~~who have rec~~overed from a disease shows that the serum possesses properties which can account for degrees of immunity without the presence of phagocytes. What it is in the serum cannot be stated, but by certain phenomena which are demonstrated it is believed that immune serum contains several substances which give it its immune properties. These protective substances are called "antibodies."

It is still not known what the chemical composition of antibodies is. It was at first believed that they were manufactured in the body out of the material of the disease germs or their products. For example, Buchner taught that an antitoxin—an antibody which neutralizes the poisonous effects of a bacterial toxin—was a substance produced by the body out of the toxin itself. Several experimental proofs against this theory have been demonstrated. In the first place, if a minute amount of toxin—much less than the amount necessary to kill—is injected into an animal, there will be produced in that animal sufficient antitoxin to protect it against many times the lethal dose of the toxin. Moreover, this amount of antitoxin may be still further increased by injecting into the animal a non-specific stimulant like pilocarpin. Again, if an animal like the horse is repeatedly injected with increasing doses of toxin, the antitoxin titre of that animal will reach a more or less stable level above which no amount of injected toxin can force it. Now, it is possible, by repeated bleedings, to remove what would amount to the total volume of blood from the animal, which would theoretically remove all the antitoxin and substance from which it is made, if Buchner's idea is correct. Instead, it is found that the horse retains a considerable amount of antitoxin in his blood. From these observations it may be assumed that antibodies are produced by the body cells out of body substance. The substances injected, disease germs or their products, are simply stimulants.

Antibodies are produced by the body cells to react against the substances which would tend to destroy them. Thus, if an individual has tetanus, the toxin tends to destroy the body cells. These cells, in turn, manufacture substances which will neutralize the toxin. The neutralizing substances are called "antibodies" or, in this case, "antitoxins." Again, if typhoid germs—which are actively motile—get into the body, the body cells will produce substances, called "agglutinins," which are able to deprive the typhoid bacteria of their motility, and to cause them to clump together. These antibodies are specific; tetanus antitoxin will not neutralize diphtheria toxin or cause Eberthella typhi to clump, nor will typhoid agglutinins agglutinate diphtheria or tetanus germs, or neutralize their toxins.

Antigens.—Antigens are substances, which, when introduced into the animal body, stimulate the formation of specific antibodies. In order to understand the subject of immunity, these two terms, "antigen," and "antibody," must be thoroughly understood. The following table will illustrate the relation between antigens and their specific antibodies:

A toxin (antigen) will stimulate the production of an antitoxin (antibody). *Example:* Diphtheria toxin will stimulate the production of diphtheria antitoxin.

An agglutinogen (antigen) will stimulate the production of an agglutinin (antibody). *Example:* Typhoid agglutinogen will stimulate the production of typhoid agglutinin.

A precipitinogen (antigen) will stimulate the production of a precipitin (antibody). *Example:* Human blood serum (precipitinogen) will stimulate the production of human serum precipitin.

A lysogen (antigen) will stimulate the production of a lysin (antibody) *Example:* Human erythrocytes will stimulate the production of human hemolysins.

An opsoninogen (antigen) will stimulate the production of opsonin (antibody). *Example:* Cholera germs (opsinogen) will stimulate the production of cholera opsonins, which will increase phagocytic action against cholera germs.

Ehrlich's Theory of Immunity.—Various theories as to the mechanism of serum reactions have been suggested. The Ehrlich theory, while it is no longer accepted by immunologists as a true explanation for the mechanism of immunity, has furnished a terminology, or language, of the science, and should be understood in order to form a foundation for further study.

Ehrlich's theory is based on the idea that the body cells have certain combining affinities or side chains likened to the valence bonds of chemical elements. By means of these chemical affinities, food substance is taken into the cell and built up into protoplasm. It is by means of these side chains, also, that toxins are able to combine with the cell substance and harm the cell.

According to Ehrlich's idea, each particular kind of substance, food, diphtheria toxin, tetanus toxin, etc. finds in the cell a particular side chain with which it, and it alone, is capable of combination. If the cells of an animal do not possess combining affinities for any particular poison, then that animal cannot be harmed by that poison. This in a way explains species immunity, or the immunity of a whole group of animals against a particular disease.

These "side chains" are called "receptors." When any particular receptor is combined with a foreign substance, more receptors of the same kind are formed by the body cells to take its place; and nature, being always prolific, produces for the cell more receptors than can remain in the cell, and the surplus float free in the blood stream.

Thus, for example, if a tetanus toxin molecule comes in contact with a cell, it will combine with only one kind of receptor in the cell—the tetanus-toxin receptor. Upon the combination of this receptor, more tetanus toxin receptors will be produced by the cell to take its place. These new receptors, although crowded off the cell into the blood stream, still have the power of combining with and neutralizing tetanus toxin. Free receptors floating in the blood stream are called "antibodies."

It soon became apparent to Ehrlich that the different phenomena which take place in the body—and in test tubes—could not be explained by the presence of a single, neutralizing antibody. He therefore conceived of a number of different kinds of antibodies which he groups into three "orders," according to the general type of reaction.

Antitoxins.—The first order of antibodies are the antitoxins. They possess one combining affinity which unites with the combining affinity of the toxin, both toxin and antitoxin being neutralized. Ehrlich calls the combining affinities of the toxin and antitoxin the *haptophore* group or radical.

It was at first supposed that the antitoxin destroyed the toxin. Later investigations showed that toxin, after combination with antitoxin, again becomes active, if the mixture is treated with hydrochloric acid or is gently heated. This indicates that in a toxin-antitoxin mixture there is a neutralization—not a destruction—of the toxin. Antitoxin is less stable than toxin.

This neutralization takes place in chemical proportions. Thus, if 1 cubic centimeter of toxin solution of a certain strength neutralizes 1 cubic centimeter of antitoxin of a certain strength, 10 or 100 cubic centimeters of this toxin solution will always neutralize 10 or 100 cubic centimeters of the same antitoxin. This phenomenon can be demonstrated in the test tube as well as in the animal body. The "Schick test" for susceptibility to diphtheria depends upon the toxin-antitoxin phenomenon.

Agglutinins, Precipitins.—The second order of receptors includes the agglutinins and precipitins. In this case, the recptors have two affinities. The one, the haptophore, combines with the haptophore group of the specific antigen, and the other, the *ergophore* or *zymophore* group, by virture of its chemical composition, is able to produce agglutination or precipitation, depending upon the antigen.

The phenomenon of agglutination is easily observed when a small amount of the serum of an animal, which has been injected with a specific antigen like typhoid germs, is mixed with a suspension of the same bacteria. The germs rapidly clump together, forming agglomerates. These clumps sink to the bottom of the tube, leaving a clear, supernatant fluid.

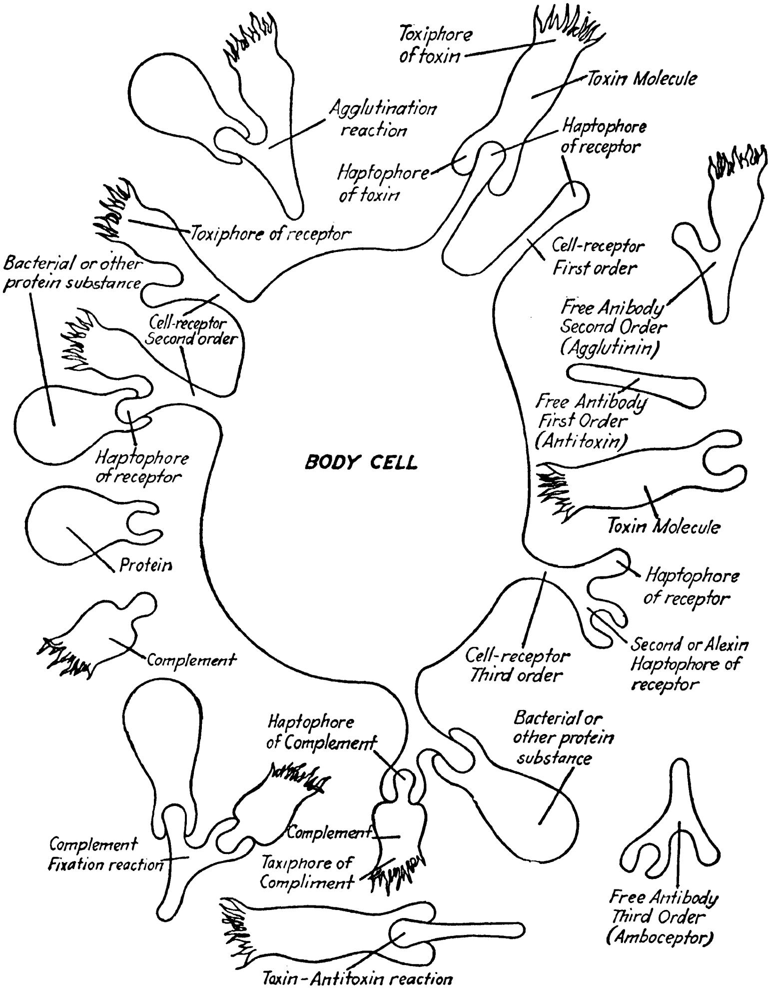

FIG. 33.—Diagrammatic representation of Ehrlich's side-chain theory

Precipitation is explained in the same way as agglutination. When an animal is injected with any dissolved foreign protein—not necessarily a "formed" protein like a bacterial cell, but white of egg, blood serum, or even vegetable proteins—the serum of that animal, when mixed with the clear solution of the specific antigen, forms a precipitate.

The necessity of the conception of a "second order" in Ehrlich's theory lies in the fact that the effect produced is much greater than can be accounted for by a simple neutralization phenomenon. We can conceive of one antitoxin molecule neutralizing one toxin molecule, but one agglutinin molecule cannot cause a single typhoid germ to clump. There must be some power conferred upon a bacterium, united with its specific agglutinin, which causes it to attract and stick to other bacteria similarly treated.

Moreover, it is possible, by heating, to destroy the zymophore group of the agglutinin molecule without the molecule losing its combining or haptophore group. If an antitoxin molecule is heated, it loses completely its neutralizing power, a fact that is indicative of a single group.

Anaphylaxis.—Precipitins and the phenomenon of "precipitation" carried out in the animal body may be the cause of the symptoms known as "anaphylaxis." If an animal, a guinea pig, for instance, be injected with a very small amount of protein, such as bacterial cells, or horse serum, and after three weeks be given an intravenous injection of a larger amount of the same protein, this latter dose, which under ordinary conditions would have little or no effect on the normal animal, will produce in the treated animal very pronounced symptoms, or even death.

At first glance, this effect may seem to be paradoxical, since it may be reasoned that the animal which had received a preliminary injection of the protein would be to a certain extent immunized and therefore better able to withstand a second injection than would a normal animal. There are two explanations for this phenomenon: One is that the body cells, being stimulated by the first small dose of antigen, produce receptors, and absorb more of the toxin substance than they do normally, which gives rise to the anaphylactic shock.[1] The second explanation is based on the "protein-splitting ferment" theory of

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[1] Von Pirquet, "Allergy," *Archives of Internal Medicine*, vol. 7, pp. 259–288, 383–436, 1911.

Vaughan, and will be discussed under that theory. Various diagnostic tests, like the luetin test for syphilis, the tuberculin tests, and the mallein test, depend on the anaphylactic phenomenon. The precipitation reaction is also used in forensic medicine to determine the source, human or animal, of blood.

Lysins, Opsonins.—The third order of receptors includes the lysins and opsonins. As mentioned under the previous discussion of opsonins, if blood from an immunized individual be washed free of all serum, the phagocytes will not ingest bacteria, but, if the serum be added, they will regain their power. The opsonins, therefore, play the part of a combining link between the phagocytes and the bacteria.

It might be well to state at this point that the action of two distinct kinds of opsonin is demonstrable. There exists in any individual a normal opsonin which is thermostable and probably specific but present in a small amount, and a non-specific thermolabile substance which is probably alexin. In an immunized individual, the thermostable opsonin or immune opsonin is greatly increased in amount, which enhances the phenomenon of phagocytosis without the aid of complement.

A lysin is an antibody which causes the dissolving of its specific antigen. Lysins (dissolvers) are produced by the injection of *any kind* of cells (bacterial cells, erythrocytes, leucocytes, spermatozoa, etc.) into an animal (as the rabbit), and are present in the serum of the immunized animal. Lysins are specific in their action against their particular antigens and are spoken of as bacteriolysins, hemolysins, etc.

The term "lysin" is really a misnomer, as these substances do not themselves cause the dissolution or breaking up of the cell. This is done by a non-specific substance called "alexin," or complement, and is found in all animal sera. Ehrlich's term, "amboceptor" (double receptor), or the term "sensitizer," is better employed to designate these bodies.

If the serum containing the lysins is heated to 56°C. for 30 minutes, the lysins will lose their lytic property. If normal serum from another animal, however, even from one of a different species, and one which does not contain any lysin—like the guinea pig—is added to the heated lysin and the lysogen, the lysin will regain its lost properties. The substance furnished by the normal guinea-pig serum is the alexin or complement. Buchner claims that the normal resistance to infection exhibited by the healthy animal and the greater resistance of an immunized animal are due to the substance alexin. The necessity of Ehrlich's "third order" lies in the "amboceptor" or double-bond conception of the antibody. Toxin neutralization, precipitation, and agglutination do not depend upon the presence of complement. Moreover, it is possible to destroy by heat or absorb agglutinins out of an immune serum and retain the bacteriolytic properties of the serum. Certain diagnostic tests, called "complement fixation" tests, are based on the combination of complement with antibodies in an immune serum. The Wassermann reaction for syphilis is one of these tests.

Vaughan's Theory of Immunity.—Victor Vaughan[1] rejects the side-chain theory of Ehrlich and claims that antibodies are simply protein-splitting enzymes. Such a ferment splits the protein (bacterial protein, toxin, etc.) into two parts, one poisonous and the other non-poisonous. The non-poisonous part stimulates the production of more of the specific protein-splitting enzyme in the blood, and, on the second injection of the protein, this increased amount of enzyme present is able to split it immediately. The poisonous part, being liberated, causes the symptoms known as anaphylaxis. When bacteria invade the body, the body cells become "sensitized," and react against them. This reaction consists of liberating the protein-splitting enzyme, which splits up the protein molecule of the bacterial protein into two portions, one poisonous and the other non-poisonous. The poisonous protein produces the apparent symptoms of the specific disease. The non-poisonous portion stimulates the body cells to produce more of the specific proteolytic enzyme. On recovery from the disease, the individual possesses an immunity, due to the storing up of this specific enzyme in the tissues. On subsequent exposure, the body cells destroy the bacteria without any noticeable reaction.

D'Herelle's Theory.—Admitting the rôle of antibodies in specific acquired immunity, F. d'Herelle[2] maintains that there is at least one other factor associated with immunity to certain diseases. This is the presence, in the intestinal canal of man, of an ultramicroscopic bacterial parasite, the bacteriophage. The bacteriophage, while normally slightly virulent to such

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[1] VAUGHAN and NOVY, "Cellular Toxins," Philadelphia, 1902.

[2] D'HERELLE, F., "Immunity in Natural Infectious Diseases," translated by G. H. Smith, p. 306, Baltimore, 1924.

pathogens as Shigella dysenteriæ, is capable of becoming extremely virulent. In the normal person, the bacteriophage may not be strong enough to protect against infection, but, if recovery takes place the virulence is so increased that future infection is impossible.

The bacteriophage plays a predominant rôle in all the phenomena of immunity. It is because of its presence that when exposed to infection an individual remains unscathed, and it is because of its presence that an individual, when sick, recovers.

Other Theories of Immunity.—A great many other theories explaining particular phases of the phenomenon of immunity have been proposed. For example, Dean believes that all the different phenomena of an immune serum, such as agglutination, precipitation, complement fixation, etc. are due to a single specific substance in the serum.

Bordet does not agree with Ehrlich that toxin and antitoxin molecules combine always one to one. If a definite amount of toxin is mixed with one-half the necessary amount of antitoxin to neutralize it, he believes that the antitoxin will be spread out over the entire amount of toxin and each toxin molecule will become one-half as poisonous.

Again, Bordet looks upon the agglutination reaction as a "two-phase" phenomenon. The agglutinin itself does not cause agglutination; it simply sensitizes the bacteria, so that, when suspended in an electrolyte, they clump. This is similar to the disturbance of a colloidal suspension by a salt.

The same worker disagrees with Ehrlich in his "amboceptor" idea of lysins. He agrees that lysin and complement are both necessary for dissolution of the cell (antigen). He looks upon the lysin, however, as a "sensitizer," or mordant. Complement cannot unite with either antigen or sensitizer alone but can only act after the union of antigen and sensitizer.

Types of Immunity.—Immunity may be considered under four heads—natural immunity, acquired immunity, active immunity, and passive immunity.

Natural immunity may be considered as inherited immunity and is possessed by species of animals toward diseases common to other species. This has been explained under the general definition of immunity. Thus, the lower animals do not suffer from such diseases as typhoid fever, syphilis, gonorrhea, etc., which are common to man. Natural immunity may be racial, as shown in the difference in susceptibility to tuberculosis between the Jews and the American Indians. Even among individuals of the same race, variations in susceptibility are observed. In the former case, it is likely that, while the phagocytes and immune substances in the body play a part, the mechanical defenses of the body, such as temperature, nutritional conditions, etc. play the essential rôle. In the case of individual immunity, inheritance may be predominant.

Acquired immunity is always considered from the point of the individual, and may be obtained in two ways. Either the individual may recover from a disease and thus acquire a resistance to that disease, or he may artificially immunize himself by injecting a substance into his body which will confer immunity.

Acquired immunity may be active or passive. Immunity acquired by recovery from the disease is always active. Substances injected artificially to produce immunity may cause active or passive immunity, depending upon the nature of the substance. The injection of these substances to produce immunity is known among physicians as "immune therapy."

Immune therapy recognizes two main classes of substances, one of which, including the vaccines and bacterins, produces active immunity, and the other, immune sera, of which antitoxins are a class, produces passive immunity. Active immunity is effected by the injection of antigens. Specific antibodies are produced by the body to counteract their effects. If another animal, as the horse, is injected with an antigen, antibodies are produced, and the horse acquires an active immunity. Now, if the immune blood is withdrawn from the horse and the serum injected into a person, that person acquires a passive immunity.

Vaccines.—Vaccines are a development of the early empirical methods for imparting immunity, first used by the Turks. These people had a custom of deliberately inoculating themselves with smallpox virus, in order to have the disease in mild form while they were in good physical condition. Smallpox vaccine, as used today, does not produce in the individual taking it even a mild attack of smallpox, since the virus used is not the cause of smallpox in man but of a somewhat similar, though infinitely milder, infection known as cowpox.

Edward Jenner (1749–1823) in 1796 first proved that immunity to smallpox could be obtained by cowpox vaccination, although its use for that purpose had been proposed some years previously. Jenner inoculated a boy with cowpox vaccine and later with a smallpox pustule, the latter without effect. The success of this experiment soon led to the general use of "vaccine." Within 10 years, the British Army and Navy had adopted its use, and it was introduced into the United States. Of course, this was before the discovery of the relation between bacteria and disease, and the practice was carried out without a true scientific understanding of its action.

Smallpox Vaccine.—All the "smallpox" vaccine used today is prepared in a few well-equipped laboratories. As a rule, young milk-fed calves are employed. The process of manufacture, while comparatively simple, is marked by the greatest possible scientific control and aseptic precautions. The calves are inspected by veterinarians for tuberculosis, and held in quarantine until they are declared free from disease. They are then clipped, and the entire body is scrubbed with an antiseptic solution and soap, and thoroughly rinsed with water.

The calves are then placed on a special operating table and their entire under surface is shaved. After this, they are wheeled on the operating table into an operating room which, for cleanliness and modern equipment, compares favorably with that of any hospital.

The shaved surface of the calf is scrubbed with soap, and this is removed by repeated washings with sterile water. The entire undersurface is then scarified with a sharp scalpel, the operator making parallel incisions and taking care not to draw blood. A stock virus (the virus of cowpox) is then rubbed over the scarified area. After vaccination, the calf is taken into a special room, where it is kept under the most rigid aseptic conditions for a period of about 8 days, or until the proper vesicles appear.

Now the calf is killed and again placed on an operating table. While the vaccine could be removed from the animal while alive, the operation would cause unnecessary pain, as the calf would have to be killed immediately afterward in order that an autopsy could be made to show that it was perfectly healthy. The body is taken to the operating room and the lymph removed. Before the vesicles containing the lymph are taken from the calf, all scabs and crusts are washed off with sterile water, and the lymph, or "pulp," is scraped off with a sterile instrument called a curette.

The "pulp" is then ground or triturated in sterile machines to a homogeneous mixture. After grinding, the lymph is mixed with a certain amount of glycerine and sterile, distilled water in order to kill off any contaminating bacteria from the air and to give it the proper consistency.

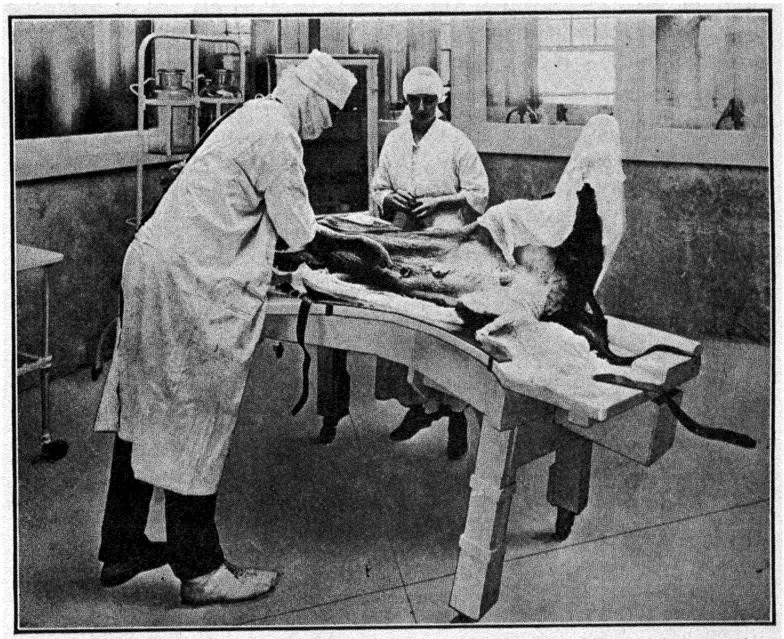

FIG. 34.—Removing smallpox vaccine from calf. Note hospital operating room conditions. (*Courtesy, H. K. Mulford Co.*)

The vaccine is now "aged" until physiological and other tests show that it is both potent and harmless. It is then put up for the market, usually in capillary tubes.

When completed, smallpox vaccine consists of a suspension of living cowpox virus in a solution of glycerine, lymph and water. It often contains other bacteria (as sterilization would kill the virus), but care is taken to be sure that these contaminations are harmless.

While a different technique is employed in its manufacture, "rabies vaccine" is also prepared from living animals, usually rabbits.

From what has been studied concerning the mechanism of immunity, it can be seen that the introduction of a vaccine into the body is really the injection of an antigen. This antigen stimulates the production of specific antibodies, which are ready to destroy any disease organisms of the same kind that may subsequently gain entrance into the body. This is an example of active immunity, since the antibodies are manufactured in the body of the now immune individual.

Bacterins, or bacterial vaccines, are simply altered—usually killed—cultures of disease germs. The proteins of the bacterial cells are agglutinogens, bacterial lysogens, etc., and, following the injection, all the various antibodies are produced. Bacterins were first introduced by Louis Pasteur, after his classical research on anthrax. A typical bacterin, typhoid bacterin, is prepared as follows:

A stock culture of Eberthella typhi which has been thoroughly tested for its antigenic properties is transferred in a physiological (0.85 per cent) saline emulsion to the surface of agar in a number of flat bottles. The bottles are placed in an incubator at 37.5°C. for 24 hours. Here the organisms grow and multiply until the culture has been increased to a large mass of bacteria. The growth is then washed off the agar with sterile physiological saline solution and collected by means of sterile bulbs into a few "concentrate" bottles. At this point, the "concentrates," which are simply thick emulsions of Eberthella typhi, are tested for purity; that is, it is determined by microscopic and cultural tests that they contain no bacteria other than Eberthella typhi. If the purity tests are satisfactory, the concentrates are heated to 53°C. for 1 hour. This treatment kills the organisms and yet does not destroy their antigenic properties.

The water bath in which the emulsion is heated is especially designed for the purpose. It consists of a large copper tank heated by gas, the flow of which is controlled by a thermoregulator. The water in the tank is kept constantly circulating by a set of propellors which have blades designed to hold the concentrate bottles. By this means, the bacterial emulsion is agitated so that all portions are evenly exposed to the temperature of the water. The surface of the bath is covered with liquid petroleum to prevent evaporation, and temperature variations. Then 0.25 and 0.3 per cent tricresol, or 0.5 per cent phenol, is added to the concentrate, to inhibit the growth of any possible contaminant which may get in, either during the subsequent treatment, or at the time of inoculation. The concentrate is now carefully tested for sterility, and a count made of the number of organisms.

The concentrate is then diluted with sterile physiological saline solution, containing 0.3 per cent tricresol, so that each cubic centimeter of the diluted bacterin will contain 1,000,000,000 killed typhoid germs.

At this point the product is again tested for sterility; a microscopic examination is made to verify the previous purity test; and the antigenic properties of the bacterin are tested on rabbits. A large dose (3 to 5 cubic centimeters) is also injected into guinea pigs as a "safety test." If all these tests are satisfactory, the bacterin is placed in vials or syringes for the market.

Sometimes a triple typhoid vaccine is made containing paratyphoid A and paratyphoid B germs besides the Eberthella typhi. This and other bacterins, except for the number of organisms put in the diluted product, are prepared in accordance with the same general principles. It is necessary to emphasize the fact that the whole process is carried out under the strictest aseptic control. Operatives are always skilled bacteriologists, who wear sterilized surgical gowns and headgear, and do all work, during which the culture is exposed, in sterilized laboratory units.

Typhoid bacterin is similar to smallpox vaccine, except that the organism is grown on an artificial culture medium instead of on the skin of a calf, and is killed before it is used. Like smallpox vaccine, it is an antigen, and, being an antigen, it stimulates the production of specific antibodies in the body of the person injected.

Wright[1] lays down the following general principles of bacterin therapy:

> 1. We must provide for conveyance of bacteriotropic substances into the focus of infection.
> 2. In case of an accumulation of stagnant fluid in the focus of infection effectually preventing the entrance of bacteriotropic substances, as a preliminary measure, we must draw off the fluid which occupies that focus.
> 3. In case there are other obstacles to the free streaming of lymph through the focus of infection, we must remove those obstacles.

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[1] Wright, A. E., *Journal American Medical Association*, pp. 470–487, Aug. 10, 1907; pp. 567–573, Aug. 17, 1907.

In other words, the injection of a bacterin will stimulate the production of bacteriolytic substances in the body of the patient, but the efficacy of these bacteriotropic substances depends upon whether or not they ever reach the focus of the infection.

In cases of non-specific local infections, it is sometimes desirable to use bacterins prepared from the microörganisms obtained from the patient. These are known as "autogenous bacterins."

Immune Sera.—As has been said, when an antigen is injected into a human being, antibodies are formed to counteract that specific antigen. This formation of antibodies, however, takes some time, and, as a matter of fact, immediately after the injection the immunity is lowered during the time the body cells are manufacturing their antibodies. This period of lowered immunity is known as the "negative phase" to immunity. If, therefore, a person already has a disease and the body cells are not producing antibodies rapidly enough to counteract the bacteria and their toxins, it would aggravate the trouble to inject an antigen in the form of a vaccine or bacterin.

To meet this condition, the antibodies are manufactured in the body of another animal, usually the horse, and the antibodies themselves are injected into the person suffering from the disease. The substances containing antibodies are called "serums."

Blood is made up of two portions; a solid portion consisting of erythrocytes and leucocytes, and a fluid portion consisting of water and various dissolved substances. The fluid portion contains a substance called "fibrinogen," which, when the blood is drawn from the body, is changed into a new substance, fibrin, which causes clotting of the cells. After clotting, the clear liquid portion of the blood is called "serum." If a salt, like sodium citrate or sodium oxalate, is added to the freshly drawn blood the blood will not clot. If the cells are now separated and removed from fluid, the clear fluid is called "plasma." Plasma has the same constituents as serum, plus the normal fibrinogen in the blood and the added dissolved salt.

Diphtheria antitoxin is a refined serum drawn from a horse which is highly immune to diphtheria toxin. Pneumonia serum is the serum of a horse which has received repeated injections of a Pneumococcus bacterin and is, therefore, highly immune to Diplococcus pneumoniæ. In other words, a vaccine or bacterin, when put into the body, stimulates the production, in the body and by the body cells, of antibodies; a serum contains these antibodies which have been produced in the body of another animal by the same process of stimulating its body cells by the injection of antigens. A vaccine or bacterin gives active immunity; a serum gives passive immunity. Active immunity lasts considerably longer than does passive immunity.

To illustrate the method of producing serum, the making of diphtheria antitoxin will be briefly described.

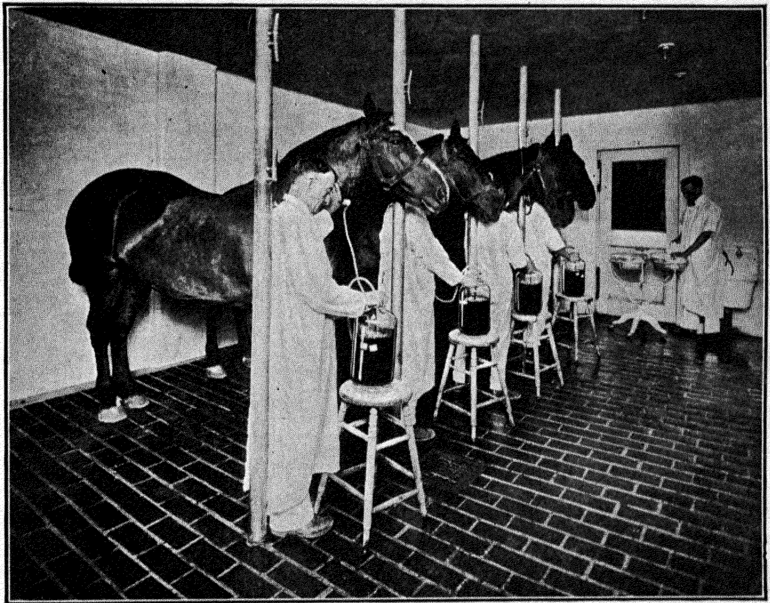

FIG. 35.—Bleeding horses for diphtheria antitoxin. (*Courtesy, H. K. Mulford Co.*)

Diphtheria Antitoxin.—The antigen used in immunizing horses for production of diphtheria antitoxin is diphtheria toxin. This is prepared by growing the diphtheria germs, Corynebacterium diphtheriæ, in bouillon, and then removing the bacteria by filtration through a Berkefeld filter candle. As the diphtheria toxin is an exotoxin, it passes through the filter and remains in the bouillon. The bacteria themselves are discarded and only the filtrate containing the toxin is used.

Healthy horses under the constant care and supervision of expert veterinarians are injected subcutaneously with this bouillon filtrate containing the diphtheria toxin. Injections are given at regular intervals, and the doses are gradually increased from a few cubic centimeters to large amounts as the horse produces more and more antitoxins.

The amount of antitoxin in the horse is measured in "antitoxic units" according to a scheme laid down by the United States government. When the course of treatment has advanced so that the horse is producing between 300 and 1,000 "units" per cubic centimeter, he is bled. A sharp cannula is introduced into the jugular vein by a veterinary surgeon, and the blood is allowed to flow into large jars through a rubber tube. Six to eight liters are drawn at a time. The blood is allowed to clot and the clear serum is drawn off. The blood serum contains the antitoxin.

In diphtheria and tetanus antitoxin, this serum is concentrated by a modification of a method known as the Gibson process. The serum is treated with a saturated solution of ammonium sulphate, which precipitates that part of the serum, the so-called pseudoglobulin, which contains the antibodies. The balance of the serum is discarded. The precipitate or "globulin," as it is now called, is redissolved in sodium chloride solution and again precipitated with acetic acid. This final precipitate contains highly purified and concentrated antitoxin. The precipitate is dried between filter papers and subjected to dialysis in running water to free it from all inorganic substances, such as chlorides, sulphates, and acetates, used in the process of concentration. The dialyzed product remaining in the dializing bags is suspended in a sodium-chloride solution of such a salt content that the solution will be "isotonic," that is, of the same salt content as human blood (approximately 0.85 per cent NaCl). The material is then filtered through Berkefeld filters to remove any bacterial contamination. Its unit value or strength is "standardized" by the method of the United States government, and it is placed into vials or syringes in definite strengths from 1,000 to 20,000 units each.

As in the other cases, the entire process of making antitoxins is under the most elaborate aseptic control. It would take chapters to describe in detail the various sterility, safety, potency, and chemical tests that these products are subjected to before they are placed in the hands of physicians.

Laboratories used for the commercial production of vaccines, bacterins, and serums are licensed by the United States government and are subject to the rigid control of the federal government through the Laboratory of Hygiene in Washington. Frequent inspections are made by government experts, and the products tested at irregular intervals from samples obtained on the open market.

Value of Immune Therapy.—The value of these products in the prevention and cure of disease cannot be overemphasized. Smallpox vaccine has practically eliminated what was at one time a universal and constant pandemic.

The incidence of typhoid (including paratyphoid) fever in the American Expeditionary Forces was less than 0.1 per cent, while it was 20 per cent in the Spanish-American War.[1]

In a paper entitled "Twenty-five Years of Antidiphtheria Serotherapy," L. Martin presented statistics showing the comparison between deaths from diphtheria before and after the introduction of diphtheria antitoxin in 1894. In 1893, there was a maximum of forty-five and a minimum of fourteen deaths each week from diphtheria in the city of Paris. In 1918, the maximum weekly deaths were four, and the minimum, one.[2]

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[1] VAUGHAN, N. C., *Journal American Medical Association*, vol. 74, p. 1145, 1920.

[2] MARTIN, L., "Vingt-cinq années de Serotherapie antidiphtherique," *Bulletin Academie de Medicine*, vol. 82, pp. 173–179, Paris, 1919.

CHAPTER XVI

HYGIENE AND SANITATION

The Greeks had a goddess of health, whom they called "Hygeia." She was supposed to be the daughter of Æsculapius, the god of medicine. The modern physician considers the daughter of first importance, and thinks of Æsculapius as an adjunct who helps out mankind when Hygeia is angry because she has not been properly worshipped. The normal or proper condition of life is health. The abnormal or improper condition of life is disease. Disease is not necessary, and only comes when the laws of Hygeia are disobeyed.

Hygiene is the science of health. The term is usually considered from the point of view of the individual. Sanitation is the science of public health and is considered from the point of view of the community or state. The laws of hygiene are those which affect the individual in his conduct toward himself; the laws of sanitation affect the individual's conduct toward his fellow man. The two concepts may be independent or they may bear directly upon each other. If an individual, through overindulgence in alcohol, lack of sleep, or improper clothing, runs down his body so that he falls a victim to tuberculosis, he has disobeyed the laws of personal hygiene, and suffers the penalty. If, having tuberculosis, he expectorates and coughs in public, he thereby exposes others to the same disease and disobeys the laws of sanitation. The laws of hygiene refer to all diseases, both constitutional and bacterial; the laws of sanitation, while to a limited extent referring to constitutional diseases, are chiefly concerned with the prevention of infections or bacterial diseases.

In previous chapters, the sources of bacterial infection and the modes of transmission have been discussed in a general way. The various lines of resistance which the body has for protection have also been pointed out, and how, by the use of immune therapy, individual resistance may be increased. The work of the sanitarian is to prevent infection, either by avoidance of exposure, or by the protection of the individual, if exposure does take place.

Personal Cleanliness.—In one measure, at least, in the control of communicable disease, the responsibility lies with the individual. That is in the matter of personal cleanliness. No amount of filth can cause a transmissible disease, if the germs of that disease are not present, but an abundance of soap and water can often remove from the body material which does contain pathogenic bacteria. A person's own hands are especially liable to be the indirect cause of his infection, and also may innocently cause the infection of his friends. The first general rule in hygiene is to keep clean. Particular emphasis must be laid upon washing the hands before meals, after using the toilet, and after handling any object which might carry disease germs. Avoidance of the common drinking cup, not using another's pipe, razor, brushes, towels, etc.; keeping away from a person who is coughing or sneezing; avoiding promiscuous kissing, are all measures in personal cleanliness which should be practiced as public health duties as well as from aesthetic considerations.

By reference to a few examples of particular types of disease, an idea may be obtained of the methods to be employed to avoid their dissemination.

Typhoid Fever.—Typhoid fever may be taken as an example of the group of intestinal diseases. Cholera, caused by Vibrio comma; dysentery, caused by Shigella dysenteriæ; and enteritis, caused by various members of the genus Salmonella, are other diseases of this group.

Typhoid fever is caused by Eberthella typhi, also known as Bacillus typhosus, Eberth's bacillus, or the typhoid bacillus. This organism closely resembles, in morphology and cultural characteristics, other members of the colon group "B. coli." As typhoid is an intestinal disease, the specific organisms are present in the excreta of typhoid patients, and the typhoid germ is always found associated with the "B. coli" group. This group has been accepted as an indicator of pollution and its presence in water shows that pollutions by intestinal forms is taking place, and that pathogenic intestinal bacteria may be present.

The disease is spread in epidemic forms by water, milk, oysters, water cress, flies, and by human carriers.

Water is probably the greatest single carrier of typhoid germs. Historic epidemics, such as in Plymouth, Pa., in 1885; Ashland, Wis., in 1893; New Haven, Conn., in 1901; and Ithaca, N. Y., in 1903 are but a few examples of the toll of death caused by typhoid-infected water in this country alone. With the introduction of properly controlled public water supplies, this disease is being reduced to a negligible factor in civil life.

Milk supplies have been the cause of typhoid epidemics, which, while not so widespread as those caused by water, have been significant in their virulence. Milk-borne typhoid epidemics are characterized by the clear-cut way in which the appearance of cases follows a particular milk route. Such cases have been traced to the washing of milk cans with polluted water, and also to the handling of the milk by carriers. The short time which elapses between the consumption of milk and the infection accounts for the high virulence of milk-borne typhoid germs.

When a person has recovered from an attack of typhoid fever, he may carry the organisms of the disease for some time without showing any symptoms of disease. In some cases, virulent germs have been isolated from the fæces of people many years after recovery from the disease. These people are known as "carriers" and constitute a real menace to any community. No person should be allowed to handle food in any capacity in a public restaurant unless he is tested and found not to be a typhoid carrier. The test is a very simple one and there is no excuse for its not being insisted upon. Many examples of typhoid caused by carriers could be cited. The historic case of "Typhoid Mary" is only one of a number. "Typhoid Mary" was a cook who worked in a number of different households. At the time it was discovered that she was a carrier, more than twenty cases of typhoid were traced to her. She was then confined in a sanitarium, but escaped and disappeared. Some time later an epidemic of typhoid broke out in a maternity hospital and the same "Typhoid Mary" was shown to be the cause. The New Jersey State Department of Health has shown evidences that a single carrier was responsible for two outbreaks of typhoid fever. The carrier, a dairy worker, was held responsible for an outbreak at St. Joseph's Villa in the fall of 1921; seventy-two cases were reported. In October, 1922, the same carrier was again found responsible for an outbreak in Newark, N. J. Here thirty cases were reported. Eberthella typhi was isolated from the stools of the carrier at six different times.[1] Numerous attempts

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[1] "Two Typhoid Outbreaks Traceable to the Same Carrier," *Public Health Report*, vol. 37, pp. 2942–2942, Washington, 1922.

have been made to "cure" typhoid carriers, but none has been especially successful.

The incubation period of typhoid fever is usually from 10 to 14, though it may be anywhere from 7 to 21 days. The period of communicability by a typhoid patient starts with the earliest symptoms and lasts throughout the illness. Carriers continue to be a source of infection long after convalescence.

Control of the disease consists in early diagnosis and extreme care that excreta and urine of patients be disinfected and kept from flies. The patient should be isolated and all articles such as drinking glasses, knives, forks, etc. which might be used by others, should be thoroughly sterilized before being again used. The real purpose of sewage treatment and the purification of drinking water is to avoid the transmission of typhoid fever and other intestinal diseases.

The signal success of prophylaxis against typhoid fever, by the use of typhoid bacterin, has been mentioned before. The repetition of a single example will suffice here. During the Spanish-American War, more American soldiers died of typhoid than as a result of Spanish bullets. In the World War, where the conditions of trench warfare made elaborate sanitary precautions impossible, less than one-tenth of 1 per cent of the American Expeditionary Forces became ill with typhoid fever. Compulsory typhoid vaccination has practically wiped out the disease in organized military life.

Smallpox is a disease that is little known today in continental America. It has been responsible for some of the great scourges of the past and there is just enough of it always present to keep sanitarians constantly on guard against epidemics.

The infectious agent of smallpox is unknown but is placed in the common dumping ground—"filterable viruses." The organism exists in the pustules on the skin of the patient, and is spread usually by contact or through the indirect means of articles of clothing, etc. It may also be carried by flies.

The incubation period for smallpox is from 8 to 16 days. The period of communicability is from the time of the earliest symptoms to the disappearance of all scabs.

Because of the high infectivity of the smallpox virus, sanitarians insist on complete isolation of all patients. All persons who have come in contact with a smallpox patient are also quarantined for a period equal to the longest incubation period.

Smallpox is another epidemic disease which has practically been eliminated in civilized countries by prophylactic vaccination. While formerly great pandemics swept the world, today a case is rarely heard of, and its incidence is the occasion of newspaper headlines.

Tuberculosis is a disease in man and other animals caused by an organism known as "Koch's bacillus," or Mycobacterium tuberculosis. It may affect the bones (caries), the lining of the abdominal cavity (tabes mesenterica, or tuberculous peritonitis), the membranes of the brain (tuberculous meningitis), the lymphatic glands (scrofula), and other parts of the body. The most common form, and by far the most dangerous from a public health standpoint, is tuberculosis of the lungs, known also as phthisis, pulmonary tuberculosis, or consumption.

Mycobacterium tuberculosis is the most resistant of the nonsporulative pathogenic bacteria. It can withstand drying and retain its virulence when not exposed to direct sunlight for as long as 6 months. As a person suffering from pulmonary tuberculosis always coughs, it may readily be seen how easily the disease may be disseminated. Inhalation of dry or wet particles of tuberculous sputum and the eating or drinking of infected material are the chief means of infection. Next to this means comes the drinking of milk from tuberculous cows.

The incubation period of the disease is unknown but variable. A tuberculous patient is infectious to others during the entire course of the disease. Extreme care should be taken to sterilize all handkerchiefs, eating utensils, and other objects which are used by the patient.

The open-air treatment for tuberculosis has been found the most satisfactory. Fresh air, day and night, forced feeding, especially milk, and absolute rest are the three essentials of this so-called "rational" treatment. The value of immune therapy, in the use of tuberculins and immune sera, has not been demonstrated. Tuberculins are antigens prepared by various methods of treating the Mycobacterium tuberculosis.

While tuberculosis is curable, especially if diagnosed early and proper treatment taken, the fact that almost 10 per cent of the total deaths in the United States every year are caused by tuberculosis indicates its seriousness as a health problem.

The prevention of tuberculosis depends in the long run on proper education—education of the infected individual in caring for himself and in safeguarding others; education of non-infected individuals as to the proper personal hygiene that is the best preventive of the serious menace; and education of the public in the necessity of safeguarding those who, either through ignorance or poverty, cannot safeguard themselves.

An individual suffering from pulmonary tuberculosis should receive full instructions not only as to the proper care of himself, but on how to avoid disseminating the disease. He should never expectorate except into a handkerchief or a cup which can be burned. He should be taught that every time he coughs without holding a cloth in his mouth he scatters millions of germs into the air, which can set up the same disease in healthy individuals. He should not kiss another person, especially a child, nor use a common drinking cup, a pipe, or any other object which could, after being infected by him, carry the germs of the disease to any other person.

It is estimated that over 90 per cent of all individuals now living have had, at some time, or will have before they die, an infection of tuberculosis. Some authorities claim that every individual on autopsy shows healed or unhealed tuberculosis lesions. This indicates that many individuals suffer from mild attacks without being aware of the fact. It also indicates that cures are effected without the knowledge that the disease has existed. Inasmuch as the proper form of treatment is known, from experience with well-developed cases, it is apparent that the prevention and the cure of incipient cases can be effected by similar means. Unless a person has constitutional defects which cannot be overcome, plenty of fresh air, avoidance of fatigue, and proper eating habits will go a long way toward the prevention of tuberculosis.

From the point of view of public education in social prevention, Professor Williams[1] has outlined a program which is a good summary of the aims of sanitarians who are interested in this problem. His program consists of six parts:

1. Education of the public, and especially the tuberculous, in the nature, course, prevention, and treatment of the disease.
2. Legislation that places tuberculosis on the list of reportable diseases.
3. Improvement of the housing conditions of the poor and of the working conditions in all industries not satisfactory.

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[1] WILLIAMS, J. F., "Personal Hygiene Applied," Philadelphia, 1924.

4. State or municipal legislation and control relating to the milk and food supply; cleanliness of streets, sleeping cars, and public places; enforcement of the ordinances against spitting.
5. Adequate hospital and sanitarium facilities to care for those who have the disease.
6. Prevention of other diseases especially predisposing, such as, in children:
    a. Measles, which is frequently followed by pulmonary tuberculosis, and
    b. Whooping cough, which predisposes to tuberculosis.

Diphtheria is an epidemic disease caused by the "Klebs-Loeffler" bacillus, Corynebacterium diphtheriæ. This organism lives on the mucous membrane of the throat of an infected person. Here it produces a characteristic lesion, a pseudomembranous inflammation. The diphtheria bacillus produces a strong toxin which affects the heart. The disease is spread from one person to another by droplet infection, direct contact, and quite frequently through the indirect means of milk which is infected by a handler who harbors the organisms.

The incubation period of diphtheria is from 2 to 5 days. A patient is liable to communicate the disease to another from the beginning of the symptoms to about 2 weeks after all soreness has disappeared from the throat. In carrier cases the period of communicability may persist as long as 6 months after cure.

Mere sanitation, in the sense of control of the water and milk supply and of living conditions, is of little value in checking the spread of the disease. The poorer classes have usually a higher immunity than the upper classes in a community. The chief dangers are carriers and active cases which have not been diagnosed. Hitchens reports that in throat cultures of students in a military training camp, nearly 1 per cent carried virulent diphtheria organisms.

The first step, therefore, in the eradication of the disease is to culture all persons handling foods—as dairymen, cooks, etc., and all convalescents from the disease. The specificity of the organisms and the ease with which it can be recognized in original smears, or at least after 24 hours' growth on Loeffler's blood-serum mixture, make it possible to identify all persons who might disseminate the disease. Immediate and complete isolation and quarantine of all such cases should follow. Unfortunately, sufficient precaution has not been taken in this regard in the past. Segregation of carriers must mean more than mere exclusion from school, if any really valuable result is to be obtained.

Next, with the introduction of the Schick test and toxin-antitoxin mixture (toxoid), it is possible to discover persons susceptible to the disease and to render them immune by prophylactic vaccination. The Schick test depends upon the principle of toxin-antitoxin combination. A minute amount of pure diphtheria toxin ($\frac{1}{50}$ M.L.D.) is injected intradermally into the forearm of a person to be tested. If that person is immune to diphtheria, his cells will contain diphtheria antitoxin, which will neutralize the toxin injected and no necrosis of the surrounding tissue will take place. On the other hand, if the individual is not immune, the toxin injected will poison the cells in the skin, causing a decided, though localized, reaction, with the ultimate death of the cells involved. Thus a positive Schick test, or a test where a noticeable reaction takes place, indicates that the individual tested is not immune to diphtheria, while a negative Schick indicates that the individual is immune.

If the individual is not immune, he may be injected with diphtheria toxin—to which antitoxin (or formalin) has been added to eliminate the poisoning effect. The toxin stimulates the production of antitoxin in the body of the person thus injected, and thereby confers an active immunity. If known carriers can be eliminated from a community, and all susceptible individuals immunized, it is clear that this disease can be kept down.

Venereal Diseases.—The word "venereal" is derived from "Venus," the name of the Roman goddess of love. Venereal diseases are diseases propagated by sexual intercourse. They are likewise diseases which ordinarily or primarily attack the sex organs. The list of such diseases is small—they are only three in number—but they make up in virulence, distribution, and far-reaching effects what they lack in numbers. Laurence Marcus[1] estimated that of the 10,000,000 males in this country between twenty and thirty years of age, approximately 3,000,000 are infected with a venereal disease. The three diseases—gonorrhea, syphilis, and chancroid—are all bacterial diseases, and each is caused by a separate and distinct bacterium.

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[1] MARCUS, LAURENCE, "Social Hygiene," vol. 7, pp. 441–456, New York, 1921.

Though it is possible to contract the diseases in other and innocent ways, the mode of transmission is almost always through sexual intercourse.

Gonorrhea affects the membrane lining the urethra, or the inner lining of the penis, in men, and the vagina in women. During sexual intercourse, a few, or many, of the germs Neisseria gonorrhœæ are implanted in the mucous membrane. These multiply and set up an inflammation. It takes several days, usually 3 to 5, for this inflammation to become severe enough to be noticeable. In rare cases, the disease may manifest itself 1 day after exposure, and sometimes not for 8 days after contact.

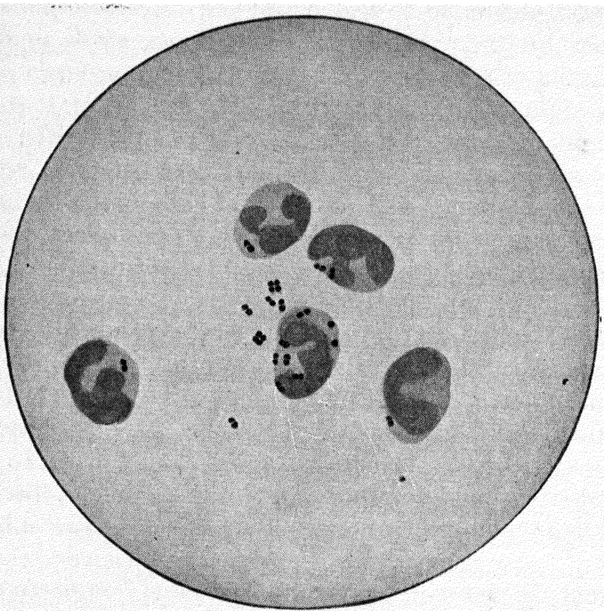

FIG. 36.—The germ of gonorrhea. Pus cells are also shown. (*Bull and Thomas, J. B. Lippincott Company.*)

The inflammation tends to spread back along the mucous membrane, and, unless checked by prompt and vigorous treatment, may extend to the bladder or even, though rarely, to the kidneys. It may also, in the case of men, spread along the vas deferens, causing a very painful inflammation of the testicle, epididymitis. In women the gonococci may spread from the vagina through the uterus into the Fallopian tubes causing so-called "pus tubes." This is one of the most frequent causes of lower abdominal operations in women, and is quite frequently fatal. It is also possible for the infection to get into the blood and be carried to distant parts of the body. When it gets into the blood it may cause two very serious conditions—gonorrheal heart disease, and gonorrheal arthritis. Gonorrheal heart disease is frequently the cause of death. Gonorrheal arthritis, although rarely fatal, is disabling and hard to cure. The effects of neither can ever be entirely eradicated and will always be a serious handicap to the individual even after the active disease has been cured.

Another condition caused by the gonococcus is gonorrheal inflammation of the eyes. Neisseria gonnorrhœæ has a particular affinity for the mucous membrane of the eye and sets up an inflammation which, if left untreated for as much as 4 days, will cause total blindness. This is one of the greatest causes of blindness in children. It is estimated that at least 25 per cent of the cases in institutions for blind children are due to gonorrhea. In children, the disease is practically always contracted from the mother at the time of birth. This condition is so serious that physicians in practically every state are required by law to give special treatment to every child whose birth they attend, in order to prevent its occurrence. Cases of gonorrheal inflammation of the eyes in adults are contracted through the carelessness of someone who has the disease. Infective material is carried from the sex organs to the eyes, either by the hands, towels, handkerchiefs, or some other article. The individual who has a case of gonorrhea is a very serious menace to the group or community in which he lives, unless he is extremely careful.

Syphilis, another of the venereal diseases, is one of the old scourges of the human race. It is a most insidious and loathsome disease. It is caused by Treponema pallidum, an organism which is hard to propagate or keep alive outside of the human body. Practically every case of syphilis is, therefore, contracted directly from some other case. The majority of cases are gotten by intercourse, but there are probably more accidental or innocent infections than in either of the other venereal diseases.

The incubation period in syphilis is usually about 3 weeks, though it may extend over a considerably longer interval. The first indication of syphilis is usually a hard sore located either in or near the genitals. The sore may appear on other parts of the body, as on the lip, when it is contracted by kissing a syphilitic individual. Usually there is only one such sore and the sore, even if not treated, may disappear after some time. There are three stages to the disease. The sore just mentioned is considered the first stage, and is frequently referred to as the initial lesion. The secondary stage generally appears within a few weeks to a few months after the appearance of the initial lesion. This secondary stage usually takes the form of skin eruptions, sore throat, and various general symptoms. If the case is treated even moderately, these secondary symptoms usually disappear in a few months. The tertiary symptoms may appear within a few months after the secondary, or they may be delayed for many years, even to as much as 20 or 25 years. These tertiary symptoms usually take the form of nervous disorders, the most prominent being locomotor ataxia and cerebrospinal syphilis. Syphilis is the greatest single agent which fills institutions for the insane.

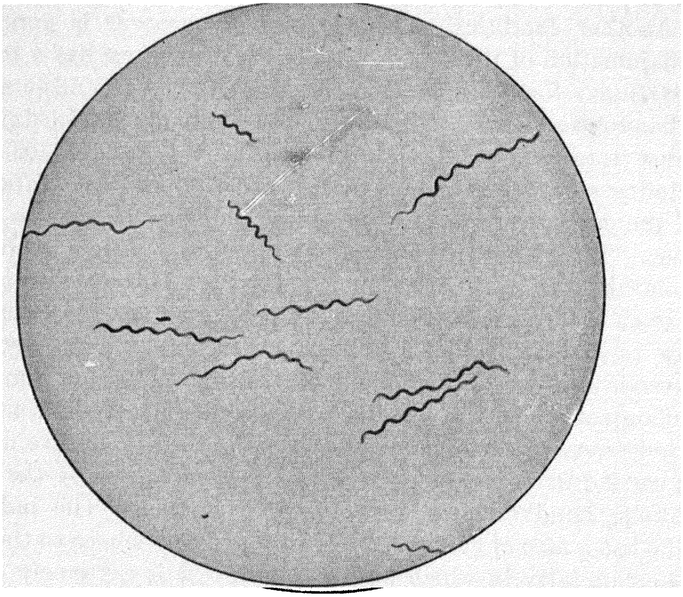

FIG. 37.—The germ of syphilis. (*Bull and Thomas, J. B. Lippincott Company.*)

Of the three venereal diseases, syphilis is possibly the greatest menace, considered from the standpoint of the innocent infections. It is possible for syphilitic parents to have children; and these children, if they manage to survive, usually are very seriously handicapped through life. Many cases of mentally deficient children are due to syphilis acquired at or before birth from syphilitic parents. Adults infrequently acquire syphilis innocently. Surgeons, nurses, and hospital attendants have been known to acquire this disease from patients in their care. It is also possible to pass syphilis on from one person to another by means of drinking cups, pipes, etc.

Patients in the first or second stage of syphilis are frequently able to continue with their occupation. This fact makes them a very serious menace to those with whom they may be working. In the tertiary stage, fortunately, the disease is not usually contagious, because there are seldom any open lesions from which the infective material can be disseminated. Also, cases of tertiary syphilis are ordinarily unable to continue with their work, and are confined, either to their homes or to an institution.

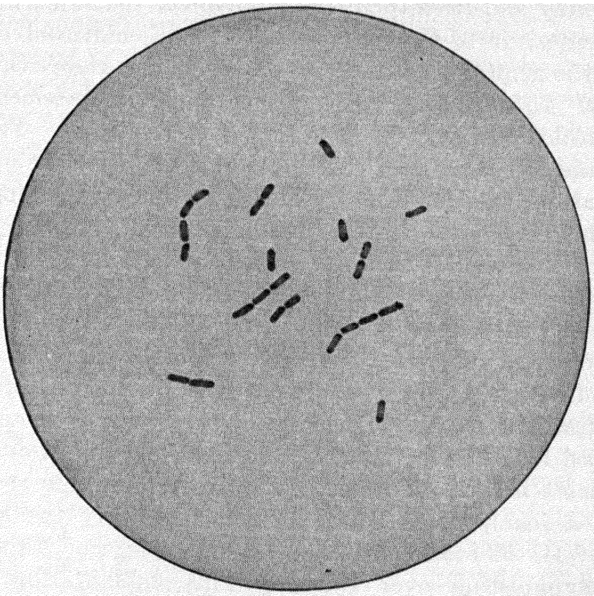

FIG. 38.—The germ of chancroid. (*Bull and Thomas, J. B. Lippincott Company.*)

Chancroid, the third venereal disease, is not as widespread as either of the others. Moreover, it does not have the far-reaching effects of the other diseases. It is caused by Hemophilus ducreyii, or the so-called bacillus of Ducrey, and is almost invariably contracted through sexual intercourse.

It appears sooner after intercourse than the other diseases, making its appearance usually within 5 days, and practically never later than 10 days, after exposure.

It appears as an ulcer, somewhat resembling at times the initial lesion of syphilis, but the lesion is a soft sore instead of the typically hard sore or hard chancre of syphilis. This leads to the name frequently given it of "soft chancre." Also, whereas the initial lesion or chancre of syphilis is usually single, the ulcer of chancroid almost always tends to be multiple. The ulcers grow rapidly and from their discharge infect the adjacent areas; consequently, it is not infrequent to find chancroid existing all over the genital organs, up on the abdomen, and back on the buttocks. In some cases in which treatment has been neglected, chancroidal ulcers may be found all over the body. The very nature and appearance of the ulcers generally lead to an early treatment of the condition, which consequently seldom gets such a hold upon the system that it becomes truly a systemic disease. Chancroid yields to treatment and can be readily cured. In this respect it is quite different from the other venereal diseases and is the least dangerous of the three.

The venereal diseases have been definitely recognized since the Middle Ages, and from the writings of men like Hippocrates and Galen it is apparent that these diseases existed and were recognized long before the Christian Era. In the Middle Ages the diseases became so widespread that at one time they constituted a real plague. In the fifteenth century the army of Charles VIII of France returned from the siege of Naples and spread syphilis all over Europe. This implantation of the disease continued to be felt for at least two centuries.

A fair estimate of the amount of venereal disease today in our male population can be gained from some of the draft figures. Colonel P. M. Ashburn of the Army Medical Corps made an intensive study of the venereal situation during the World War and has presented a number of statistics, a few of which are of interest. During the month of August, 1917, approximately two hundred thousand white men were inducted into the service; of these 3.22 per cent had venereal disease. During the same month, about eighty thousand Negroes were inducted into the service, and 20.87 per cent showed venereal disease. This gives some idea as to the prevalence of venereal disease in the population of this country, both white and colored.

It is believed that the general venereal situation will become very much better in the course of the next generation. One of the reasons for this is the education of the public. In the past the existence of venereal disease has always been covered up and has never been a matter for general knowledge and discussion. The subject is now being brought into the open. The venereal problem is finding expression in the laws placed on the statute books of the various states and is being openly discussed in many civic organizations. Women's clubs have taken up the matter and have lecture courses on venereal disease and its control, in which the subject is discussed freely and openly and without any attempt at disguising or concealing the true state of affairs. This does not mean that people are considering venereal disease any less loathsome and vile, but rather that they recognize that the venereal situation is a real problem, and desire to learn the facts and to take steps to improve conditions.

Malaria.—This is a disease caused by several species of protozoa of the genus Plasmodium. The organism lives in the blood stream of the diseased individual and in the body of the mosquito. It is transmitted by a single species of mosquito.

The incubation period of the disease varies with the infecting organism, but is usually about 14 days. The control of the disease is essentially an engineering problem, to be solved by the eradication of the mosquito. Other factors are the protection of individuals against the mosquitoes by the proper screening of sleeping quarters, the segregation of malarial victims so that non-infected mosquitoes cannot become infected and thus transmit the disease, and the curing of infected individuals for the same reason.

Sanitation as a science is demanding the attention of all educated people. It is not confined to a community, state, or nation, as it is realized that, for the eradication of disease, all foci of infection must be wiped out. In a plea for an international health service, the late Professor Whipple[1] indicated three main channels into which world sanitation must be directed: (1) the safeguarding of home life, (2) the safeguarding of industrial life, and (3) the prevention of transmission of disease.

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[1] WHIPPLE, "World Sanitation: A Twentieth Century Possibility," *International Journal of Public Health*, vol. 1, p. 38, Geneva, 1920.

CHAPTER XVII

BACTERIA IN INDUSTRY

Industrial bacteriology as a science may be said to have started in 1857 when Louis Pasteur first explained the cause of fermentation. Prior to this, various industrial processes, such as the manufacture of wines, vinegar, indigo, etc., were carried out in an empirical manner, without any knowledge of why certain changes took place or of the causative agents involved. Through the present knowledge of bacteriology it is now possible to control these processes, assure the proper conditions for the greatest yield, and, by means of pure cultures of the bacteria involved, avoid the production of impurities during manufacture.

The industrial processes in which bacteria are employed in the manufacture of foods, such as cheese, butter, etc., were described in the chapter of Bacteria in Foods. In this chapter will be described a few of the other industrial processes in which bacteria play a part.

Industrial Alcohol.—In the discussion on the fermentation of the various fruits and grains for wines, liquors, etc., it was pointed out that yeasts were responsible for the conversion of certain sugars into alcohol. In these cases, certain other substances were also considered essential to give each finished product its characteristic taste and aroma. Because of its chemical and physical properties and especially because of its great value as a solvent, however, the manufacture of ethyl alcohol on a commercial scale is a large industry.

The process always depends on the fermentation of dextrose or other hexose, with alcohol and carbon dioxide as the end products. The formula: $C_6H_{12}O_6 = 2CH_3CH_2OH + 2CO_2$ would apparently indicate the reaction. Yet when we consider the structural symbol for a hexose and the nature of the intermediate products formed it may be seen that the reaction is not so simple. The following equations, known as the Newberg reactions will show this:

$$C_6H_{12}O_6 = 2CH_3{\cdot}CO{\cdot}CHO \text{ (methyl glyoxal)} + 2H_2O$$

$$CH_3{\cdot}CO{\cdot}CHO + H_2O = CH_3{\cdot}C(OH)_2{\cdot}CHO \text{ (hydrated methyloxal)}$$

$$\begin{array}{lcl} CH_3{\cdot}C(OH)_2{\cdot}CHO & H_2 & CH_2OH{\cdot}CHOH{\cdot}CH_2OH \text{ (glycerol)} \\ & + \;|\; = & \\ CH_3{\cdot}C(OH)_2{\cdot}CHO & O & CH_3C(OH)_2{\cdot}COOH \text{ (hydrated pyruvic acid)} \end{array}$$

$$CH_3C(OH)_2{\cdot}COOH = CH_3CO{\cdot}COOH \text{ (pyruvic acid)} + H_2O$$

$$CH_3CO{\cdot}COOH = CH_3CHO \text{ (acetaldehyde)} + CO_2$$

$$\begin{array}{lcl} CH_3{\cdot}CHO & H_2 & CH_3{\cdot}CH_2OH \text{ (ethyl alcohol)} \\ \text{(acetaldehyde)} + & | \; = & \\ CH_3{\cdot}CO{\cdot}CHO & O & CH_3CO.COOH \text{ (pyruvic acid)} \\ \text{(methyl glyoxal)} & & \end{array}$$

In order to manufacture alcohol profitably for industrial purposes, the sugar source cannot have a commercial value greater than alcohol and its cost of manufacture. For example, cane sugar is too valuable to candy manufacturers to warrant its use in making alcohol. Sugar-beet molasses, inferior grain crops, potatoes, sawdust, and other waste forms of cellulose furnish cheap sugar sources.

When the alcohol is obtained from a direct sugar source like molasses, the raw material is diluted with water and placed into fermenting vats. The fermentation is carried on by means of a yeast, Saccharomyces cerevisiæ.

Various starchy substances, like potato, corn, rice, grain, etc. are first converted into dextrose by enzyme action and the addition of water. The raw materials are thoroughly boiled or cooked under a high steam pressure and then malt is added to furnish the hydrolytic enzymes. The reactions which take place are as follows:

$$2(C_6H_{10}O_5)n + 2nH_2O = 2nC_{12}H_{22}O_{11}$$

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6.$$

In the amylo process of starch inversion, the molecular change is brought about by the use of a mold, Amylomyces rouxii. A corn mash is inoculated with the organism, and when the dextrose formed is at the maximum, a yeast is added which converts it into alcohol.

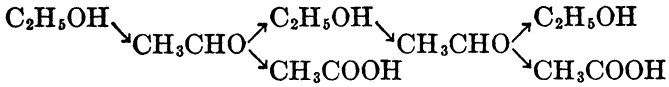
Cellulose, as sawdust, is hydrolyzed into hexose by digestion under steam pressure, with hydrochloric or sulphuric acid.

In every process where sugar is not the direct source, a hexose is obtained before the yeast is added. The process now continues as in the direct sugar fermentation, the hexose molecule being ultimately converted into alcohol by means of the yeast. The solution of alcohol obtained is distilled and a product containing 95 per cent alcohol in water is readily obtained. To remove the last traces of water and obtain absolute alcohol, it is necessary to distill the alcohol repeatedly in the presence of some dehydrating agent like calcium oxide.

Acetic Acid.—The formation of vinegar from wines depends on the chemical change of the alcohol of the wine into acetic acid:

$$C_2H_5OH + O_2 = CH_3COOH + H_2O.$$

This change is brought about by the enzymes of a bacterium, Acetobacter acetus. The "Cannizzarore action" between two aldehyde molecules, in which one is reduced and the other oxidized, takes place,



$$\begin{array}{lllll} C_2H_5OH\searrow & & \nearrow C_2H_5OH\searrow & & \nearrow C_2H_5OH \\ & CH_3CHO & & CH_3CHO & \\ & & \searrow CH_3COOH & & \searrow CH_3COOH \end{array}$$

Vinegar is made from a number of different substances. Wines are common sources, as also is cider (apple juice). Other fruits, such as pears, peaches, etc., and sugar-containing substances like honey, molasses, and maple syrup are sometimes used. In every case, alcohol is first formed. The acetic-acid fermentation starts with alcohol as the raw material.

On a commercial scale, vinegar is usually manufactured by what is known as the German, or "quick," process. A dilute solution of alcohol in water is passed through a cylinder containing wood shavings which have been inoculated with Acetobacter acetus. As the organisms are obligate aërobes, air is forced upward through the cylinder. As can be seen from the formula, the process is one of oxidation.

The domestic method, known as the "Orleans process," is to fill a vat with a weak wine and vinegar. The bacteria in the vinegar gradually convert the alcohol into acetic acid. The acid content cannot go above 14 per cent, as the Acetobacter cannot exist in a greater acidity.

Mother of vinegar is a zoöglœal mass of Acetobacter acetus, or other species of the same genus, which secrete a gelatinous material holding the cells together. Sometimes a small nematode worm, called "vinegar eel" may be present in vinegar. It plays no part in the formation of vinegar, and is not detrimental to the product.

A British patent was issued in 1918 for the production of acetic acid by cellulose fermentation. A mass of cellulose material, such as straw, is inoculated with fermenting vegetable matter, *e.g.*, stable manure, and maintained under aërobic conditions at a temperature between 25° and 60°C. The cellulose undergoes acetic fermentation. Calcium carbonate or some other suitable substance is added to neutralize the acid as it is formed.

Acetic acid is also manufactured commercially by purely chemical means. Wood shavings or sawdust are distilled dry in iron retorts at a low temperature. A liquid is obtained which, when treated with calcium oxide, yields calcium acetate. The calcium acetate is treated with concentrated hydrochloric acid to liberate acetic acid. By treatment with potassium permanganate to oxidize impurities, plus distillation, a relatively pure commercial product is obtained.

Glycerine.—Glycerine or glycerol ($CH_2OH{\cdot}CHOH{\cdot}CH_2OH$) is a by-product in the manufacture of soap, which is simply a salt of a fatty acid. Thus, when stearin is heated with sodium hydroxide, the fat is decomposed into the sodium salt of the fatty acid (stearic) and glycerine.

Glycerine may also be manufactured by bacteriological means in fermenting molasses. To molasses, in which the sugar content is 17 to 20 per cent, is added a culture of Saccharomyces ellipsoideus, and sufficient sodium carbonate to neutralize. The material is incubated at 30 to 32°C. About 20 to 25 per cent of the sugar is converted into

$$(C_6H_{12}O_6 \rightarrow \underset{\text{acetaldehyde}}{CH_3CHO} + CH_2OH{\cdot}CHOH{\cdot}CH_2OH + CO_2)$$

glycerine, and practically all the remainder into alcohol and carbon dioxide.[1] The addition of sodium sulphide exerts a very favorable influence in the production of glycerine and decreases the amount of alcohol. The reaction is as follows:

$$C_6H_{12}O_6 + Na_2SO_3 + H_2O = C_3H_8O_3 + CH_3CHO{\cdot}NaHSO_3 + NaHCO_3$$

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[1] King, A. R., "The Production of Glycerol from Molasses," *Journal Society Chemical Engineers*, review, vol. 38, pp. 175–177, 1919.

During the war, Germany produced more than 1,000 tons of glycerol by bacteriological means.

Citric Acid.—Citric acid ($CH_2COOH{\cdot}C(OH)COOH{\cdot}CH_2COOH$) is usually made from the juices of "runts" in the lemon packing industry. The juices are extracted from the pulp of the peeled lemons by spontaneous fermentation. The extracted juice, which contains citric acid, is neutralized with calcium carbonate and the resultant calcium citrate collected. Citric acid can be made artificially from sugar solutions by a fermentation process. A mold, Penicillium citrinum, is the active agent. Giltner[1] states that ". . . as high as 4 per cent citric acid may be produced from sugar solutions by the action of these molds."

Lactic Acid.—Lactic acid ($CH_3CHOH{\cdot}COOH$) is an organic acid formed by the fermentation of sugar of milk. Its formation in food products has been discussed in the chapter on Bacteria in Foods. The following equation represents the reaction:

$$C_6H_{12}O_6 = 2CH_3CHOH{\cdot}COOH.$$

Among the earliest methods of obtaining lactic acid on a commercial scale was that proposed by Beusch:[2]

> . . . 6 pounds of cane sugar and ½ ounce of tartaric acid (which serves to convert the cane sugar into dextrose) are dissolved in 26 pounds of boiling water. After 2 days, 2 pounds of chalk are added, together with 4 ounces of putrid cheese suspended in 8 pounds of sour milk. The mixture is then set aside at a temperature between 30 and 35°C. and well stirred every day until, in the course of 6 or 8 days, it is converted into a thick paste of calcium lactate. This paste is boiled for ½ hour with ½ ounce of quicklime and 20 pounds of water, the solution strained through cheesecloth and evaporated to a syrup. The crystalline mass which first forms is pressed by itself, then three or four times after having been each time stirred up with ⅒ part of cold water, and the lactate of calcium thus purified is dissolved in twice its weight of boiling water. To 32 parts of the solution of the calcium salt is added a mixture of 7 parts of sulphuric acid and 7 parts of water. The lactic acid thus formed is strained, while still hot, through linen, to separate it from the sulphate of lime, and boiled with 1⅜ parts of zinc carbonate for 15 minutes. Lastly, 1 part of the zinc salt is dissolved in 7½ parts of boiling water and treated with sulphuretted hydrogen until zinc sulphide ceases to precipitate. The filtrate, containing the pure lactic acid, is evaporated on the water bath.

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[1] GILTNER, W., "General Microbiology," p. 264, Philadelphia, 1928.

[2] BEUSCH, *Annales Chemie Pharm.*, vol. 61, p. 174.

Butanol and Acetone.—Butyl alcohol (butanol) ($CH_3CH_2CH_2CH_2OH$) and acetone ($CH_3CO{\cdot}CH_3$) production by a bacteriological process has become a large industry since the war. In fact, one of the largest whisky distilleries in the United States, located at Terre Haute, Ind., is now being used for this purpose. The organism used is a spore-forming anaërobic bacterium isolated from corn by Dr. Charles Weizman. It resembles very closely Clostridium welchii, the organism causing gas gangrene. The fermenting process is carried out in large vats containing a fairly concentrated mash of corn (1:15). Active fermentation starts in about 7 hours and continues vigorously for about 36 hours. It then falls off rapidly and stops in about 48 hours. The end products of fermentation are butyl alcohol, acetone, ethyl alcohol, and carbon dioxide, with some lactic, butyric, and acetic acids. The intermediate steps in the process are: (1)the preparation and hydrolization of the mash, in which the starch in the corn is hydrolized to sugar; (2) the fermentation, in which the sugar is converted into butyric ($CH_3(CH_2)_2COOH$), acetic (CH_3COOH), and lactic ($CH_3CHOHCOOH$) acids, and later reduced to the alcohols (as $CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}COOH \rightarrow CH_3CH_2CH_2CH_2OH$) and acetone; (3) the fractional distillation of the fermented material with the recovery of the desired end products. Approximately 5½ pounds of butyl alcohol, 3 pounds of acetone, and 1½ pounds of ethyl alcohol are obtained from a bushel of corn.

The ketone, acetone, was formerly produced by dry distillation of calcium acetate.

Solvents used in the manufacture of rubber goods, pyroxylin, varnishes, photographic films, artificial silk, leathers, perfumes, etc. are made from butyl alcohol and its derivatives.[1]

Gases.—The collection of by-product gases in the fermentation industries is proving to be of considerable value. Hydrogen, and carbon dioxide are given off in the manufacture of butanol. In the industrial-alcohol plant, carbon dioxide is a by-product. Both of these gases have a high commercial value. Besides its use in filling balloons, hydrogen is employed as a fuel when

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[1] Hall, H. S., "Solvents Produced by Bacteria," *Chemical Age*, March, 1921; Speakman, H. B., "The Production of Acetone and Butyl Alcohol by a Bacteriological Process," *Journal Society Chemical Industry*, vol. 38, pp. 155–161, 1919; Nathan, F., "The Manufacture of Acetone," *Journal Society Chemical Industry*, vol. 38, p. 271, 1919; Killefer, D. H., "Butanol and Acetone from Corn," *Industrial and Engineering Chemistry*, vol. 19, p. 46, 1927.

extremely high temperatures are necessary, as in the working of platinum. Carbon dioxide is used in making "soda water" and for securing low temperatures. It is also used as a fire extinguisher.

Leather Manufacture.—The various processes by which the hides or skins of animals are treated in the manufacture of leather are known as salting, soaking, fleshing, unhairing, bating, drenching, tanning, and finishing. In all of these procedures, except tanning and finishing, bacteria play a part, either harmful or beneficial.

In the preliminary process of salting, bacterial action is responsible for so-called "salt stains"[1] a common defect which greatly lowers the market value of the leather. This may be overcome by the use of some material like sodium carbonate, sodium fluoride, or zinc chloride, which will check bacterial growth.

The purpose of soaking and fleshing of skins is to remove the layer of fat and flesh next to the skin. Here again defects which show in the finished leather may be traced, to some extent at least, to bacteria. Proctor[2] has suggested the use of sulphurous acid in the soak water to assist in the absorption of water by the skins, and check bacterial growth.[3]

In unhairing, the skins, after being freed from adipose tissue, are treated by different methods for the removal of the hair and epidermal layers from the true skin. In one of these, sweating, the skins are hung in a warm, humid room until the hair slips easily. During this state, bacterial action may cause serious loss, although, as a rule, its effect may be considered as an aid to the process. Skins to be made into softer leathers are limed rather than sweated. Liming consists of exposing the skins to a solution of calcium hydroxide for from 1 to 3 weeks. This dissolves the outer layer of epidermis and loosens the hair, at the same time separating the fibers of the lower layer or corium. Wood and Law[4] regard the growth of bacteria as the

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[1] Paessler, J., "Salting of Hides and Skins," *Ledertech. Rundschau*, p. 137, 1912.

[2] Proctor, "Principles of Leather Manufacture," 2nd ed., p. 161, 1912.

[3] The danger of transmission of anthrax to persons employed in the manufacture of leather has led to the precaution of adding a disinfectant like mercuric chloride to the soak. This kills any Bacillus anthracis that may have been present on the skins.

[4] Wood, J. T., and D. J. Law, "Light Leather Liming Control," *Collegium*, p. 121, 1912.

principal factor in unhairing by the liming process. The action is due to saprogenic organisms through the production of proteolytic enzymes.

Bating is a very old process for preparing unhaired skins for tanning. Probably the greatest value of bating lies in the fact that during this process the fibers of the skin are opened so that later tannin can be thoroughly impregnated into the skin. This particular phase of the process is called "plumping." While artificial bating is now employed to a large extent, the procedure originally was to keep the skins in a warm infusion of dog or fowl dung. Here bacteria are instrumental in removing lime from the skins and thereby adjusting the reaction. Certain proteolytic, amylolytic, and steatolytic enzymes produced by the bacteria improve the skins during the bating process.

After bating, some skins are submitted to the process of drenching. This process is carried out just before tanning, and consists of immersing the skins in a bran mash and allowing the mixture to ferment. This is entirely a bacteriological procedure. The organisms convert the carbohydrates in the bran into various organic acids, such as formic, acetic, butyric, and lactic, with the liberation of gases.

Sometimes the fermentation may not proceed in the usual manner and the liquor, instead of becoming acid, turns slightly alkaline, frequently becoming bluish black, due to the presence of chromogenic bacteria. Under these conditions the skin is rapidly attacked by proteolytic organisms, but may be saved if transferred in time to a solution of acid and salt.[1]

Tanning is a process whereby the proteins of the skins are combined with tannin, obtained from the barks of trees, etc., to form leather. The process is purely chemical, and bacteria play no part, except, occasionally, a harmful one, due to proteolytic action.

Various methods of finishing are employed, depending upon the type of leather desired. Chamoising, currying, dressing, scouring, whitening, waxing, sizing, etc. are terms applied to different operations, all of which are physical.

Rubber.—The proper control of the factors influencing spontaneous coagulation of the juice of the rubber tree (Hevea

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[1] WILSON, JOHN A., "The Chemistry of Leather Manufacture," p. 200, New York, 1923.

brasiliensis), from which rubber is prepared, is one of the oldest procedures known in connection with the making of rubber. Denier and Vernet[1] have studied the bacteria found in the latex, and have isolated one which is commonly present and appears to aid proper coagulation.

Acid Phosphate.—The use of bacteria in the manufacture of acid phosphate (monocalcium phosphate), a fertilizer, was first suggested by Lipman in 1914. A mixture of sulphur and rock phosphate is seeded with a sulphur-oxidizing organism and the mass incubated for about 15 weeks. By the end of this time, the sulphur has been oxidized, and about 50 per cent of the phosphate is rendered soluble.

Sizing Cotton.—In the process of sizing cotton and other fabrics, a large amount of starch is added. Starch is also present in appreciable amounts in the internal layer of the fiber itself. The getting rid of this starch, or desizing, which was previously done by boiling the cotton in an alkali solution, can be accomplished by means of a diastatic enzyme, such as is produced by Aspergillus oryzæ.[2]

Flax, Hemp, Jute.—These are vegetable fibers used for making twine, cord, and rope. The industry is very important in all parts of the world. Before the World War, most of the flax was grown in Russia, but the finest finished products come from Belgium. Courtrai, on the river Lys, produces the highest grade. The bacteriologist is interested in that part of the process of producing rope known as "retting." This is the first step in the separation of the fiber from the stems of the flax, hemp, and jute plants. The process is one of fermentation or the action of bacteria in dissolving a resin, which binds the fiber together and to the woody part of the stem. The bacteria used are found naturally in the plant, and all that is necessary is the proper control of the temperature of the water used and the time taken in fermentation.

Natural retting is of two varieties. Dew retting is accomplished by spreading the plant stems out on the ground for from 4 to 6 months. River retting or water retting is the placing

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[1] Denier and Vernet, "Etude Bacteriologique de la coagulation Naturelle du latex de l'Hevea brasiliensis," *Comptes rendus Academie de Sciences*, pp. 123, 126, 165, Paris, 1917.

[2] Waksman, S. A., "The Industrial Application of Enzymes of Aspergillus oryzæ," *Abstracts of Bacteriology*, authors' abstract, vol. 4, p. 7, 1920.

of the stems in still water and allowing them to soak for about 2 weeks. Several patents have been taken out, for example the Legrand patents, for special apparatuses for controlling the retting.

In the Rossi process, the flax is boiled for 40 minutes and then treated with a pure culture of Bacillus comesii, a member of the butyric-acid group, for about 40 hours at 30°C. The retting vats are aërated from below.[1]

Tea is known on the market under various names indicating its source, such as India tea, Ceylon tea, Pekoe, etc. It is produced from the leaves of plants of the genus Thea. There are two main classes of tea—green tea, in which no fermentation takes place, and black tea, in which the leaves are dried slowly. In the former, the original color of the tea leaves is preserved by destroying the ferment by quick drying, while in the latter, the fermentation is taken advantage of.

Fowler[2] describes the processes which are involved in the manufacture of Indian black tea.

1. *Withering.*—Withering of the leaf, which consists of exposure to the sun on fine basket-work trays.
2. *Rolling.*—Rolling by machine, which has the effect of pressing out a certain amount of the juice of the leaves. The soft leaves are often made into balls which are used to absorb the juice.
3. *Fermentation.*—These balls are broken up and allowed to ferment and then spread out to dry in the sun.
4. *Firing.*—This takes place in a chest of shallow firing drawers, the bottoms of which are made of fine wire gauze.
5. *Sorting.*—In this process, various qualities of leaf are sorted by sieving, etc.

Dr. Mann's researches have been concerned primarily with the changes going on during the fermentation and withering processes, and the relation of these to the quality of the tea.

The quality of tea appears to depend on the following factors:

a. The flavor, caused principally by an essential oil.

b. Pungency, caused in greatest measure by the unfermented tannin.

c. Color of liquor, caused chiefly by the fermented tannin.

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[1] LOESER, R., *Journal Society Chemical Industry*, vol. 38, p. 407A, London.

[2] FOWLER, G. J., "Bacteriological and Enzyme Chemistry," New York, 1911.

d. Body of liquor, measured principally by the total soluble matter, of which a large part is tannin both fermented and unfermented.

It was found that the fermentation is the result of the action of an enzyme present in the tea leaf. The presence of bacteria during the fermentation process is distinctly injurious, rendering the tea sour and unfit for consumption. In order to prevent deleterious changes of this sort, it is necessary that the fermentation should be carried on under aseptic conditions, that is, scrupulous cleanliness must be maintained throughout the process. The use of antiseptics is injurious to the natural enzyme as well as to the microörganisms. If the temperature also is kept at about 80°F., the change is found to be mainly enzymic. The chemical change which takes place during fermentation consists essentially in an oxidation of the tannin. It has been found, indeed, that there are two enzymes present. The chief one is an oxidase, causing the darkening of tea juice. The flavor improves in proportion to the amount of enzyme in the leaf. It would appear that in the tea leaf the tannin is combined with sugar; during fermentation, this compound is split up and the tannin is oxidized to brown products. This oxidized tannin combines with other substances in the leaf, forming compounds, some of which are insoluble in water. There is, therefore, a decrease in soluble tannin. It is possible for this to go too far and the pungency of the tea to be injuriously affected.

The enzyme increases during the withering of the leaf, and one of the most important results of Dr. Mann's investigations is the possibility of the exact control of the withering process. The object of withering is twofold—to soften the leaf in preparation for rolling, and to produce the greatest amount of enzyme. Under normal conditions these two changes are practically simultaneous, but in very dry weather the leaf may be physically ready to roll before sufficient enzyme is developed; and, on the other hand, in very wet weather the leaf may be chemically ready for rolling before it is properly withered. It may be possible, therefore, to control the time of withering, either retarding it by heaping up the leaves, or quickening it, *e.g.*, by means of fans, and so obtaining the necessary conditions for the production of the best tea.

It is of further interest that the amount of enzyme in the leaf has been shown to depend on the percentage of phosphoric acid used in manuring the plants; further, much more enzyme is present in leaves plucked at 6:30 a.m. than at 6 p.m., which supports the suggestion made below with regard to the indigo plant.

Coffee.—The coffee bean is also subjected to a "spontaneous" fermentation to remove the extraneous fibrous matter surrounding the "bean" proper.

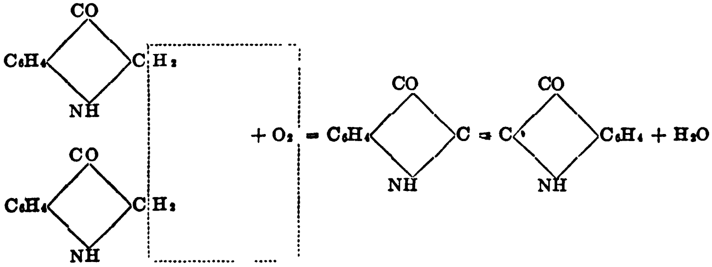
Cocoa.—Cocoa is prepared from the seed of several tropical trees of the genus Theobroma. The seeds are found in a pod about 10 inches long and number from fifty to one hundred. The seeds are bitter and indigestible when collected, but are "cured" by placing them in earthen jars, or in heaps covered with clay, or in large boxes. An aërobic fermentation takes place which destroys the surrounding pulp. The beans are then roasted and prepared for the market.

Indigo is a dye derived from a substance which occurs in nature in various plants. Until recently, the dye had been prepared for use by a fermentation process. Indigo occurs in the plants in the form of indican, a glucoside, which, upon exposure to a suitable ferment such as is furnished by Bacillus indigogenus, is oxidized into indigotin, an insoluble blue dye.

In practice, the indigo plant is cut down and placed in bundles in a vat. The vat is filled with water and the plants allowed to steep for about 12 hours at 25°C., when the extract or tea, known as indigo white, is run into fresh vats and exposed thoroughly to the air by violent stirring. The indican is thus oxidized and the indigotin precipitates as a "mud." This is collected and dried. For commerce it is usually cut into small cubes.

According to Fowler, the indican is fermented during the steeping process into indoxyl and glucose. In the subsequent exposure to air, the indoxyl is oxidized to indigotin.

$$C_{14}H_{17}O_6N + H_2O = C_8H_7ON + C_6H_{12}O_6.$$



Indoxyl + Oxygen = Indigotin (or indigo) + water (2 molecules).

Commercially, most indigo is now prepared from napthalene, a coal-tar product.

Tobacco.—In the preparation of tobacco, two distinct processes are involved, both of which depend to some extent upon the action of microörganisms. After the leaves of the tobacco plant are cut, they are bound into sheaths and hung in special rooms where the temperature, moisture, and ventilation can be carefully controlled. This process is known as "curing," and takes from 4 to 6 weeks. The changes which take place in the leaves are the same as those which ordinarily are known as "wilting," in other leaves. David Ellis[1] describes these changes as: (1) a change of the starch in the leaves to sugar (inversion); (2) a partial disappearance of the sugar; (3) a decomposition of the protein matter, with formation of amino acids; (4) a decrease of fatty matter and of tannin.

The second process, known as "sweating," is purely a fermentation process. The leaves are loosely packed and moistened. The temperature of the pack is maintained below 50°C. by changing the position of the leaves and admitting air. The fermentation consists of oxidation of the tannin and volatile alkaloids, such as nicotin. Just what causes these changes to take place is not definitely known. Some authorities claim that the enzymes which cause the sweating are normally in the tobacco leaves. On the other hand, certain bacteria, like Bacillus subtilis and Bacillus mycoides, are always present during sweating.

Sugar.—In the extraction and purification of sugar, microörganisms play a destructive rôle. It is estimated that the annual loss to the sugar industry through the deterioration, or inversion, of the saccharose runs into millions of dollars. This inversion, which is the change of dextro-rotary cane sugar into levorotary invert sugar, is carried out by molds, yeasts, and in some cases, bacteria.

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[1] Ellis, David, "Outlines of Bacteriology," New York, 1909.

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