GENERAL MICROBIOLOGY

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Preface

This edition is a rather complete revision of *General Bacteriology* first written by the late Professor D. B. Swingle in 1940 and revised in 1947. The new title *General Microbiology* has been adopted to indicate a broader scope of the field. However, we have still followed Professor Swingle's basic philosophy of presenting this phase of biology in such a manner as to encourage a beginning student to relate his daily living to the activities of the microbial world and at the same time to acquire a scientific approach which would permit him to pursue more advanced courses in microbiology.

Some of the more recent aspects of bacterial cytology, genetics, and nutrition have been presented not with the idea of emphasizing the controversial nature, but to indicate the increasing importance of bacteria themselves. A chapter on mutualisms has been added to illustrate some of the recent interest in symbiotic relationships as well as germfree studies. Since students are vitally interested in the effects of microorganisms on their well-being, the sections on pathogenic bacteria and viruses have been expanded.

The pronunciation of scientific names is frequently a stumbling block to the beginning student and in the chapter on classification an attempt has been made to incorporate pronouncing aids. Professor Robert S. Breed was most helpful in this endeavor. We are also indebted to Professor C. E. Skinner of the State College of Washington for his suggestions on the chapters on molds and yeasts, and to our colleagues at Montana State College including Dean J. A. Nelson for his reading of the chapters relating to the dairy field, and Miss Berenice Bayliss and Dr. L. DS. Smith for their constructive criticisms on the last chapters relating to immunology and pathogenic microorganisms.

The authors are appreciative and indebted to the organizations and individuals which have provided many illustrations; Dr. Harry E. Morton, Chairman of the Committee on Materials for Visual Instruction in Microbiology for the Society of American Bacteriologists, was most helpful. We are indebted to *Biochimica et Biophysica Acta* for Fig. 4-10, the *Journal of Bacteriology* for Figs. 4-13, 4-14, 9-1, 11-20 and 11-27, the *Journal of Infectious Diseases* for Fig. 17-2, the *Proceedings of the Society for Experimental Biology and Medicine* for Fig. 11-6 and *The Scientific Monthly* for Fig. 8-4.

Particular gratitude is extended to Professor F. B. Cotner, Dean of the Division of Science at Montana State College for his assistance and encouragement throughout this undertaking and to Miss Elizabeth DeFrate for her critical reading of much of the manuscript.

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February, 1955

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THE MICROSCOPIC WORLD

In the great advance that living things have made through evolutionary processes, some have reached the exalted position of higher plants and animals; but other lines have lagged so far behind that after millions of years they are still only one-celled beings. These, and others only slightly larger and more complex, are included under the term *microorganisms.*

Living Things. In botany and zoology we learn that living things have certain characteristics in common. (1) *Metabolism.* This includes all of the chemical reactions carried on by organisms in order to live. Probably the surest criterion of life is *respiration,* whereby, as a result of oxidation, energy is stored and released as needed. (2) *Growth.* As a result of metabolic processes the individual is increased in size and usually changed somewhat in form. In this growth process the individual manufactures the material by which its size is increased. This is unlike the growth of nonliving things—crystals, for example—in which enlargement takes place from material added by outside agencies. (3) *Reproduction.* The living individual is capable, *through its own activities,* of producing new individuals of its own kind, either alone or with the aid of another individual. (4) *Irritability.* This is the capacity of living things for responding to environmental or external stimuli such as heat, light, gravity, pressure, etc.[1]

Plant vs. Animal Characteristics. There is no single character that distinguishes plants from animals; indeed there are some species of microorganisms—e.g., *Euglena viridis*—that possess both plant and animal characters and are on the border line of the two kingdoms. Certain characters, however, are more common in animals and others more common in plants, as we usually classify them. Locomotion, formerly classed as an exclusively animal characteristic, is the most distinctive, but a few plants have it, e.g., bacteria and some unicellular algae. Most animals have cell walls that are plastic and contain nitrogenous materials rather than cellulose, but the cell walls in the exoskeleton of insects are rigid. Animals are all dependent upon other organisms, but so are the plants that lack chlorophyll—yeasts, molds, and other

[1] Biologists fully recognize that in the nonliving world there are certain phenomena that have a superficial resemblance to those here described; but the fact that each living individual has all of these properties and that it takes a more active part in their accomplishment than do nonliving things makes a distinction possible as a rule.

fungi. Plants generally have firm and very definite cell walls wholly distinct from their protoplasm and usually containing cellulose or other carbohydrates. Their mature cells, as a rule, contain large central vacuoles. The presence of chlorophyll is a plant character, although many, particularly the fungi, lack it. Generally, plants have more synthetic power than animals and are able to manufacture protoplasm from simpler food materials—water, carbon dioxide, nitrates, etc.

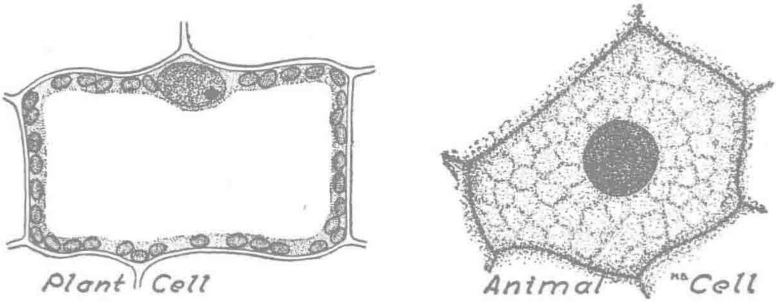

Fig. 1-1. Left, cell from a leaf of *Elodea canadensis;* right, epidermal cell of salamander. To show comparison of plant cell, with its definite cellulose wall, chloroplasts, and central vacuole, with animal cell which lacks these structures.

In studying the following table the reader must keep in mind the exceptions mentioned above.

PLANT AND ANIMAL CHARACTERISTICS

Plants	*Animals*
Possess chlorophyll	Have no chlorophyll
Carry on photosynthesis	Lack photosynthesis
Have distinct cell walls	Have not distinct cell walls
Central vacuoles in cells	No central vacuoles in cells
Have no locomotion	Have locomotion

Kinds of Microorganisms

One-celled organisms, and multicellular individuals so small that a microscope is required for their study, are rather numerous, making a miscellaneous group of many thousand species—some plants and some animals.

The Protozoa. These are unicellular animals, usually lacking chlorophyll and possessing locomotion. *Ameba, Paramecium,* and *Vorticella* are familiar examples. Many of them are saprophytes, living in water and obtaining their food from nonliving organic matter. A large number consume other microorganisms, and others live parasitically in the bodies of higher animals where they cause important diseases such as malaria and African sleeping sickness. Few, if any, cause diseases of plants.

The Molds. There are several classes of lower plants lacking chlorophyll which make up the heterogeneous group, fungi. The largest of these are the puffballs, mushrooms, and bracket fungi. Those that live saprophytically on fruits, vegetables, etc., are given the common name of *mold*. *Penicillium*, the blue or green mold of oranges, grapes, etc., is the most familiar example. The molds have a vegetative body or *mycelium* composed of a mass of branching threads easily visible to the unaided eye. On maturity they reproduce by enormous numbers of microscopic *spores* which are usually one-celled. Some species, including the Penicillia, produce their spores on stalks, pinching them

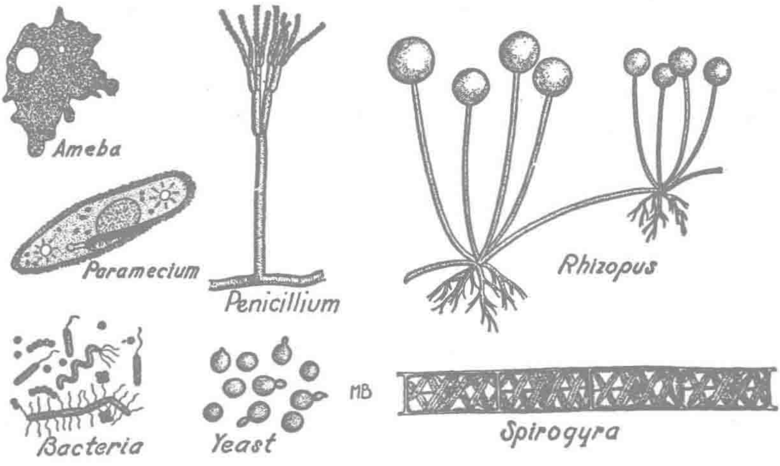

Fig. 1-2. Microorganisms representing various groups.

off by constriction from the tips of branches. Other kinds, including the bread mold *Rhizopus*, produce their spores in sacs or *sporangia*, cutting them out of the protoplasm of the young sporangium by *cleavage*. The formation of spores by these methods represents *asexual* reproduction and goes on very rapidly, producing enormous numbers in the course of a day. Each spore under favorable conditions forms a new plant like its parent by germinating and sending out branches that form a mycelium. Most kinds of mold also have sexual reproduction in which gametes form and conjugate to make a *zygote*, which grows into a new plant. The fungi have well-developed nuclei with mitotic division.

Considering the fungi as a whole, there are thousands of species, many of which are saprophytic; many others are parasitic on plants, where they form rusts, smuts, mildews, etc.; but only relatively few are parasitic on higher animals. Athletes' foot and a few other human diseases are, however, caused by fungi.

The Yeasts. Certain fungi appear to have degenerated in that they have lost all or nearly all of the ancestral mycelium, having left only round or oval cells that serve for both vegetative growth and reproduction. These are the yeasts. Most of them reproduce by budding, and some also form spores—four or eight in each cell. Very few species of yeast are parasitic; most of them thrive best in solutions containing sugar, which they change to alcohol and carbon dioxide. They are more closely related to the molds than to the bacteria.

The Bacteria. The microorganisms that will receive the most attention in this book are the bacteria. The described species number about 1200. They are usually considered the simplest and lowest of all forms of life now on the earth. Some bacteria are so small that they are scarcely visible with the highest magnification of the microscope, while others are much larger and can easily be seen with the low powers. Most bacteria are colorless and very simple in structure. The nucleus is not visible by ordinary methods. Many kinds of bacteria are parasitic on man or higher animals, many are parasitic on plants, but many others are saprophytic, while a few are independent of other organisms or their products.

The bacteria will be discussed more in detail in later chapters of this book.

The Algae. Radically different from the other microorganisms are the algae, which possess chlorophyll and manufacture carbohydrates by photosynthesis, thus being independent of the saprophytic or parasitic life of fungi and most bacteria. For the most part they are aquatic, living in nearly all natural waters, both fresh and salt, and on the moist surfaces of rocks, soil, etc.

Four classes of algae are recognized under the common names of *blue-green algae, green algae, brown algae,* and *red algae.* The blue-green algae are of especial interest here, as their nuclear structure and their lack of sexual reproduction strongly suggest a relationship to the bacteria. Algae of the other three classes have both asexual and sexual reproduction. Their asexual spores are borne inside the large, old, mother cells by cleavage. The species of algae are few in number compared with those of the fungi.

The Viruses. There is evidence for the existence of living things that are too small to be seen even with the best light microscopes. The juices from plants affected with bushy stunt or mosaic diseases and fluids from the diseased tissues of human beings with poliomyelitis, yellow fever, or influenza, for example, are capable of reproducing the same disease if even a tiny amount is injected into another plant or animal of the same kind. If the liquids are passed through the finest clay filters, which will remove all known bacterial organisms, the filtrate still contains ultramicroscopic particles that will cause the disease. Such materials are called plant and animal viruses, and such diseases are known as viral diseases.

In 1915 a French bacteriologist, d'Herelle, found that something was killing his cultures of the dysentery organisms. Further investigation showed that a tiny portion from a killed culture transferred to a healthy one would cause the bacteria in it to die also, and this destructive inoculation could be continued from culture to culture indefinitely. The filtrate from such a diseased culture was likewise capable of killing healthy ones, although it showed no microscopic organisms. D'Herelle concluded that he had discovered an ultramicroscopic organism which was a strict parasite on bacteria and he named it *Bacteriophagum intestinale*, or bacteriophage, meaning "to eat bacteria." Today these forms are considered to be bacterial viruses.

Many of the plant, animal, and bacterial viruses have been observed with the electron microscope, and some have been obtained in crystalline form. All of the viruses have the power of increasing in living cells which are necessary in order to cultivate them. Also, they have the ability to mutate.

In this chapter, only a brief sketch has been given of the great, miscellaneous group that is composed of microorganisms, in order to orient the student in this field and give him a working basis for the course. A more detailed discussion of them is given in later chapters.

Review Questions

At the close of each chapter a set of review questions is given. The student should be able to answer all of those pertaining to one chapter before going on to the next. As a rule the answers can be found in the text of that chapter, but purposely a few questions are asked that call for wider reading.

1. Name the characteristics or properties found in all living things that, taken collectively, are not found in any nonliving things.

2. Why could not the burning of coal be classed as respiration? How does the enlargment of a crystal in a solution of the same substance differ from the growth of a living thing? As a result of weathering, rocks break up into small fragments of the same kind. Why not call this reproduction? In a mechanical thermometer the needle on the dial swings back and forth when the instrument is heated and cooled. How does this differ from an irritable response?

3. Which character used to distinguish between plants and animals has the fewest exceptions? Name an organism that has both plant and animal characters to such an extent that it is truly intermediate.

4. What is the relationship between the terms "molds" and "fungi"?

5. How do algae differ from fungi? Which class of algae seems most closely related to the bacteria?

2

The Rise of Microbiology

There can be no doubt that man suspected the presence of organisms too small to be seen with the unaided eye long before microscopes were invented. Such a possibility was occasionally expressed by early writers. For example, Varro, a Roman who lived a century before the Christian era, made this remarkable guess: "Certain minute invisible animals develop which, carried by the air, may enter the body through mouth or nose and cause serious ailments." It was natural for thinking people to reason that since known plants and animals vary greatly in size, some being so tiny that they are barely visible, there may exist others that are below the limit of human vision.

Also, the effects of microorganisms were known centuries before the living causes were seen. The fermentation of sugary liquids, the decay of foods and refuse, and such diseases as tuberculosis and leprosy were known but not understood. Not until the invention of lenses and microscopes could the causes of these phenomena be seen.

Discovery of Bacteria

Bacteria were first seen in connection with the making of lenses and the testing of their qualities. The compound microscope was invented by J. and Z. Janssen in 1590, but for nearly a century it remained a crude affair that had little practical value; and if during that time it ever revealed microorganisms, no record of the fact was made.

Anton van Leeuwenhoek. In Delft, Holland, in the seventeenth century, there lived a man who in his mature years ground lenses. They were double convex lenses equipped with focusing devices, and some of them magnified as much as 300 diameters, which is considerably higher than the present-day compound microscope with 8-mm objective and 10× eyepiece. He found, however, that lenses having about half that magnification gave better results, as they were less difficult to use. Leeuwenhoek, who made about four hundred of these lenses, was quite correct in his opinion that the best of them were more serviceable than the compound microscopes of that time (Fig. 2-1).

As his interest lay in making better and better lenses it was natural that he should test them on all sorts of objects. In so doing he made many valuable discoveries. He appears to have been the first to see yeasts, red blood corpuscles, spermatozoa, various details of muscle and nerve tissue, and some of the protozoa, as well as bacteria. These last he found in rain water, in decaying matter, and in scrapings from his own teeth (Fig. 2-2).

All these valuable discoveries might have received only local attention and been lost to science, but by good fortune he was able through a friend to present his findings by letter to the Royal Society of England. His first report of microscopic discoveries, in 1674, was followed by many others, including one on bacteria in 1683 which was illustrated with a picture. Because of their motility he thought them to be tiny animals, as did also zoologists for more than a century thereafter.

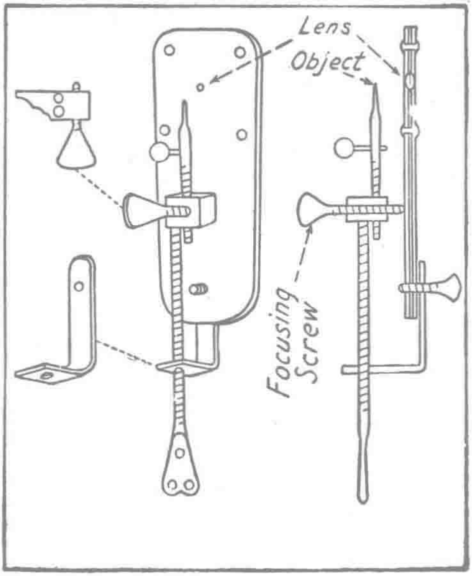

Fig. 2-1. One of Leeuwenhoek's microscopes. Some of these microscopes were held in both hands, others were set in a block for support. The object was mounted on the sharp point and focusing was done with one of the screws. The broad metal portion in which the lens was set helped to exclude vagrant light from the eye. From Bulloch's *History of Bacteriology*. Oxford University Press.

We now realize that Leeuwenhoek was a remarkable man. He had unusual powers of observation, patience, skill, and ingenuity. Wholly without scientific training, he nevertheless had the true scientific spirit which prompted him to study and learn for the sake of revealing truths for their own sake, regardlesss of their utility to man.

At that time no one suspected that these interesting little microorganisms had any economic significance, that some of them later would be included among man's greatest benefactors and others among his worst enemies. Nearly two centuries passed before these facts were brought to light by Pasteur and his contemporaries.

Origin of Cultural Methods. At the present time studies in bacteriology are made largely by the use of cultures in which the organisms are grown under controlled conditions in artificial culture media.

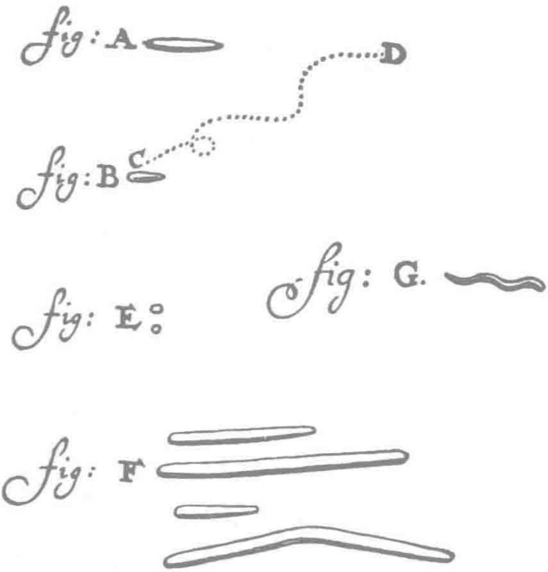

Fig. 2-2. Pictures of bacteria seen with a simple lens, as drawn by Leeuwenhoek.

These methods originated in an attempt to settle the old controversy over spontaneous generation—the theory that living things sometimes come into existence without antecedents, from nonliving materials. This theory, accepted by Aristotle and many others, had been pretty thoroughly discredited in its application to higher forms of animal life and even for insect larvae; but the fact that clear liquids such as beef broth and vegetable infusions became cloudy on standing, from the presence of myriads of bacteria, yeasts, etc., was perplexing.

John T. Needham, an Irish priest, in some pioneer experiments carried out from 1745 to 1750, revived the argument. In his simple experiments he heated decoctions of wheat and barley grains, meat, etc., in stoppered bottles and found that microorganisms promptly developed, although the heating had been supposed to sterilize them. An Italian abbot, Lazzaro Spallanzani, challenged Needham's work on the grounds that the heating was insufficient and the sealing imperfect. He repeated Needham's experiments, using carefully sealed flasks and heating for hours in boiling water, and was thus able to preserve meat broth indefinitely. He, therefore, claimed that Needham's evidence of spontaneous generation was faulty. Spallanzani's experiments, in turn, were criticized on the ground that they had excluded free access of air, which would be necessary for the development of organisms to take place. In the bitter controversy that ensued it became evident that a method must be devised for admitting sterile, unchanged air into the flasks. Schultze, in 1836, tried forcing the air into the sterile flasks through strong acids and bases, and Schwann the next year admitted it through hot tubes, but these methods were criticized on the false supposition that the air was so changed that it would not support life. H. Schroeder and Th. von Dusch met this criticism in 1854 by drawing the air into flasks of heated infusions through cotton wool, which could not possibly alter it other than by filtering out the dust and germs (Fig. 2-4). Thus was born a method of plugging culture flasks and tubes so satisfactory, cheap, and rapid, that it is in universal use today and makes possible a great variety of cultural studies.

Fig. 2-3. Lazzaro Spallanzani (1729-1799). From Frobisher's *Fundamentals of Bacteriology.* W. B. Saunders Co.

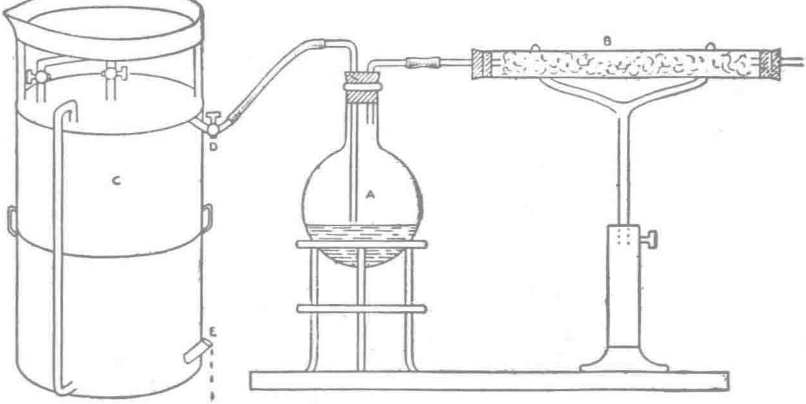

Fig. 2-4. Device used by Schroeder and Dusch for filtering air through cotton. From Bulloch's *History of Bacteriology.* Oxford University Press.

If any doubts remained that growth would not start in sterile liquids by spontaneous generation, they were dispelled by Chevreuil and Pasteur, who reported in 1865 that by the use of flasks with necks drawn out and bent downward to exclude dust they were able to preserve sterile broth for years without stoppers or sealing of any kind (Fig. 2-5).

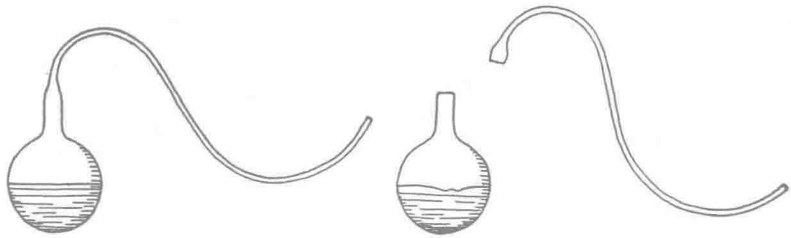

Fig. 2-5. Pasteur's flasks, constructed to admit air but exclude microorganisms.

Early Canning Demonstration. How often purely scientific experiments are followed by demonstrations of their practical utility! In 1810 a Frenchman, Nicolas Appert, showed that, by the processes of heating and sealing, food could be preserved in bottles indefinitely. Thus, slowly through the home, and then rapidly in factories, the great canning industry of the present day has come into being.

Agricultural Bacteriology. In the days of Pasteur, and largely through his efforts, many phenomena not previously understood were explained on the basis of chemical change brought about by bacteria, yeasts, and other microorganisms. Thus were explained the alcoholic fermentation of sugary solutions by yeasts and processes of decay by bacteria and molds. The prolonged and heated controversy between Pasteur and the great German chemist, von Liebig, as to whether microorganisms play the part of cause in fermentation and decay, or are merely innocent bystanders, resulted in a complete victory for Pasteur in his claim that these phenomena have a biological basis. The souring of milk, long shrouded in superstition, was shown by Fuchs, Pasteur, Lister, von Hessling, and others to be caused by certain bacteria.

That the change of organic forms of nitrogen and ammonia into nitrates is caused by bacteria was shown in 1873 by the French chemists Schlösing and Müntz, and in 1889 the Russian bacteriologist, Serge Winogradsky, isolated the species responsible. Likewise the German chemists, Hellriegel and Wilfarth, in 1887 gave proof that bacterial organisms in the nodules of leguminous plants can fix atmospheric nitrogen, and the next year the Dutch bacteriologist, Martinus Beijerinck, isolated pure cultures of these organisms. He also found another kind of organism that could fix nitrogen in the soil without the aid of legumes.

Medical Bacteriology. A suspicion that some diseases were caused by invisible living organisms had been expressed centuries before the existence of such organisms was demonstrated with the microscope. The opinion of Varro as to disease-producing germs in the air has already been mentioned (page 6). More definite suspicions that such diseases as bubonic plague, smallpox, and tuberculosis are caused by minute forms of life came from observing their spread from person to person, as though something capable of increase passed from the sick to the well. The idea that such a *contagion,* or spread by contact, actually takes place was forcefully stated by an Italian scholar, H. Fracastorius, in 1546. Neither he nor anyone else, however, had at that time seen bacteria, and he did not make clear whether his theoretical "*seminaria*" (sometimes translated as "seeds" or "germs") were living things or nonliving exhalations.

Fig. 2-6. Louis Pasteur (1822-1895). From Bulloch's *History of Bacteriology.* Oxford University Press.

A century later, but before the discoveries of Leeuwenhoek, a German priest, A. Kircher, declared dogmatically that diseases spread through the air through the agency of minute living beings. His contention proved nothing but served to focus attention on this possibility.

Except for the remarkable work of an English physician, Benjamin Marten, published in 1720 as *A New Theory of Consumptions*—which received little attention and was soon practically forgotten—little was added to the doctrine that bacteria produce disease until 1840. Then the German pathologist, Jacob Henle, stated the germ theory of disease in essentially its present form and gave some sound advice on establishing proofs that a given disease is caused by a given organism, thus paving the way for Koch's postulates given below. This announcement was followed closely by the work of Louis Pasteur and his contemporaries, who rapidly changed the theory into established fact.

By this time it was generally agreed that diseases of many kinds had much in common with fermentation and decay, and the establishment of the germ theory for the one helped to establish it for the other.

Early Lines of Evidence. It is interesting to consider here the kinds of evidence that were used by the pioneer bacteriologists to establish the germ theory of disease.

The character of the spread of epidemics from person to person caused the Italian physician, Fracastorius, as early as 1546, to propose his germ theory of disease; and in 1762 an Austrian physician, Marcus von Plenciz, improved on the theory by the claim that each disease was caused by a *different* species of organism.

Improvement of the microscope enabled Pollender in 1849 and Davaine in 1850 actually to see the causative organism of anthrax in the blood of animals sick with that disease.

The use of disinfectants on the hands of obstetricians to prevent puerperal, or child-bed, fever was recommended by Oliver Wendell Holmes in 1843 and by an Austrian physician, Semmelweiss, in 1847, on the theory that the disease was caused by an organism introduced by the hands and that the disinfection prevented it.

In 1787 Wollstein of Vienna reported the transfer of glanders in horses by inoculation with pus from the inflamed nasal passage of a diseased animal. In 1857 Brauell reported that he had been able to transfer anthrax to sheep by inoculation, and in 1865 Davaine, following up his earlier microscopic observations, reported the successful transfer of the disease with infected blood.

These direct inoculations with infected materials were soon superseded by the more refined method of inoculation with pure cultures as practiced by Pasteur and especially by his young German rival, Robert Koch. This use of pure cultures brings in another line of evidence, the obtaining of the causative organism from the body of the sick person or animal and the production of the same disease by inoculation with it.

Koch's Postulates or Rules of Pathogenicity. While yet a young man, Robert Koch urged the desirability of a more refined technic; and, although he realized that all the evidences given above had value, he sensed the fact that dependence on any one of them may lead to error in establishing the cause of any disease. He therefore published four rules,[1] which, if successfully followed, offer convincing proof that a given species of organism is the cause of a given disease. These rules, published in 1882, are as follows:

[1] First formulated by Jacob Henle, one of Koch's teachers in anatomy.