Chapter 2 The History of Microbiology

OUTLINE

History is the story of the achievements of men and women, but it records relatively few outstanding names and events. Many important contributions were made by people whose names have been forgotten and whose accomplishments have been lost in the longer and deeper shadows cast by those who caught the fancy of the chroniclers. It has been said that in science the credit goes to the one who convinces the world, not to the one who first had the idea. So, in the development of microbiology, the outstanding names are often of those who convinced the world—who developed a technique, a tool, or a concept that was generally adopted, or who explained their findings so clearly or dramatically that the science grew and prospered.

Antony van Leeuwenhoek's lucid reports on the ubiquity of microbes enabled Louis Pasteur 200 years later to discover the involvement of these creatures in fermentation reactions and allowed Robert Koch, Theobald Smith, Pasteur, and many others to discover the association of microbes with disease. Koch is remembered for his isolation of the bacteria that cause anthrax and tuberculosis and for the rigid criteria he demanded before a specific bacterium be held as the cause of a disease. His important contributions to the creation of the science of microbiology won him the 1905 Nobel prize.

The building of the Panama Canal dramatized Walter Reed's studies of the epidemiology of yellow fever, but historians remember that Theobald Smith's work on transmission of Texas fever pointed the way for Walter Reed's subsequent work.

In diagnosis by laboratory methods, G. F. I. Widal and August von Wasserman presented those who followed them with tools and ideas with which to work. Paul Ehrlich's discovery of a chemical compound that would destroy the syphilis spirochete in the human body without injury to tissue cells paved the way for future developments in the use of chemicals in treating disease. For this he shared the Nobel prize in 1908 with Elie Metchnikoff, who discovered a system in the human body that combated infection.

Though of relatively short duration, the history of microbiology is filled with thrilling achievements. We have won many battles with microorganisms and have learned not only to make them work for us but also to control some of those that work against us.

THE MICROSCOPE

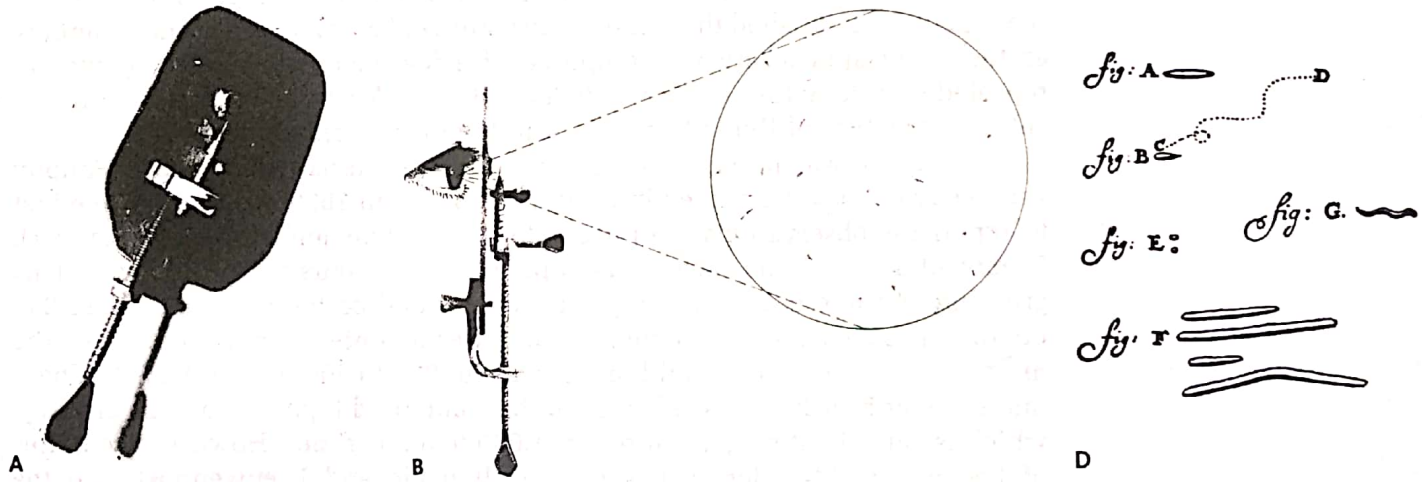
Microbiology began when people learned to grind lenses from pieces of glass and combine them to produce magnifications great enough to enable microbes to be seen. During the thirteenth century Roger Bacon (1220–1292) postulated that disease is produced by invisible living creatures. This suggestion was made again by Girolamo Fracastoro of Verona (1483–1553) and Anton von Plenciz in 1762, but these people had no proof. As early as 1658, a monk named Athanasius Kircher (1601–1680) referred to "worms" invisible to the naked eye in decaying bodies, meat, milk, and diarrheal secretions. Although his description lacked accuracy, Kircher was the first person to recognize the significance of bacteria and other microbes in disease. In 1665 Robert Hooke's description of cells in a piece of cork established the fact that the bodies of "animals and plants, complex as they may appear, are yet composed of a few elementary parts frequently repeated"—a quotation not from Hooke but from Aristotle's description of the cellular structure of living things back in the fourth century B.C.

Although he was probably not the first to see bacteria and protozoa, Antony van Leeuwenhoek, who lived in Delft, Holland, from 1632 to 1723, was the first to report his observations with accurate descriptions and drawings (Fig. 2-1). Leeuwenhoek had the means and opportunity to pursue his hobby of lens grinding and microscope making. During his lifetime he made more than 250 microscopes consisting of home-ground lenses mounted in brass and silver, the most powerful of which would magnify about 200 to 300 times (Fig. 2-2). These microscopes bear little resemblance to the compound light microscope of today, which is capable of magnifications of 1,000 to 3,000 times. However, the lenses of Leeuwenhoek's microscopes were well made and Leeuwenhoek had the openness of mind that is so very important in an investigator. His descriptions of protozoa were so accurate that many of the forms he described are easily recognized today.

Leeuwenhoek carefully recorded his observations in a series of letters to the British Royal Society. In one of the first letters, dated September 7, 1674, addressed to Henry Oldenburg, Secretary of the Royal Society, he described the "very little animalcules" which we recognize as free-living protozoa. On October 9, 1676, he wrote:

In the year 1675, I discovered living creatures in rain water which had stood but a few days in a new earthen pot, glazed blue within. This invited me to view this

Figure 2-1. Antony van Leeuwenhoek (1632–1723), a Dutch student of natural history whose hobby was making microscopes, is shown here with one of the more than 250 microscopes that he made. His best lenses were capable of magnifications up to ×270, and he was the first person to report descriptions of microorganisms in detail. *(Courtesy of Lambert-Hudnut, Division Warner-Lambert Pharmaceutical Company.)*



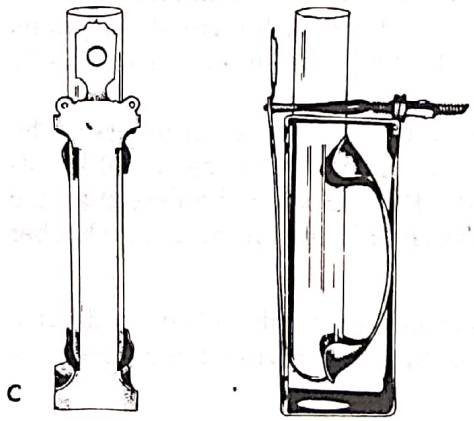


Figure 2-2. (A) The Leeuwenhoek microscope. Replica of a simple microscope made in 1673 by Leeuwenhoek. *(From the collection of the Armed Forces Institute of Pathology, Washington, D.C.)* (B) Side view of a Leeuwenhoek microscope, illustrating the manner in which observations of specimens were made. (C) Front and side views of Leeuwenhoek's brass aquatic microscope. Lenses could be slotted in at the front and focused by the butterfly nut at the rear. *(Erwin F. Lessel, illustrator.)* (D) Leeuwenhoek's sketches of bacteria from the human mouth, from letter of September 17, 1683. Note particularly shapes of cells and relative sizes. The dotted line between **C** and **D** indicates motility (movement) of a bacterium. *(Courtesy of C. Dobell, Antony van Leeuwenhoek and His "Little Animals," Dover, New York, 1960.)*

water with great attention, especially those little animals appearing to me ten thousand times less than those . . . which may be perceived in the water with the naked eye.

He described his little animals in great detail, leaving little doubt that he saw bacteria, fungi, and many forms of protozoa. For example, he reported that on June 16, 1675, while examining well water into which he had put a whole pepper the day before:

I discovered, in a tiny drop of water, incredibly many very little animalcules, and these of divers sorts and sizes. They moved with bendings, as an eel always swims with its head in front, and never tail first, yet these animalcules swam as well backwards as forwards, though their motion was very slow.

His enthusiastic letters were read with interest by the British scientists, but the importance of his discoveries evidently went unappreciated. The talents and astuteness of this remarkable man can best be appreciated by reading Dobell's biography of Leeuwenhoek.

Before the time of Pasteur, microorganisms were studied mainly to satisfy curiosity concerning their characteristics and their relationships to higher living forms, without awareness of their importance in fermentation and disease.

SPONTANEOUS GENERATION VERSUS BIOGENESIS

The discovery of microbes spurred interest in the origin of living things, and argument and speculation grew. As far as human beings were concerned, the Greek explanation that the goddess Gaea was able to create people from stones and other inanimate objects had been largely discarded. But even the astute Aristotle (384–322 B.C.) taught that animals might originate spontaneously from the soil, plants, or other unlike animals, and his influence was still strongly felt in the seventeenth century. About 40 B.C., Virgil (70–19 B.C.) gave directions for the artificial propagation of bees. This was but one of many fanciful tales of a similar nature that persisted into the seventeenth century. For example, it was accepted as a fact that maggots could be produced by exposing meat to warmth and air, but Francesco Redi (1626–1697) doubted this. Proof that his skepticism was well founded came from an experiment in which he placed meat in a jar covered with gauze. Attracted by the odor of the meat, flies laid eggs on the covering, and from the eggs maggots developed. Hence the experiment established the fact that the origin of the maggots was the flies and not the meat. This experiment and others involving mice and scorpions appear to have settled the matter so far as these forms of life were concerned. But microbes were another matter; surely such minute creatures needed no parents!

There appeared champions for and challengers of the theory that living things can originate spontaneously, each with a new and sometimes fantastic explanation or bit of experimental evidence. In 1749, while experimenting with meat exposed to hot ashes, John Needham (1713–1781) observed the appearance of organisms not present at the start of the experiment and concluded that the bacteria originated from the meat. About the same time, Lazaro Spallanzani (1729–1799) boiled beef broth for an hour and then sealed the flasks. No microbes appeared following incubation. But his results, confirmed in repeated

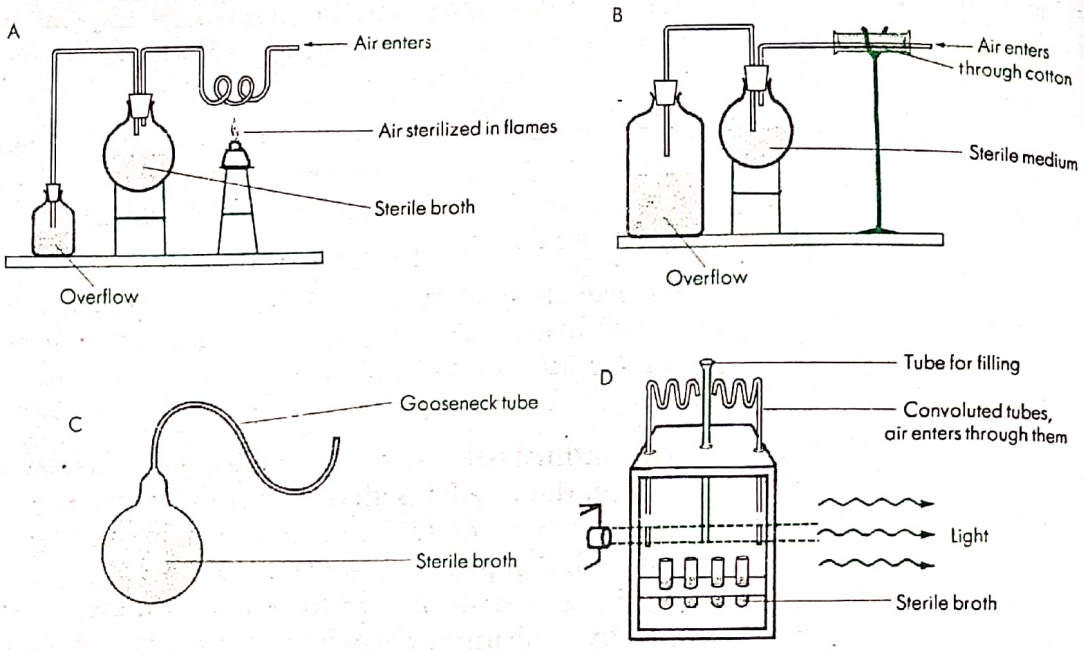


Figure 2-3. The theory of spontaneous generation was disproved with the devices illustrated here, all of which eliminated airborne bacteria. Schwann heat-sterilized the air which flowed through the glass tube to his culture flask (A). Schröder and von Dusch filtered the air entering the culture flask through cotton (B). Simple goose-necked flasks (C) were devised by Pasteur. Tyndall constructed a dust-free incubation chamber (D).

experiments, failed to convince Needham, who insisted that air was essential to the spontaneous production of microscopic beings and that it had been excluded from the flasks by sealing them. This argument was answered some 60 or 70 years later independently by two other investigators, Franz Schulze (1815–1873) and Theodor Schwann (1810–1882). Schulze passed air through strong acid solutions into boiled infusions, whereas Schwann passed air into his flasks through red-hot tubes (Fig. 2-3A). In neither case did microbes appear. But the die-hard advocates of spontaneous generation were still not convinced. Acid and heat altered the air so that it would not support growth, they said. About 1850, H. Schröder and T. von Dusch performed a more convincing experiment by passing air through cotton into flasks containing heated broth (Fig. 2-3B). Thus the microbes were filtered out of the air by the cotton fibers so that growth did not occur, and a basic technique of plugging bacterial culture tubes with cotton stoppers was initiated.

The concept of spontaneous generation was revived for the last time by Felix-Archimede Pouchet (1800–1872), who published in 1859 an extensive report "proving" its occurrence. But Pouchet reckoned without the ingenious, tireless, and stubborn Pasteur (1822–1895). Irritated by Pouchet's logic and data, Pasteur performed experiments that ended the argument for all time. He prepared a flask with a long, narrow gooseneck opening (Fig. 2-3C). The nutrient solutions were heated in the flask, and air—untreated and unfiltered—could pass in or out; but the germs settled in the gooseneck, and no microbes appeared in the solution.

Pasteur reported his results with a great flourish at the Sorbonne in Paris on April 7, 1864. His flasks would yield no sign of life, he said:

> For I have kept from them, and am still keeping from them, that one thing which is above the power of man to make; I have kept from them the germs that float in the air. I have kept from them life.

In his exuberance, Pasteur sent a few darts at those he disagreed with:

> There is no condition known today in which you can affirm that microscopic beings come into the world without germs, without parents like themselves. They who allege it have been the sport of illusions, of ill-made experiments, vitiated by errors which they have not been able to perceive and have not known how to avoid.

Finally, John Tyndall (1820–1893) conducted experiments in a specially designed box to prove that dust carried the germs (Fig. 2-3D). He demonstrated that if no dust was present, sterile broth remained free of microbial growth for indefinite periods.

FERMENTATION

Louis Pasteur (Fig. 2-4) began his brilliant career as professor of chemistry at the University of Lille, France. A principal industry of France being the manufacture of wines and beer, Pasteur studied the methods and processes involved in order to help his neighbors produce a consistently good product. He found that fermentation of fruits and grains, resulting in alcohol, was brought about by microbes. By examining many batches of "ferment," he found microbes of different sorts. In good lots one type predominated, and in the poor products another kind was present. By proper selection of the microbe, the manufacturer might be assured of a consistently good and uniform product. Pasteur suggested that the undesirable types of microbes might be removed by heating—not enough to hurt the flavor of the fruit juice, but enough to destroy a very high percentage of the microbial population. He found that holding the juices at a temperature of 62.8°C (145°F) for half an hour did the job. Today **pasteurization** is widely used in fermentation industries, but we are most familiar with it in the dairy industry.

Figure 2-4. Louis Pasteur in his laboratory. *(Courtesy of Institut Pasteur, Paris.)*

THE GERM THEORY OF DISEASE

Even before Pasteur had proved by experiment that bacteria are the cause of some diseases, many observant students had expressed strong arguments for the germ theory of disease. Fracastoro of Verona suggested that diseases might be due to invisible organisms transmitted from one person to another. In 1762 von Plenciz not only stated that living agents are the cause of disease but suspected that different germs were responsible for different diseases. That the concept of parasitism was becoming quite general is reflected in the following bit of doggerel written by Jonathan Swift (1667–1745) early in the eighteenth century:

So naturalists observe, a flea
  Hath smaller fleas that on him prey;
And these have smaller fleas to bit 'em;
  And so proceed ad infinitum.

This is better known in the colloquial version:

Big bugs have little bugs,
  Upon their backs to bit 'em;
And little bugs have smaller ones,
  And so ad infinitum.

Oliver Wendell Holmes (1809–1894), a successful physician as well as a scholar, insisted that puerperal fever, a disease of childbirth, was contagious and that it was probably caused by a germ carried from one mother to another by midwives and physicians. He wrote *The Contagiousness of Puerperal Fever* in 1842. At approximately the same time, the Hungarian physician Ignaz Philipp Semmelweis (1818–1865) was pioneering in the use of antiseptics in obstetrical practice. Deaths due to infections associated with childbirth were reduced in the cases handled according to his instructions, which minimized chances for infection. As part of his crusade he published *The Cause, Concept and Prophylaxis of Childbed Fever* in 1861. Still, most physicians ignored his advice, and it was not until about 1890, when the work of Joseph Lister in England had become known, that the importance of antisepsis was fully appreciated by the medical profession.

Pasteur's success in solving the problem of fermentation led the French goverment to request that he investigate pebrine, a silkworm disease that was ruining an important French industry. For several years Pasteur struggled with this problem, heartaches and disappointments following one after another. Eventually he isolated the parasite causing the disease. He also showed that silkworm farmers could eliminate the disease by using only healthy, disease-free caterpillars for breeding stock.

Turning from silk to wool, Pasteur next tackled the problem of anthrax, a disease of cattle, sheep, and sometimes human beings. He grew the microbes in laboratory flasks after isolating them from the blood of animals that had died of the disease. Meanwhile Robert Koch (1843–1910) was busy with the anthrax problem in Germany. Koch, a quiet, meticulous physician, sometimes neglected his medical practice to play with the fascinating new science of bacteriology. It was he who discovered the typical bacilli with squarish ends in the blood of cattle that had died of anthrax. He grew these bacteria in cultures in his laboratory, examined them microscopically to be sure he had only one kind present,

and then injected them into other animals to see if these became infected and developed clinical symptoms of anthrax. From these experimentally infected animals he isolated microbes like those he had originally found in sheep that died of anthrax. This was the first time a bacterium had been proved to be the cause of an animal disease. (Pebrine is caused by a protozoan rather than by a bacterium.) This series of observations led to the establishment of **Koch's postulates**, which provided guidelines to identify the causative agent of an infectious disease. Koch's postulates are: (1) A specific organism can always be found in association with a given disease. (2) The organism can be isolated and grown in pure culture in the laboratory. (3) The pure culture will produce the disease when inoculated into a susceptible animal. (4) It is possible to recover the organism in pure culture from the experimentally infected animal.

LABORATORY TECHNIQUES AND PURE CULTURES

As we have previously stated, microorganisms occur in nature in extremely large populations made up of many different species. In order to study the characteristics of a particular species it is first necessary to separate it from all other species. Laboratory procedures have been developed that make it possible to isolate microorganisms representing each species and to grow (cultivate) each of the species separately. The growth of a mass of cells of the same species in a laboratory vessel (such as a test tube) is called a **pure culture.**

Pure cultures of bacteria were first obtained by Joseph Lister in 1878 using serial dilutions in liquid media. With a specially constructed syringe he diluted a fluid (probably milk) containing a mixture of bacteria until a single organism was delivered into a container of sterile milk. After incubation, bacteria in this container were of a single kind, identical to the parent cell. Lister named the organism *Bacterium lactis.*

Meanwhile Koch was carefully refining methods for the study of bacteria. He found that by smearing bacteria on a glass slide and adding certain dyes to them, individual cells could be seen more clearly with the microscope. He added gelatin and other solidifying materials such as agar to media in order to obtain isolated growths of organisms known as **colonies**, each of which contained millions of individual bacterial cells packed tightly together. From these colonies, pure cultures could be transferred to other media. The development of a liquefiable solid-culture medium was of fundamental importance.

Using techniques he had devised, Koch studied with painstaking care material taken from patients with pulmonary tuberculosis. After performing a series of rigid tests, as he had done with the anthrax bacillus, he announced the discovery of the microorganism that causes tuberculosis.

The importance of pure cultures to the development of the science of microbiology cannot be overestimated, since by using pure-culture techniques the microorganisms responsible for many infections, certain fermentations, nitrogen fixation in soil, and other activities were isolated and identified. However, strict adherence to pure-culture techniques and Koch's postulates sometimes led investigators up dead-end streets. Early investigators did not know about viruses, nor did they know about the cooperation of two or more microorganisms in causing disease or in bringing about a desirable fermentation such as we find in the ripening of cheese. Today we are as much interested in mixed microbial

populations and the effects they produce as we are in pure cultures. Further advances in marine microbiology, rumen microbiology, microbiology of the intestinal tract, and many other systems will depend upon understanding first the physiology of individual microorganisms in pure culture and then upon the ecological relationships of the total microbial populations in a given environment.

PROTECTION AGAINST INFECTION: IMMUNITY

Pasteur continued to make discoveries concerning the cause and prevention of infectious diseases. About 1880 he isolated the bacterium responsible for chicken cholera and grew it in pure culture. Here again, the practical Pasteur made use of the fundamental techniques devised by the more theoretical Koch. To prove that he really had isolated the organisms responsible for chicken cholera, Pasteur arranged for a public demonstration where he repeated an experiment (Fig. 2-5) that had been successful in many previous trials. He inoculated healthy chickens with his pure cultures, but to his dismay, the chickens failed to get sick and die! Reviewing each step of the experiment, Pasteur found that he had accidentally used cultures several weeks old instead of the fresh ones grown especially for the demonstration. Some weeks later he repeated the experiment, using two groups of chickens. One of these groups had been inoculated at the first demonstration with the old cultures that had proved ineffective, and the second had not been previously exposed. Both groups received bacteria from fresh young cultures. This time the chickens in the second group got sick and died, but those in the first group remained hale and hearty. This puzzled Pasteur, but he soon found the explanation. In some way bacteria could lose their ability to produce disease, i.e., their **virulence**, after standing and growing old. But these **attenuated** (having decreased virulence) bacteria still retained their capacity for stimulating the host to produce substances, i.e. **antibodies**, that protect against subsequent exposure to virulent organisms.

This demonstration explained the principle involved in Edward Jenner's successful use of cowpox virus, in 1798, to immunize people against smallpox (Fig. 2-6). Pasteur next applied this principle to the prevention of anthrax, and again

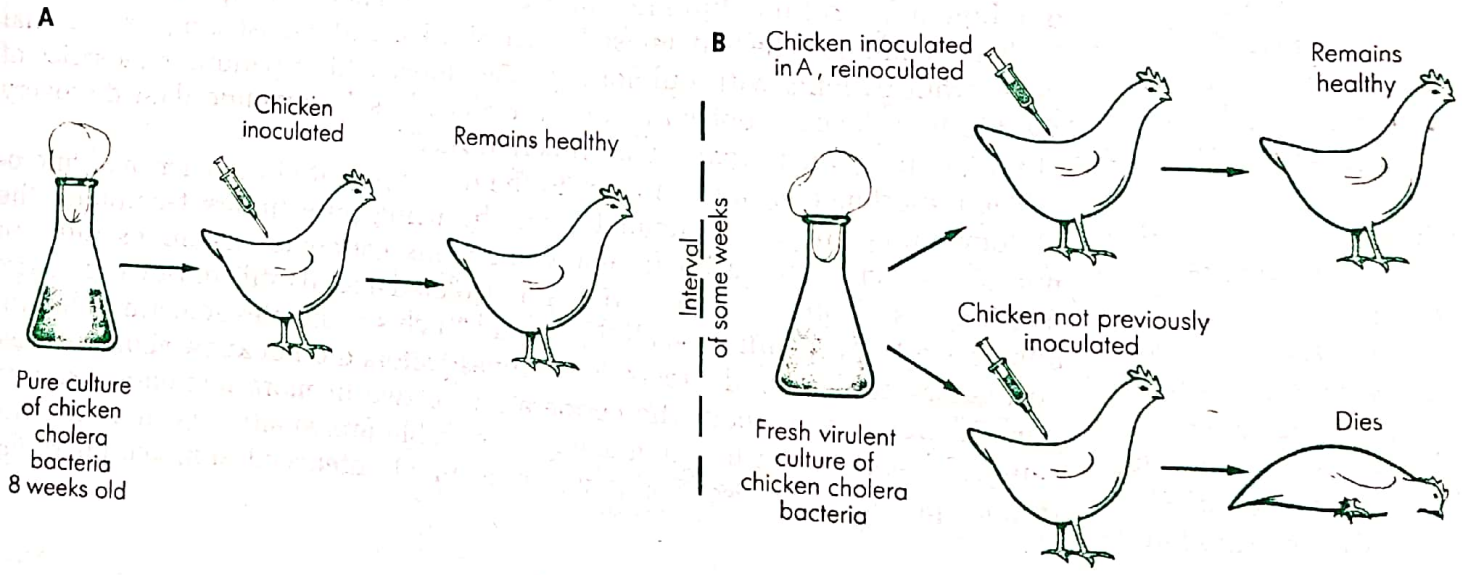


Figure 2-5. The principle of immunization was demonstrated by Pasteur when he inoculated chickens with cultures of chicken cholera bacteria several weeks old and the chickens remained healthy. They did not become sick when inoculated with a fresh culture several weeks later although this fresh culture killed chickens that had not received the attenuated (old) culture.

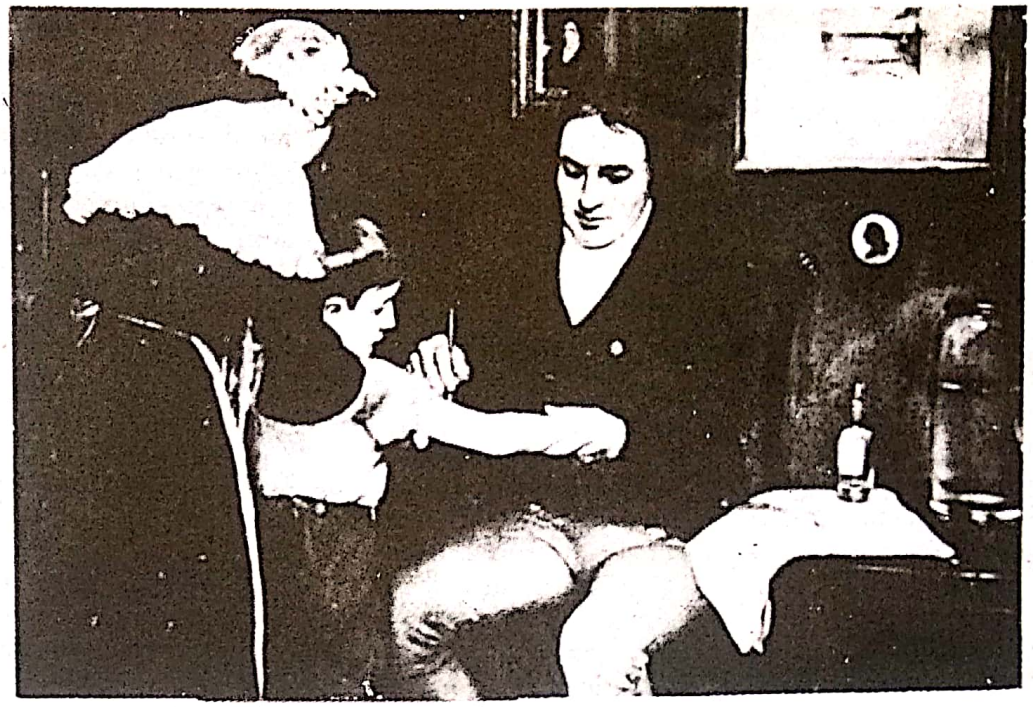
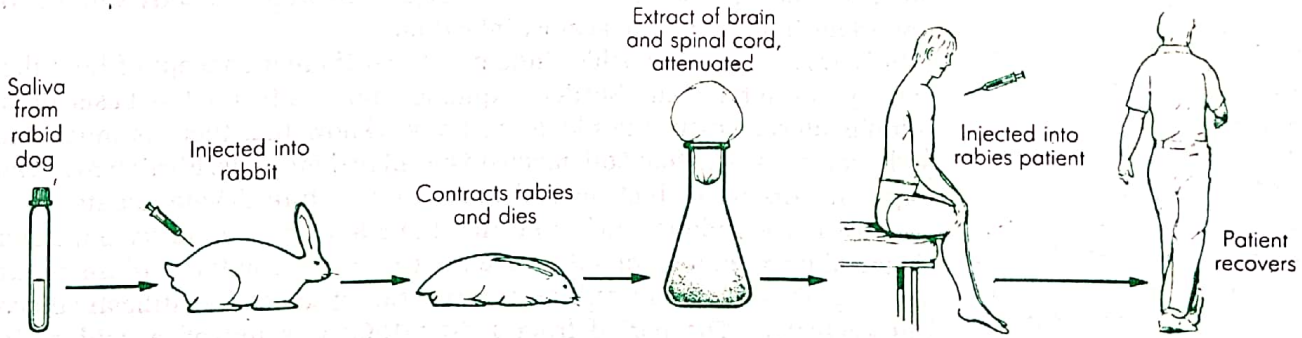


Figure 2-6. Edward Jenner vaccinating (inoculating) James Phipps with cowpox material, which resulted in development of resistance to smallpox infection. *(Courtesy of Culver Pictures, New York.)*

it worked. He called the attenuated cultures **vaccines**, a term derived from the Latin *vacca*, meaning "cow." Pasteur was honoring Jenner when he applied the term *vaccination* to immunization with attenuated cultures of bacteria that had no connection with cows.

Pasteur's fame was by now well established throughout France, and the belief became prevalent that he could work miracles with bacteria and the control of infections. It was not surprising, then, that he was given an even greater challenge: he was asked to work on a disease affecting human beings. As he was a chemist and not a physician, studying a human disease might prove risky. But Pasteur again accepted the challenge to be of service to humanity and set out to make a vaccine for hydrophobia, or rabies, a disease transmitted to people by bites of dogs, cats, and other animals. Because it was invariably fatal, when a boy named Joseph Meister was bitten by a mad wolf, his family did not hesitate to take the one chance in thousands that Pasteur could make a vaccine that would save him.

Figure 2-7. Rabies vaccine is made by inoculating a rabbit with saliva from a rabid dog. Virus in the extract of the rabbit's spinal cord is attenuated before injection into a patient.



For example, bacteria known as bdellovibrios are predatory on other bacteria, and viruses called bacteriophages can infect and destroy bacterial cells.

Ecological Characteristics

The habitat of a microorganism is important in characterizing that organism. For example, microorganisms normally found in marine environments generally differ from those in freshwater environments. The microbial population of the oral cavity differs from that of the intestinal tract. Some kinds of microorganisms are widely distributed in nature, but others may be restricted to a particular environment. The relation of an organism to its environment is often complex and may involve special characteristics of the organism that are not yet known.

MICROBIAL CLASSIFICATION, NOMENCLATURE, AND IDENTIFICATION

Once the characteristics of microorganisms have been determined and appropriately catalogued, the process of classification can begin.

Classification

In microbiology, taxa are initially constructed from strains. A strain is made up of all the descendents of a pure culture; it is usually a succession of cultures derived from an initial colony. Each strain has a specific history and designation.

Taxonomic Groups (Taxa)

For example, strain ATCC 19554 is a strain of spirilla isolated originally from pond water in Blacksburg, Virginia in 1965 by Wells and Krieg, and cultures of this strain are maintained at the American Type Culture Collection (ATCC), Rockville, Maryland. Cultures of the same species that were isolated from other sources would be considered different strains.

The basic taxonomic group (taxon) is the species, i.e., a collection of strains having similar characteristics. Bacterial species consist of a special strain called the type strain together with all other strains that are considered sufficiently similar to the type strain as to warrant inclusion in the species. The type strain is the strain that is designated to be the permanent reference specimen for the species. Unfortunately, it is not always the strain that is most typical of all the strains included in the species, but it is the strain to which all other strains must be compared to see if they resemble it closely enough to belong to the species. Therefore, type strains are particularly important and special attention is given to their maintenance and preservation, particularly by national reference collections such as the ATCC in the United States or the National Collection of Type Cultures in England. Many other culture collections are maintained throughout the world.

In the definition just given for a bacterial species, the phrase "considered sufficiently similar to the type strain" indicates that the definition contains an element of subjectivity. In other words, the criteria which one taxonomist believes to constitute "sufficient similarity" may be quite different from those used by another taxonomist. At present there are no specific criteria for a bacterial species that are universally accepted. However, certain criteria based on DNA homology experiments (described later in this chapter) are probably more widely accepted today than any others and eventually may lead to a unifying concept for defining a species.

Just as a bacterial species is composed of a collection of similar strains, a

bacterial **genus** is composed of a collection of similar species. One of the species is designated the **type species**, and this serves as the permanent example of the genus; that is, other species must be judged to be sufficiently similar to the type species to be included with it in the genus. Unfortunately, there is even less agreement about the criteria for a bacterial genus than there is for a bacterial species.

Taxonomic groups of higher rank than genus are listed below, and the same considerations about subjectivity apply here as well:

Family	A group of similar genera
Order	A group of similar families
Class	A group of similar orders
Division	A group of similar classes
Kingdom	A group of similar divisions

The Goals of Classification

Taxonomists strive to make classifications that have the following two qualities:

1 ***Stability.*** Classifications that are subject to frequent, radical changes lead to confusion. Every attempt should be made to devise classifications that need only minor changes as new information becomes available.
2 ***Predictability.*** By knowing the characteristics of one member of a taxonomic group, it should be possible to assume that the other members of the same group probably have similar characteristics. If this cannot be done, the classification has little value.

General Methods of Classifying Bacteria

Three methods are used for arranging bacteria into taxa:

The Intuitive Method. A microbiologist who is thoroughly familiar with the properties of the organisms he or she has been studying for several years may decide that the organisms represent one or more species or genera. The trouble with this method is that the characteristics of an organism that seem important to one person may not be so important to another, and different taxonomists may arrive at very different groupings. However, some classification schemes based on the intuitive method have proved to be quite useful.

Numerical Taxonomy. In an effort to be more objective about grouping bacteria, a scientist may determine many characteristics (usually 100 to 200) for each strain studied, giving each characteristic equal weight. Then using a computer he or she calculates the % **similarity** (%S) of each strain to every other strain. For any two strains, this is:

$$\%S = \frac{NS}{NS + ND}$$

where NS is the number of characteristics that are the same (positive or negative) for the two strains, and ND is the number of characteristics that are different. (The method is sometimes made more rigorous by making NS equal to the number of *positive* characteristics that are the same for the two strains, since what organisms can do may be more important than what they cannot do.) Those strains having a high %S to each other are placed into groups; those

groups having a high %S to each other are in turn placed into larger groups, and so on (see Fig. 3-1). The degree of similarity needed to rank a group as a species, genus, or other taxon is a matter of judgment on the part of the taxonomist. This method of classification has great practical usefulness as well as being relatively unbiased in its approach; it also yields classifications that have a high degree of stability and predictability.

Genetic Relatedness. The third and most reliable method of classification is

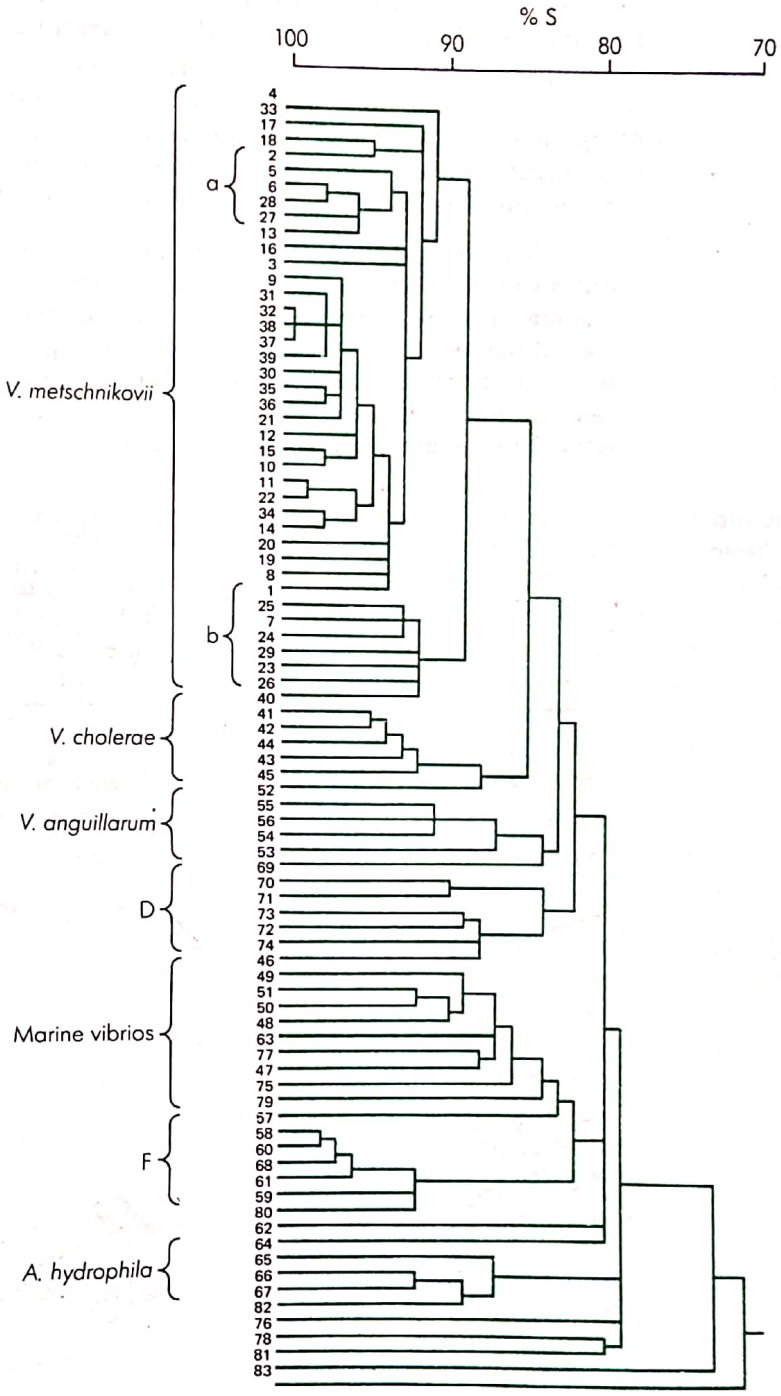


Figure 3-1. Diagram showing the arrangement of 83 strains of oxidase-positive, vibriolike bacteria according to a numerical taxonomic study. Some of the resulting groups represent species (e.g., *Vibrio metschnikovii, V. cholerae*, etc.); others are designated only by vernacular names (Marine vibrios, Group D, etc.). *Courtesy of J. V. Lee, T. J. Donovan, and A. L. Furniss, Int J Syst Bacteriol* **28**:*99–111, 1978.*)

based on the degree of genetic relatedness between organisms. This method is the most objective of all and is based on the most fundamental aspect of organisms, their hereditary material (DNA). In the 1960s the development of that branch of science known as molecular biology provided techniques by which the DNA of one organism could be compared with that of other organisms. At first only crude comparisons could be made, based on mol% G + C values. It is true that two organisms of the same or similar species that are very closely related will have very similar mol% G + C values, and it is also true that two organisms having quite different mol% G + C values are not very closely related. *However, it is important to realize that organisms that are completely unrelated may have similar mol% G + C values.* Therefore, much more precise methods of comparison were needed—namely, methods by which the DNA molecules from various organisms could be compared with respect to the *sequence of their component nucleotides*. This sequence is the most fundamental characteristic of an organism. Modern techniques have now made it possible to make such a comparison. The basic principles can be described briefly as follows:

1 ***DNA homology experiments.*** The double-stranded DNA molecules from two organisms are heated to convert them to single strands. The single strands from one organism are then mixed with those from the other organism and allowed to cool. If the two organisms are closely related, **heteroduplexes** will form. In other words, a strand from one organism will pair with a strand from the other organism (see Fig. 3-2). If the two organisms are not closely related, no heteroduplexes will form. This method is most useful at the species level of classification.

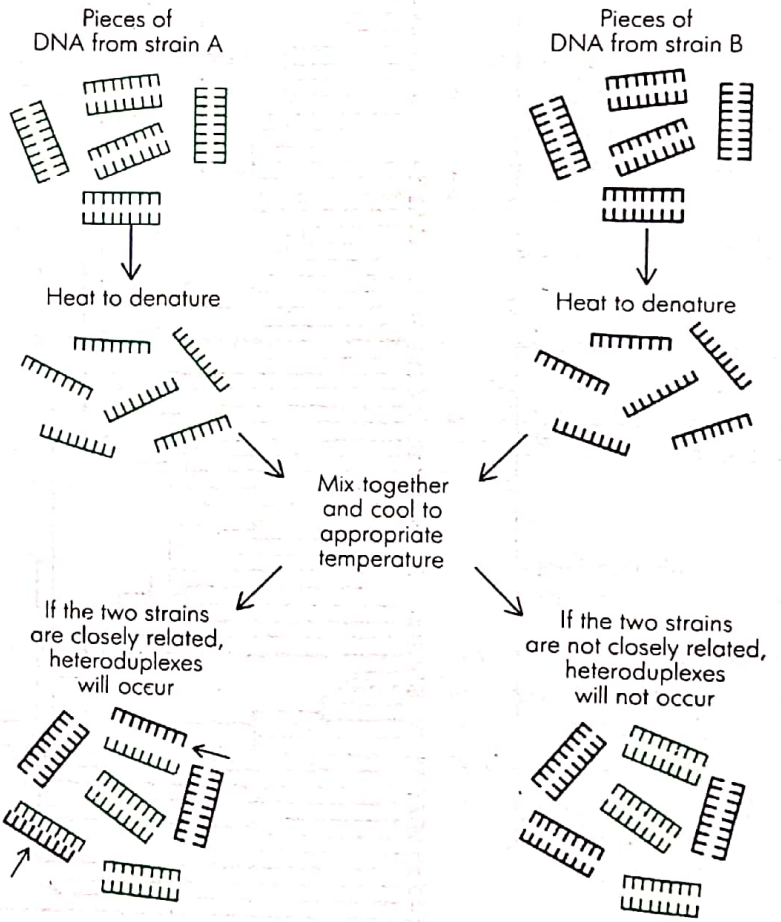


Figure 3-2. Schematic diagram illustrating the basic principle behind DNA homology experiments.

2 ***Ribosomal RNA homology experiments and ribosomal RNA oligonucleotide cataloging.*** Two organisms may not be so closely related as to give a high level of DNA homology, yet they may still have some degree of relatedness. Ribosomes, the small granular-appearing structures within the cell which manufacture proteins, are composed of proteins and RNA. The ribosomal RNA (rRNA) is coded for by only a small fraction of the DNA molecule, the rRNA cistrons. In all bacteria so far studied, the nucleotide sequence of these rRNA genes has been found to be highly conserved; that is, during evolution, the nucleotide sequence has changed more slowly than that of the bulk of the DNA molecule. This means that even if two organisms are only distantly related and show no significant DNA homology, there still may be considerable similarity in the nucleotide sequences of their rRNA cistrons. The degree of similarity that exists can therefore be used as a measure of relatedness between organisms, but at a level beyond that of species (at the level of genus, family, order, etc.). RNA homology experiments and RNA oligonucleotide cataloging are two modern methods used to determine the degree of similarity between the rRNA cistrons of different organisms. The techniques are complex and are being used by only a few laboratories.

Classifications based on genetic relatedness come the closest to achieving the taxonomic goals of stability and predictability. Moreover, the data obtained for such classifications allow microbiologists to infer the way in which bacteria have evolved, so that the present-day bacterial genera and species can be arranged in a hierarchy that reflects their ancestral relationships, i.e., in a phylogenetic classification. Much of the work is still fragmentary, but some of the results, especially those obtained by Dr. C. R. Woese of the University of Illinois and his colleagues, have already revolutionized current thinking about how bacteria have evolved and how they are related to one another. In fact, it is now apparent that present-day bacteria evolved by at least two very different major routes from an early ancestral form and that they now comprise two very large groups: the eubacteria (which are the traditional, familiar ones that have received the most study) and the archaeobacteria (consisting of methane-producers, extreme halophiles, and thermoacidophiles). It has been proposed that these two groups be considered as two separate kingdoms of life, and, indeed, they do seem as distantly related to each other as they are to eucaryotic organisms. Although the kingdom question is still debatable, data obtained from rRNA oligonucleotide cataloging nevertheless make it clear that the archaeobacteria are separated from other bacteria by a great phylogenetic gulf (see Fig. 3-3).

Nomenclature

Each species of microorganism has only one officially accepted name, by international agreement. This system provides for precise communication. If an organism were to be called *Escherichia coli* in one country and *Coprobacterium intestinale* in another, chaos would result. It would be difficult to know that the same organism was being studied.

The name of a species is merely a convenient label. It is not necessarily even descriptive, although some names are. For example, *Micrococcus luteus* means "yellow berry" in Latin, and *Proteus vulgaris* is Latin for "common organism of many shapes." Some species are named after persons: for example, *Escherichia coli*—the organism of the colon, named after Theodor Escherich (a German bacteriologist); or *Clostridium barkeri*—the spindle-shaped organism,

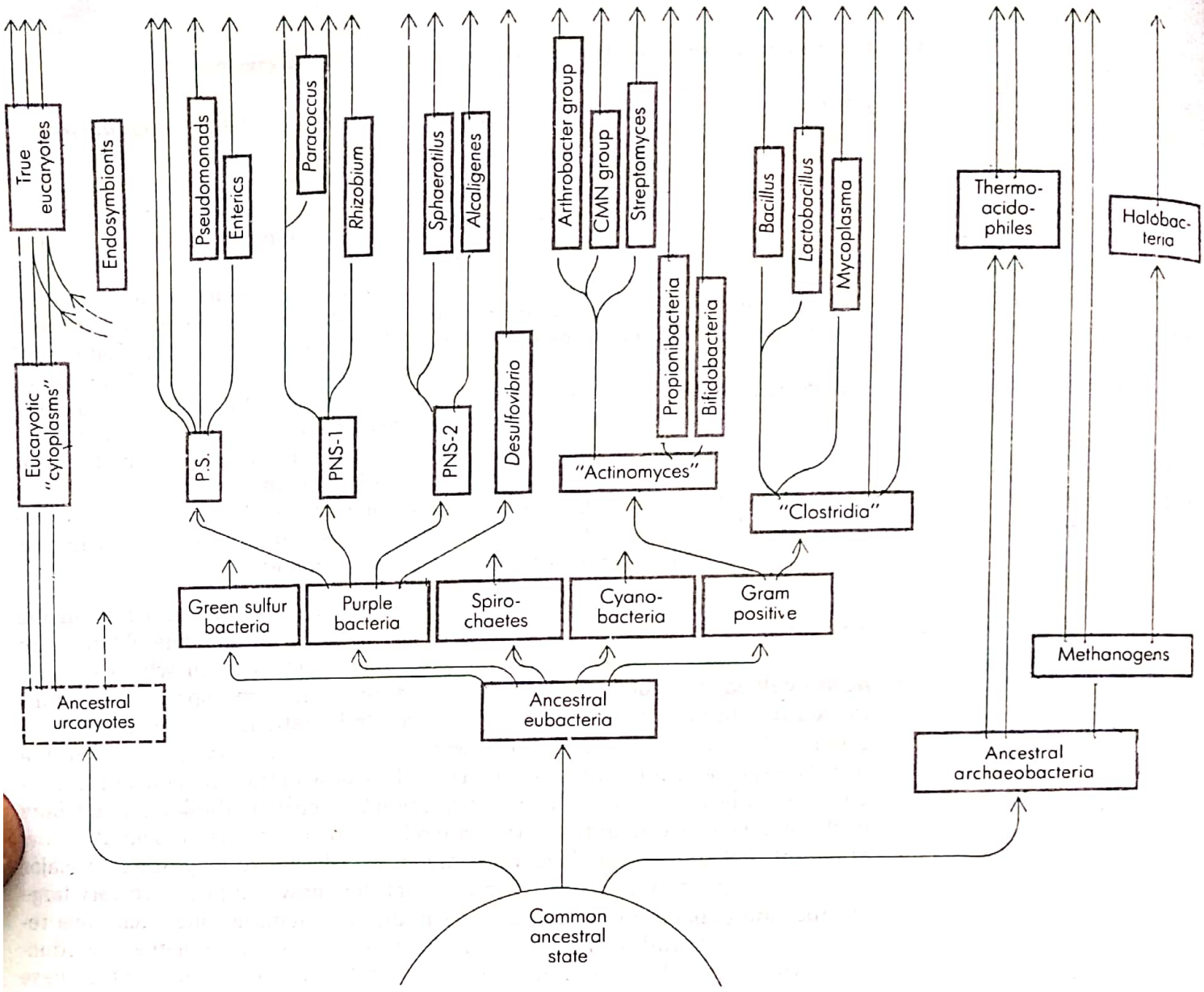


Figure 3-3. Schematic representation of the major lines of procaryotic descent, based on rRNA oligonucleotide cataloging. The archaeobacteria arose from an early ancestral form according to the pathway at the center. (A third line of descent which led to eucaryotic organisms is depicted at the left.) *(Courtesy of G. E. Fox et al., Science* **209:***457–463, 1980.)*

named after H. A. Barker (an American biochemist). Some names are even nonsensical [e.g., *Runella slithyformis*—"the organism whose shape resembles runes (characters of an ancient alphabet) and which is slithy," the latter term being taken from Lewis Carroll's poem "Jabberwocky" from *Alice in Wonderland*]. The important point is that names are only convenient designations. For example, instead of referring to "the rod-shaped, acid-fast bacterium that is slow-growing, is stimulated by glycerol, causes pulmonary tuberculosis in humans, is spread mainly by airborne droplets, forms buff-colored colonies, synthesizes niacin, reduces nitrate to nitrite, and is pathogenic for guinea pigs," it is much more convenient simply to say "*Mycobacterium tuberculosis*."

Although it might seem that microbial names could be constructed almost at random, the fact is that certain rules must be followed. Bacteria, for example, are named according to rules set down in the *International Code of Nomenclature of Bacteria*; other codes govern the naming of algae, fungi, and viruses.

One rule in bacteriological nomenclature is that a name must be written as a Latin or latinized **binomial** (two words) and must follow certain rules of Latin grammar. The first word in the binomial is the genus name and is always capitalized. The second word is the specific epithet and is never capitalized. Both the genus name and specific epithet are given in italics (or underlined, which means "italics" to a printer). Bacteria are sometimes referred to by **common** or **colloquial** names, which have no official standing in nomenclature and are never italicized (for example, the "colon bacillus," which is *E. coli*, or the "tubercle bacillus," which is *M. tuberculosis*). Such names do not lead to precise communication; for instance, many bacteria occur in the colon besides *E. coli*, and other organisms besides *M. tuberculosis* can cause tuberculosis.

Those bacterial names which have official standing in microbiology were published in the *Approved Lists of Bacterial Names* in January, 1980. Any new or additional names must be published in the *International Journal of Systematic Bacteriology* in order to achieve official recognition.

The International Code of Nomenclature of Bacteria was developed with reference to the much earlier established International Codes of Zoological and Botanical Nomenclature. All of these codes incorporate certain common principles as listed below.

1 Each distinct kind of organism is designated as a species.
2 The species is designated by a Latin binomial to provide a characteristic international label (binomial system of nomenclature).
3 Regulation is established for the application of names.
4 A law of priority ensures the use of the oldest available legitimate name.
5 Designation of categories is required for classification of organisms.
6 Requirements are given for effective publication of new specific names, as well as guidance in coining new names.

Identification

An organism must be classified before it can be **identified**—that is, given a name. This is true even if the classification is merely the recognition that the organism is different from any known organism. (For example, this occurred with the Legionnaires' disease agent, which caused the famous pneumonia epidemic in 1976 in Philadelphia; this organism was unlike bacteria of any established species; it has now been classified in a new bacterial genus, *Legionella*, and has been assigned the species name *L. pneumophila*.) Once an organism is classified, a few of its characteristics are selected by which it can be identified by other microbiologists. In order to be useful for identification, the combination of characteristics chosen must occur only in that particular kind of organism and in no other. The characteristics chosen should also be ones that are easy to determine, such as shape, staining reactions, and sugar fermentations. For example, DNA homology experiments, while very useful for classifying an organism, would be quite unsatisfactory for the routine identification of an organism because of the complexity of the procedure.

Many identification schemes are in the form of **keys**, which give identifying characteristics arranged in a logical fashion. **Identification tables** are also useful and generally contain more characteristics than do keys, with the information arranged in an easy-to-read, summarized form.