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MICROBIOLOGY

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（内部交流）

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with 128 illustrations

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PREFACE

Continued research adds new data and corrects older basic data that may be included in a textbook. It is therefore necessary to keep the student up to date in the field of microbiology since an ever-increasing amount of new knowledge is being produced. New antibiotics are being introduced, and the improved methods of testing them have increased their value in treating disease. The explosion of knowledge in the field of transplant immunology has given us a much better understanding of this subject as well as adding impetus to its practical applications. The science of virology has been going forward to a point where a more intimate understanding of this field is a must for everyone acquainting themselves with microbiology. Newer concepts of the relationships of viruses to cancer are exciting, and hopefully a breakthrough in this area will eventually arrange all the pieces of the jigsaw puzzle so that in the future, cancer will be more easily diagnosed, treated, and prevented.

Louis P. Gebhardt
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INTRODUCTION TO MICROBIOLOGY

The science of microbiology (Gr., *micro,* small; *bio,* living) deals with the study of living microorganisms, particularly microscopic plants—the bacteria, molds, yeasts, viruses, and rickettsiae. This science also includes the microscopic animals such as the parasitic animals. The latter, however, are generally studied in zoology and parasitology courses. Viruses can be visualized only by the aid of the electron microscope. Although the complete intact cells of bacteria and other microorganisms may be readily seen by use of the oil immersion lens of a light microscope, the electron microscope is necessary to visualize the structural details.

We are frequently unaware of the presence of these microscopic forms of life. However, their metabolic activities often make their presence evident; for example, the souring of milk, food spoilage, or the development of an illness.

Although microbiology encompasses many subspecialties, it is one of the branches of the larger science of biology. This book will deal only with those microscopic and submicroscopic living forms that have been categorized in the science of microbiology. No attempt will be made to discuss the complete field of microscopic plants and animals.

Not all, in fact very few, microorganisms are disease producing or pathogenic; the majority of these living microscopic plants are frequently helpful to man and his environment. Many assist in soil fertilization; others help in the production of foods such as cheeses and bread, medicines such as antibiotics, and a host of industrial products.

The majority of the bacteria, yeasts, and molds are easily grown on inexpensive food; thus they can be produced in large quantities in lifeless foodstuff (such as meat broth), serum, and milk. Certain yeasts are used to produce protein foodstuffs, which are competitive with other protein in cost.

Since most of the saprophytic (nondisease-producing) microorganisms grow in simple foodstuff, they are found almost everywhere—in the soil, water, air, foods, milk, etc. They may be transported on dust particles for many miles; thus microorganisms are present on any article on which dust is deposited.

Likewise, microorganisms are ever present on the skin of all living things and also

in the mouth and gastrointestinal tract of man, lower animals, insects, etc. Some of those found on the skin and in the mouth and throat as well as those in the intestinal tract may be capable of disease production. Some of the microorganisms found constantly in the intestinal tract are frequently helpful, aiding in the digestion of food, assisting in the production of vitamins, and frequently maintaining a balance so that harmful microorganisms may be prevented from damaging the host when they gain access to the gastrointestinal tract.

Microorganisms have played an important role as tools for the study of the cell nucleic acids and the details of genetic material and have helped immeasurably in understanding the genetic code.

The science of microbiology may be subdivided into numerous specialty areas, each of which requires individual study for a student to become an expert in any particular field.

Basic microbiology. Basic microbiology involves a basic understanding of cultivation, staining, growth characteristics, environment, physiology, biochemistry, morphology, antigenic structure, sterilization, disinfection, and classification. The relationship of closely related microorganisms to each other as well as their relationship to unrelated genera and species must be understood. Thus basic microbiology is a necessary part of all specialty areas.

Agricultural microbiology. The specialty area of agricultural microbiology deals with the soil microorganisms and their role in agriculture—curing of silage, soil fertilization, and diseases of trees, plants, vegetables, cereal grains, fruits, and other crops.

Dairy microbiology. The dairy microbiologist is interested in the manufacture of cured and cottage cheese, spoilage microorganisms, and procedures to rid milk of possible disease-producing microorganisms (e.g., pasteurization). Safe dairy products are the goal of his specialty training.

Veterinary microbiology. The veterinarian must deal with microbial, fungal, and viral diseases of lower animals, and the veterinary microbiologist may pursue this specialty.

Food microbiology. Individuals trained in food microbiology are the ones who protect our food from spoilage, attest to its safety, and maintain rigid testing to be absolutely sure no disease-producing microorganisms are present in the food we eat. This area includes the control of all food-processing and canning industries and proper refrigeration control.

Industrial microbiology. Specialists in the area of industrial microbiology control industrial chemical production by microorganisms (e.g., antibiotics, alcoholic beverages, organic acids, vitamins, and various alcohols), help prevent deterioration of fabrics, and are concerned with oil and geological microbiology and a host of other industrial activities in which microorganisms play a role.

Sanitary microbiology. Individuals trained in sanitary microbiology study the bacteriology of water and assist in the purification of water so that it is safe from disease-producing microorganisms and free from unpleasant odors and tastes. Sanitary microbiologists inspect restaurants for proper food-handling procedures and sanitization of dishes and eating implements; they also regulate other areas of food serving, including the sampling of food and milk and the testing of food handlers to eliminate carriers of disease microorganisms. This specialty area is also concerned with sewage control, making sure that sewage does not contaminate food or water supplies and that sewage treatment is properly carried out.

Research microbiology. Individuals trained in research microbiology may cover all areas of microbiology, from general basic microbiology to the more exotic area of molecular and genetic microbiology.

Medical microbiology. Medical microbiology deals primarily with disease-producing microorganisms, diagnostic microbiology,

medical bacteriology research, and immunological and immunochemical research. These individuals perform diagnostic work in hospitals, clinical laboratories, public health laboratories, research laboratories, and other areas where pathogenic microorganisms are studied.

Mycology. The area of mycology deals with the yeasts and molds. The majority of these microorganisms are useful and beneficial to man. Only a few are pathogenic or harmful to man. Bread making, certain types of cheese production, antibiotic production, beer, and wine production, etc. all depend on an understanding of these microorganisms.

Virology. Virology deals with the smallest of microorganisms, none of which are visible by means of a light microscope but require an electron microscope to enable one to see them. This group of microorganisms, unlike the bacteria, yeasts, molds, etc., require living tissue cells for growth.

• • •

The specific microorganisms alluded to will be discussed more fully as the student progresses.

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HISTORICAL DEVELOPMENT

Prehistoric men faced a fearsome struggle for existence in a harsh and adverse environment. To the struggle for food, shelter, and protection against hostile beasts were no doubt added the constant ravages of infectious diseases. Abnormalities still evident in ancient skeletons bear mute witness of bone infections and arthritis, and the unexplained disappearance of entire tribes and the discovery of burial grounds filled with youthful bodies suggest the havoc wrought by epidemics.

To combat these unseen causes of death, the shaman, or medicine man, came into existence. Diseases were attributed to evil spirits, and the shaman, gruesomely attired and wailing in ghastly tones, sought to drive away the killer demons. The medicine man still exists in many ethnic groups and exerts a powerful influence on tribal activities.

With the dawn of recorded history, written records carried many references to *pestilentia,* the epidemics of disease for which no cause was known. The periods of fear and horror that accompanied the great epidemics often drove entire populations into headlong exodus from stricken areas. Philosophers searched in vain for an explanation of these great surges of illness and death. Hippocrates, the father of medicine, attributed the outbreaks to unhealthy seasonal changes in the atmosphere. Others explained that filth, odors, miasmas, or approaching comets were the cause of these deadly periodic disasters.

Biblical writings suggested that leprosy could be spread by contact (Lev. 14 and Num. 12) and advocated segregation or banishment of an afflicted leper during his period of uncleanliness. In most countries in which leprosy exists, banishment of lepers from society is still practiced.

Numerous observations have been recorded by lay writers regarding the cause of the plague or Black Death epidemic of Italy. Benedetti (1493) in *De Observatione in Pestilentia, Venetiis,* pointedly expressed conviction that to touch a sick person invited disease in a well person, and he further suggested that clothing must be cleaned or purified after it had been worn by a sick person. No prima facie evidence, however, was forthcoming as to how disease was transmitted by touch. The great Italian physician-poet Fracastoro (1546) was perhaps the first to conceive the existence of actual disease-producing agents, seeds of

disease that were probably capable of being transferred from one person to another. He suggested that direct contact, distance contact (probably sneezing and coughing), and articles touched or worn by an ill person containing the seeds of disease could all spread contagion. Fracastoro also wrote on the contagiousness of specific diseases and particularly on the disease syphilis. A sheepherder by the name of Syphilis had contracted the disease we now know as syphilis, and Fracastoro wrote a poem about the man and the disease. However, knowledge of the actual cause of disease was at best speculative and philosophical.

ERA OF THE DISCOVERY OF MICROORGANISMS

Roger Bacon in 1267 described a lens and Salvino D'Armati (d. 1317) was said to have been the inventor of spectacles. However, the microscope did not come into existence until nearly three centuries later. At some time during the period 1591-1608, Zaccharias Janssen (or Jensen), while working with his father (a spectacle maker), placed two lenses together to make a crude compound microscope. This instrument had no provisions for focusing. However, a compound microscope made by Galileo Galilei in 1610, which he termed the *occiale,* had the tube mounted so that it could be focused. Under it, according to his own description, a water flea looked as large as a hen. The name *microscope* was first proposed by Faber (or Fabri) in 1625. In 1650 Huygens invented the eyepiece lens system that bears his name; it is essentially the same as that used in most modern microscopes.

Hooke, the great British microscopist, in *Micrographia* (1665) first applied the term *cell* to the boxlike structures he observed in thin sections of cork when they were examined by means of a compound microscope.

With these developments, the stage was set for the revealing of the world of microorganisms. However, until Antony van Leeuwenhoek began to squint through his simple microscope, the existence of microorganisms was largely a matter of philosophical postulation.

It was about 1670 that Antony van Leeuwenhoek (1632-1723), a draper of Delft, Holland, started his hobby of making simple microscopes. His microscope had only a single lens, but he was able to obtain magnifications up to 160 diameters (some commentaries say 270). This is no doubt higher than that obtainable with the contemporary low-power lens of the compound microscopes. The success of his instrument probably lay in the fact that he devised a system of water immersion by which a drop of water was placed between the lens and the object.

For him nothing was too sacred for observation. He turned his microscope on a great variety of substances. These he described in letters addressed to the Royal Society of London. He was the first to describe spermatozoa, scales of the outer skin, the crystalline lens of the eye, and striations of voluntary muscles. His eighteenth letter to the Royal Society, dated October 9, 1676, contained a description of tiny "animalcules" in water in which peppercorns had been soaked. We recognize that he was describing the now familiar rods, spheres, and spirals that are the characteristic bacterial shapes. Dated September 17, 1683, his thirty-ninth letter described similar forms in material scraped from teeth. He was amused at their active motion and illustrated his letter with drawings of what he saw. Later he observed and described yeast cells found in the dregs of a beer vat.

While Leeuwenhoek may not have been the first to see bacteria under the microscope, certainly he was the first to describe these forms accurately and was probably the first to recognize the value of the microscope as a laboratory instrument.

Fig. 2-1. Modern binocular microscope with three objective lenses: low dry, high dry, and oil immersion.

THE MICROSCOPE

Since Leeuwenhoek's simple water immersion lens microscope, great strides have been made in the construction of compound microscopes that increase the magnification from a few hundred to nearly 2,000 diameters.

The mechanics of using the microscope are not difficult to master, but the student should handle this instrument with great care, for although sturdily built, it is a precision instrument and can be damaged by carelessness.

The component parts of the compound microscope are (1) the eyepiece, or ocular, (2) the objective (lens next to object on microscope slide), and (3) the substage condenser, which cencentrates the light from the mirror or microscope lamp on the object being viewed. Eyepieces are available that provide different magnifications. For example, a 5× eyepiece magnifies the image from the objective five times; the 10×, ten times; etc. The highest practical eyepiece magnification is 15×, although 20× eyepieces are available. However, with the latter only a small circle in the center of the field is in clear focus. The compound microscopes commonly used in microbiology teaching laboratories are usually equipped with 10× eyepieces. Monocular microscopes are equipped with a single eyepiece, whereas the binocular microscope (Fig. 2-1) has a matched pair of eyepieces that magnifies the objective image projected to them through a prism system. The use of dual eyepieces was first developed by Greenough in 1897.

Most microscopes have three objectives mounted in a revolving nosepiece—the low-power (10×), the high-power (40 to 45×), and the oil immersion (95 to 100×) objectives. Since the eyepiece magnifies the image formed by the objective, it follows that total magnification = eyepiece magnification × objective magnification. Thus a 10× objective with a 10× eyepiece would give a magnification of 100 times.

The diameter of the real field observed under the low-power objective is about 1.5 mm, the high-power field is about 0.35 mm, and the oil immersion field is only about 0.15 mm. The oil immersion objective works very close to the microscope slide, and consequently a wide cone of light is required. Unfortunately, as light rays pass through the top surface of the microscope slide, they are bent sufficiently to cause much of the cone of light to be dispersed beyond the objective. This loss can be overcome by placing on the top of the microscope slide a drop of oil with the same refractive index as the slide and the objective lens. The objective is then immersed into the oil, light dispersion does not occur, and a clear image is obtained.

Table 2-1. Characteristics of microscope objective lenses

Common name of objective lens	*Magnification*	*Equivalent focus in millimeters*	*Working distance in millimeters*	*Numerical aperture*
Low power	10×	16.0	6.8	0.25
High power	40–50×	4.0	0.73	0.65
Oil immersion	95–100×	1.8	0.12	1.25

In addition to the magnification values, objectives commonly carry one or two other identifying markings. These are (1) equivalent focus and/or (2) numerical aperture (NA) (Table 2-1).

The numerical aperture furnishes an indication of the resolving power of a given objective since the higher the numerical aperture value, the greater will be the resolving power of a given objective. Magnifying power alone does not determine the effectiveness of a microscope. To provide a clear image of the object being viewed, (1) the objective lens must have sufficient resolving power to show clearly the desired details, (2) the magnification must be adequate to enlarge the fine details enough so they will be easily visible to the eye, (3) the image should be of high quality, and (4) the light should be bright enough to allow the eye to operate at its greatest efficiency.

By using the numerical aperture value of a given objective, the student can easily estimate its resolving power. In a microscope equipped with a substage condenser and with the iris opened to the point that the entire field is illuminated, the resolving power may be calculated by the formula that follows.

$$\text{Resolving power} = \frac{\text{Wavelength of light used}}{2 \times \text{Numerical aperture}}$$

Resolving power is defined as the ability to separate (show clearly) two closely placed points or fine details. When the point is not clearly resolved, it appears as an indistinct disk or the fine details of two small objects close together appear as a blur. A simple explanation of this phenomenon would be to test our own eyes on the headlights of an automobile traveling a straight piece of road. At first, at a great distance away there is a single blob of light. As the car approaches, there is a suggestion of two lights; the nearer the lights get, the more the single blob of light becomes partly separated, until you are able actually to see two distinctly separated headlights.

The wavelength of visible light falls between 8,000 and 4,000 angstrom units (Å). Converted to micrometers (1 μm = 1/1,000 mm), this becomes 0.8 to 0.4 μm. As a simple example of resolving power calculation, select a monochromic band of light with a wavelength of 5,000 Å and a low-power objective with a numerical aperature of 0.25. Then,

$$\text{Resolving power} = \frac{5{,}000 \text{ Å } (0.5 \ \mu\text{m})}{2 \times 0.25} = 1 \ \mu\text{m}$$

Therefore any object smaller than 1 μm could not be clearly resolved. Since many of the smaller rod- and sphere-shaped bacteria are smaller than this limit, it is evident that the low-power objective would be of little value in attempting to study such small forms. The resolving power of an oil immersion lens in a compound microscope is about 0.25 μm.

Dark-field microscope

In the operation of the compound microscope the beam of light passes from the condenser directly through the object on the slide and upward through the objective and eyepiece (Fig. 2-2). Relatively transparent bacteria such as the spirochetes of

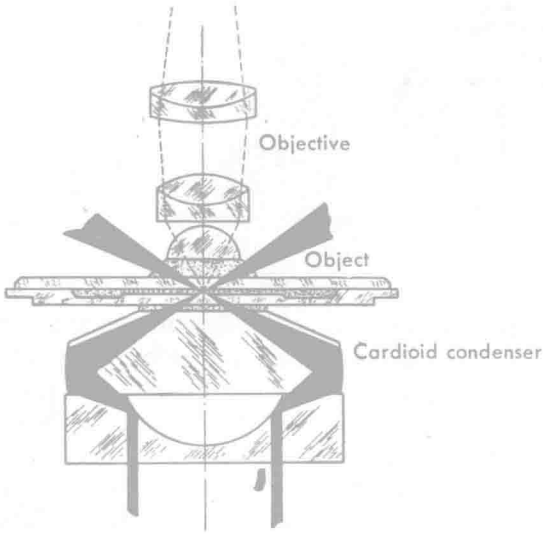

Fig. 2-2. Paths of light rays in a dark-field microscope. (Courtesy Bausch & Lomb Optical Co., Rochester, N. Y.)

syphilis and yaws, which refract light to only a meager degree, are not visible by such direct illumination. Use of a modified condenser system that illuminates obliquely (from the sides) rather than directly makes it possible to observe these forms. Condensers of this type are known as dark-field condensers and may be either cardioid or paraboloid in construction. Dark-field condensers are equipped with an opaque stop in the center that prevents the direct passage of light except around the periphery of the stop. An object on the microscope slide is thus illuminated from the sides and appears as a scintillating self-luminous body against a dark (nearly black) background. The lighted object resembles in appearance the particles of dust seen when a beam of sunlight passes through a darkened room. In addition to being especially useful for the observation of spirochetes, the dark-field microscope is also of value in examining other microorganisms and suspended colloidal particles. Although not as effective as a regular dark-field condenser, the dark-field type of illumination can be obtained by inserting a simple dark-field disk in the filter carrier at the base of the substage condenser.

Phase microscope

Many biological structures, particularly within the cell, are not visible with the ordinary light microscope because they differ only slightly in density or refractive index from the rest of the cell. However, such structures can often be seen with the phase-contrast or phase microscope (Fig. 2-3). This instrument differs from the usual microscope in two respects: (1) a condenser with annular diaphragms is substituted for the usual condenser and (2) the objectives are equipped with diffraction plates. In use, the annular diaphragm is centered so that its image coincides with the diffraction plate of the objective. An essentially oversimplified explanation of the optics is that the incident (in-phase) rays pass through and the diffracted or out-of-phase rays are eliminated. The end result is that objects or structures otherwise invisible are brought into view. The greatest use of the phase microscope is in the examination of living material.

Fluorescence microscope

Certain dyes fluoresce with ultraviolet light—that is, dyes that are one color under ordinary light show a different and often brightly glowing color under ultraviolet light. This phenomenon is utilized in the fluorescence microscope. With this technique the material to be examined is stained with a fluorescent dye and is then observed through a microscope with an ultraviolet light source and a set of filters to absorb the visible light. The stained material appears as a brightly glowing object; the color varies with the filter system used. One use of this microscope is in the diagnosis of tuberculosis. Because of the strong affinity of the fluorescent dye auramine for the waxy components of the tuberculosis organism, selective staining

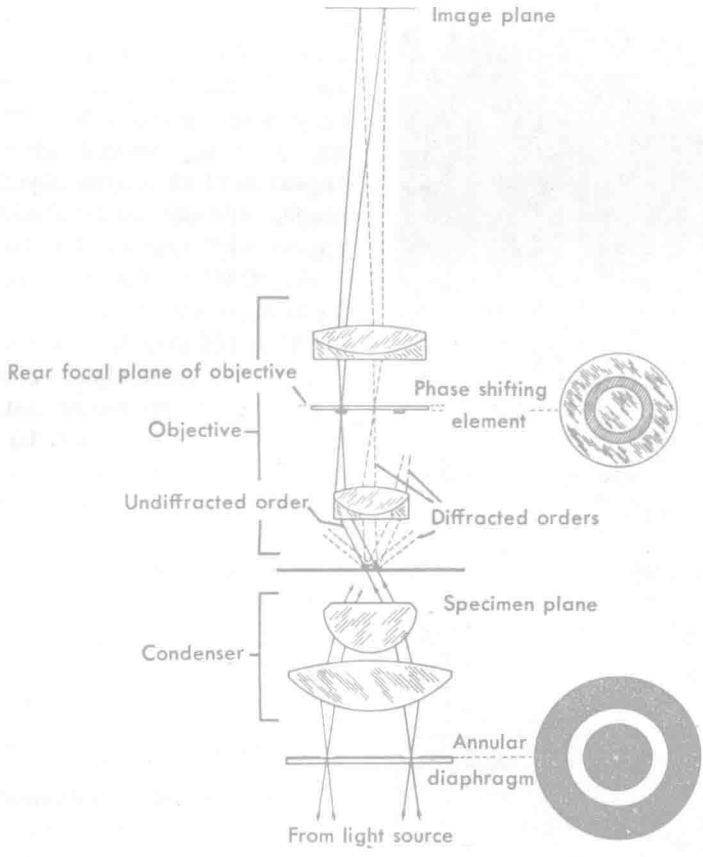

Fig. 2-3. Paths of light rays in a phase microscope. (Courtesy Bausch & Lomb Optical Co., Rochester, N. Y.)

occurs. When examined with the fluorescence microscope, the tuberculosis organism stands out strongly against the nonfluorescing background. Also, by binding fluorescent dyes to specific antibodies, bacteria specific for the antibodies can be detected in the presence of other bacteria. Many antigen-antibody complexes can thus be quickly identified by this ultraviolet system.

Electron microscope

The first electron microscope was developed by Knoll and Ruska in 1932. Its design was based on the earlier observation that a beam of electrons, when passing through a vacuum, is bent by a magnetic field. By the use of a tube that could be evacuated, 60,000 volts to activate the electron source, and properly placed electromagnets to focus the beam, the components of an electron microscope were available. The electron source was analogous to the light source, the electromagnet between the light source and the object being viewed functioned as a substage condenser, and additional electromagnets corresponded in their function to the objective and eyepiece lenses. The electron stream image was made visible with a fluorescent screen, and the image could then be photographed. From this beginning has come the highly effective electron microscopes of today with resolving powers below 10 Å (Fig. 2-4).

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