

4

MICROBIAL DIVERSITY

PART 1 | Acellular and Procaryotic Microbes

CHAPTER OUTLINE

INTRODUCTION

ACELLULAR MICROBES

Viruses

- Origin of Viruses*
- Bacteriophages*
- Animal Viruses*
- Latent Virus Infections*
- Antiviral Agents*
- Oncogenic Viruses*
- Human Immunodeficiency Virus*

Mimivirus

Plant Viruses

Viroids and Prions

THE DOMAIN BACTERIA

Characteristics

- Cell Morphology*
- Staining Procedures*
- Motility*
- Colony Morphology*
- Atmospheric Requirements*
- Nutritional Requirements*
- Biochemical and Metabolic Activities*

Pathogenicity

Genetic Composition

Unique Bacteria

- Rickettsias, Chlamydias, and Closely Related Bacteria*

Mycoplasmas

- Especially Large and Especially Small Bacteria*

Photosynthetic Bacteria

THE DOMAIN ARCHAEA

LEARNING OBJECTIVES

AFTER STUDYING THIS CHAPTER, YOU SHOULD BE ABLE TO:

- Describe the characteristics used to classify viruses (e.g., DNA vs. RNA)
- List five specific properties of viruses that distinguish them from bacteria
- List at least three important viral diseases of humans
- Discuss differences between viroids and virions, and the diseases they cause
- List various ways in which bacteria can be classified
- State the three purposes of fixation
- Define the terms diplococci, streptococci, staphylococci, tetrad, octad, coccobacilli, diplobacilli, streptobacilli, and pleomorphism
- Define the terms obligate aerobe, microaerophile, facultative anaerobe, aerotolerant anaerobe, obligate anaerobe, and capnophile
- State key differences among rickettsias, chlamydias, and mycoplasmas
- Identify several important bacterial diseases of humans
- State several ways in which archaea differ from bacteria

INTRODUCTION

Imagine the excitement that Anton van Leeuwenhoek experienced as he gazed through his tiny glass lenses and became the first person to see live microbes. In the years that have followed his eloquently written late 17th to early 18th century accounts of the bacteria and protozoa that he observed, tens of thousands of microbes have been discovered, described, and classified. In this chapter and the next, you will be introduced to the diversity of form and function that exists in the microbial world.

As you will recall, microbiology is the study of microbes, which are too small to be seen by the naked eye. Microbes can be divided into those that are truly cellular (bacteria, archaea, algae, protozoa, and fungi) and those that are acellular (viruses, viroids, and prions). The cellular microorganisms can be subdivided into those that are procaryotic (bacteria and archaea) and those that are eucaryotic (algae, protozoa, and fungi). For a variety of reasons, acellular microorganisms are not considered by most scientists to be living organisms. Thus, rather than using the term microorganisms to

describe them, viruses, viroids, and prions are more correctly referred to as acellular microbes or infectious particles.

ACELLULAR MICROBES

Viruses

Complete virus particles, called *virions*, are very small and simple in structure. Most viruses range in size from 10 to 300 nm in diameter, although some—like Ebola virus—can be up to 1 μm in length. The smallest virus is about the size of the large hemoglobin molecule of a red blood cell. Scientists were unable to see viruses until electron microscopes were invented in the 1930s. The first photographs of viruses were obtained in 1940. A negative staining procedure, developed in 1959, revolutionized the study of viruses, making it possible to observe unstained viruses against an electron-dense, dark background.

No type of organism is safe from viral infections; viruses infect humans, animals, plants, fungi, protozoa, algae, and bacterial cells (Table 4-1). Many human diseases are caused by viruses (refer back to Table 1-1). Many of the viruses that infect humans are shown in Figure 4-1. Some viruses—called oncogenic viruses or

oncoviruses—cause specific types of cancer, including human cancers such as lymphomas, carcinomas, and some types of leukemia.

Viruses are said to have five specific properties that distinguish them from living cells:

- The vast majority of viruses possess *either* DNA or RNA, unlike living cells, which possess both.
- They are unable to replicate (multiply) on their own; their replication is directed by the viral nucleic acid once it has been introduced into a host cell.
- Unlike cells, they do not divide by binary fission, mitosis, or meiosis.
- They lack the genes and enzymes necessary for energy production.
- They depend on the ribosomes, enzymes, and metabolites (“building blocks”) of the host cell for protein and nucleic acid production.

A typical virion consists of a genome of either DNA or RNA, surrounded by a *capsid* (protein coat), which is composed of many small protein units called *capsomeres*. Together, the nucleic acid and the capsid are referred to as the *nucleocapsid* (Fig. 4-2). Some viruses (called *enveloped viruses*) have an outer envelope composed of lipids and polysaccharides (Fig. 4-3). Bacterial viruses may also have a tail, sheath, and tail fibers. There are no ribosomes for protein synthesis or sites of energy production; hence, the virus must invade and take over a functioning cell to produce new virions.

Viruses are classified by the following characteristics: (a) type of genetic material (either DNA or RNA), (b) shape of the capsid, (c) number of capsomeres, (d) size of the capsid, (e) presence or absence of an envelope, (f) type of host that it infects, (g) type of disease it produces, (h) target cell, and (i) immunologic or antigenic properties.

There are four categories of viruses, based on the type of genome they possess. The genome of most viruses is either double-stranded DNA or single-stranded RNA, but a few viruses possess single-stranded DNA or double-stranded RNA. Viral genomes are usually circular molecules, but some are linear (having two ends). Capsids of viruses have various shapes and symmetry. They may be polyhedral (many sided), helical (coiled tubes), bullet shaped, spherical, or a complex combination of these shapes. Polyhedral capsids have 20 sides or facets; geometrically, they are referred to as icosahedrons. Each facet consists of several capsomeres; thus, the size of the virus is determined by the size of each facet and the number of capsomeres in each.

TABLE 4-1

Relative Sizes and Shapes of Some Viruses

VIRUSES	NUCLEIC ACID TYPE	SHAPE	SIZE RANGE (nm)
Animal Viruses			
Vaccinia	DNA	Complex	200 \times 300
Mumps	RNA	Helical	150–250
Herpes simplex	DNA	Polyhedral	100–150
Influenza	RNA	Helical	80–120
Retroviruses	RNA	Helical	100–120
Adenoviruses	DNA	Polyhedral	60–90
Retroviruses	RNA	Polyhedral	60–80
Papovaviruses	DNA	Polyhedral	40–60
Polioviruses	RNA	Polyhedral	28
Plant Viruses			
Turnip yellow mosaic	RNA	Polyhedral	28
Wound tumor	RNA	Polyhedral	55–60
Alfalfa mosaic	RNA	Polyhedral	18 \times 36–40
Tobacco mosaic	RNA	Helical	18 \times 300
Bacteriophages			
T2	DNA	Complex	65 \times 210
L	DNA	Complex	54 \times 194
F _x -174	DNA	Complex	25

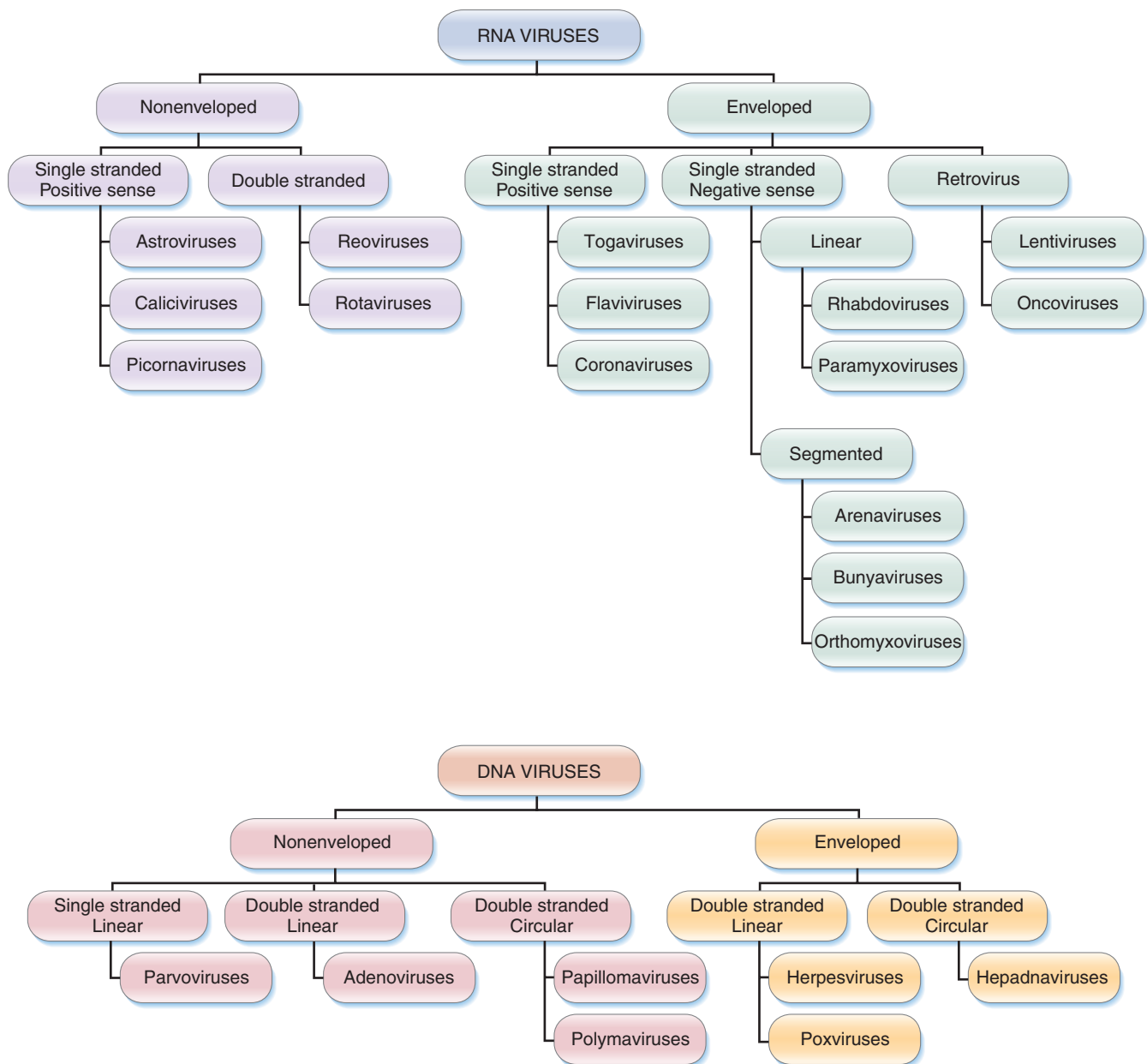


FIGURE 4-1. Some of the viruses that infect humans. Note that some viruses contain RNA, whereas others contain DNA, and that the nucleic acid that they possess may either be single or double stranded. Within the host cell, single-stranded positive sense RNA functions as messenger RNA (mRNA), whereas single-stranded negative sense RNA serves as a template for the production of mRNA. Some of the viruses possess an envelope, whereas others do not. (From Engleberg NC et al. *Schaechter's Mechanisms of Microbial Diseases*, 4th ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

Frequently, the envelope around the capsid makes the virus appear spherical or irregular in shape in electron micrographs. The envelope is acquired by certain animal viruses as they escape from the nucleus or cytoplasm of the host cell by budding (Figs. 4-4 and 4-5). In other words, the envelope is derived from either the host cell's nuclear membrane or cell membrane. Apparently, viruses are then able to alter these membranes by adding protein fibers, spikes, and knobs that enable the virus to recognize the next host cell to be in-

vaded. A list of some viruses, their characteristics, and diseases they cause is presented in Table 4-2. Sizes of some viruses are depicted in Figure 4-6.

Origin of Viruses

Where did viruses come from? Two main theories have been proposed to explain the origin of viruses. One theory states that viruses existed before cells, but this seems unlikely in view of the fact that viruses require cells for their replication. The other theory states that cells came first and

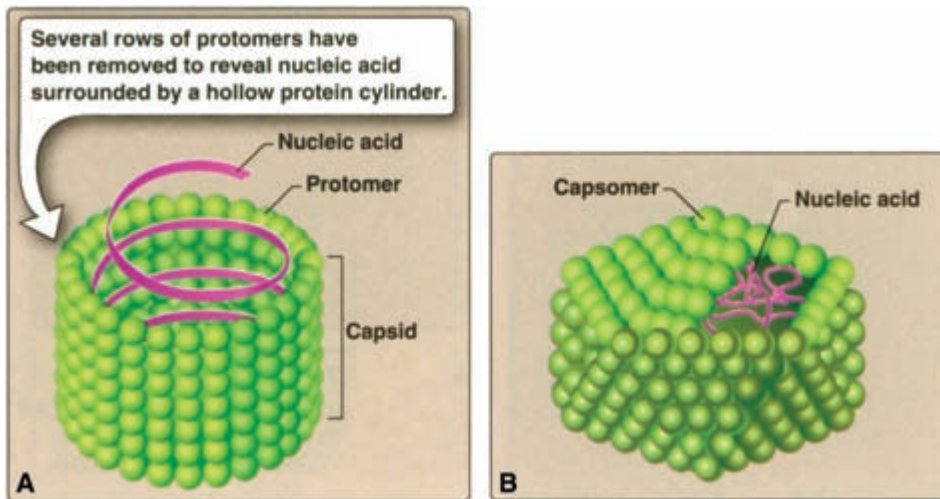


FIGURE 4-2. Viral nucleocapsids. (A) Nucleocapsid of a helical virus. (B) Nucleocapsid of an icosahedral virus. (From Harvey RA et al. Lippincott's Illustrated Reviews: Microbiology, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

that viruses represent ancient derivatives of degenerate cells or cell fragments. The question of whether viruses are alive depends on one's definition of life and, thus, is not an easy question to answer. However, most scientists agree that viruses lack most of the basic features of cells; thus, they consider viruses to be nonliving entities.

Bacteriophages

The viruses that infect bacteria are known as *bacteriophages* (or simply, *phages*). Like all viruses, they are obligate intracellular pathogens, in that they must enter a bacterial cell to replicate. There are three categories of bacteriophages, based on their shape:

- Icosahedron bacteriophages: an almost spherical shape, with 20 triangular facets; the smallest icosahedron phages are about 25 nm in diameter.
- Filamentous bacteriophages: long tubes formed by capsid proteins assembled into a helical structure; they can be up to about 900 nm long.

- Complex bacteriophages: icosahedral heads attached to helical tails; may also possess base plates and tail fibers.

In addition to shape, bacteriophages can be categorized by the type of nucleic acid that they possess; there are single-stranded DNA phages, double-stranded DNA phages, single-stranded RNA phages, and double-stranded RNA phages. From this point, only DNA phages will be discussed.

Bacteriophages can be categorized by the events that occur after invasion of the bacterial cell: some are virulent phages, whereas others are temperate phages. Phages in either category do not actually enter the bacterial cell—rather, they inject their nucleic acid into the cell. It is what happens next that distinguishes virulent phages from temperate phages.

Virulent bacteriophages always cause what is known as the *lytic cycle*, which ends with the destruction (lysis) of the bacterial cell. For most phages, the whole process (from attachment to lysis) takes less than 1 hour.

The steps in the lytic cycle are shown in Table 4-3.

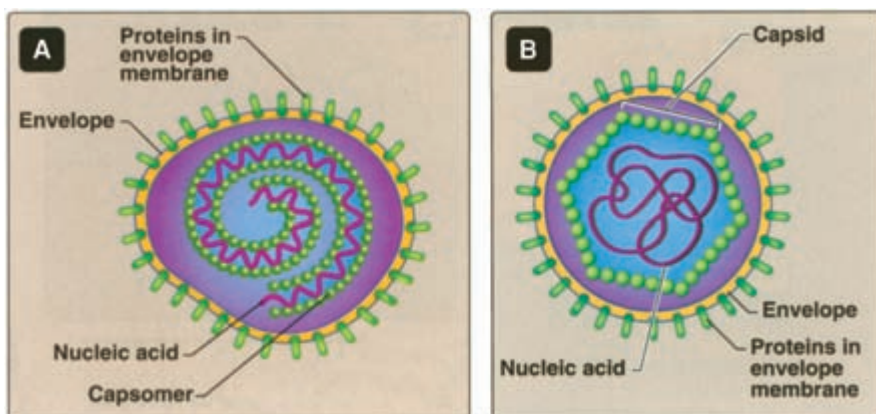


FIGURE 4-3. Enveloped viruses.

(A) Enveloped helical virus.

(B) Enveloped icosahedral virus.

(From Harvey RA et al. Lippincott's Illustrated Reviews: Microbiology, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

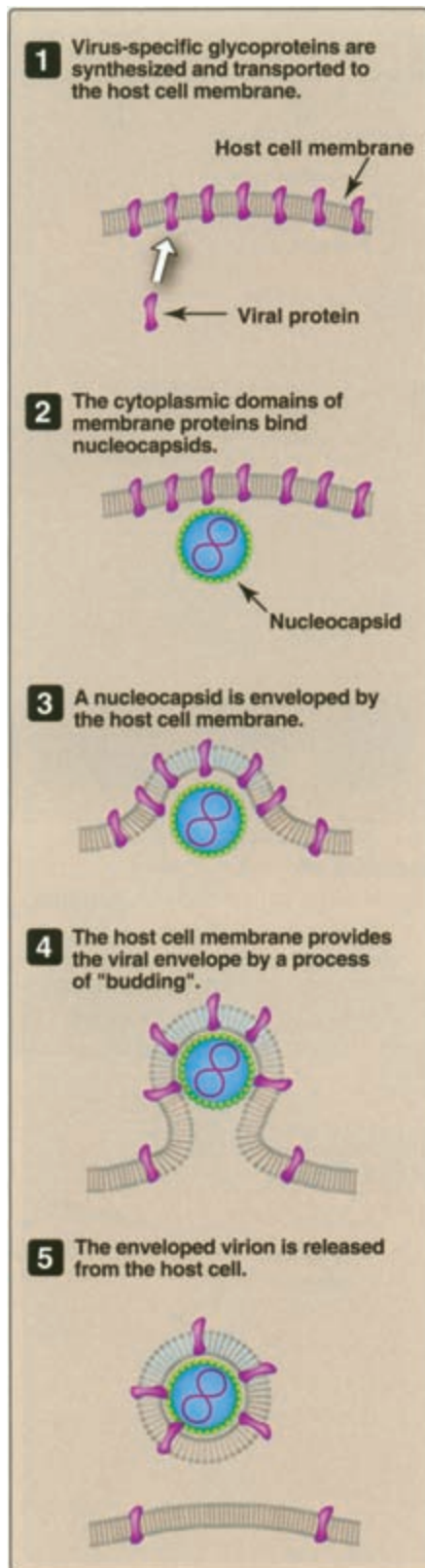


FIGURE 4-4. Virus particle becoming enveloped in the process of budding from a host cell.

(From Harvey RA et al. Lippincott's Illustrated Reviews: Microbiology, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

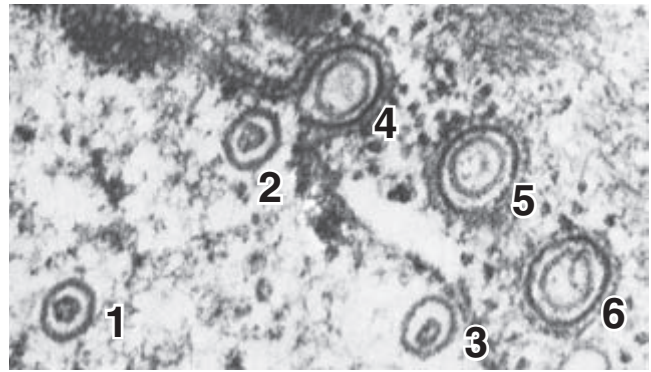


FIGURE 4-5. Herpesviruses acquiring their envelopes as they leave a host cell's nucleus by budding. (1-3) Viruses within the nucleus. (4) Virus in the process of leaving the nucleus by budding. (5, 6) Viruses that have already acquired their envelopes. (From Volk WA, et al. Essentials of Medical Microbiology, 5th ed. Philadelphia: Lippincott-Raven, 1996.)

The first step in the lytic cycle is *attachment* (adsorption) of the phage to the surface of the bacterial cell. The phage can only attach to bacterial cells that possess the appropriate receptor—a protein or polysaccharide molecule on the surface of the cell that is recognized by a molecule on the surface of the phage. Most bacteriophages are species- and strain-specific, meaning that they only infect a particular species or strain of bacteria. Those that infect *Escherichia coli* are called coliphages. Some bacteriophages can attach to more than one species of bacterium. Figure 4-7 shows numerous bacteriophages attached to the surface of a *Vibrio cholerae* cell.

The second step in the lytic cycle is called *penetration*. In this step, the phage injects its DNA into the bacterial cell, acting much like a hypodermic needle (Fig. 4-8). From this point on, the phage DNA “dictates” what occurs within the bacterial cell. This is sometimes described as the phage DNA taking over the host cell’s “machinery.”

The third step in the lytic cycle is called *biosynthesis*. It is during this step that the phage genes are expressed, resulting in the production (biosynthesis) of viral pieces. It is also during this step that the host cell’s enzymes (e.g., DNA polymerase and RNA polymerase), nucleotides, amino acids, and ribosomes are used to make viral DNA and viral proteins. In the fourth step of the lytic cycle, called *assembly*, the viral pieces are assembled to produce complete viral particles (virions). It is during this step that viral DNA is packaged up into capsids.

The final step in the lytic cycle, called *release*, is when the host cell bursts open and all of the new virions

TABLE 4-2

Selected Important Groups of Viruses and Viral Diseases

VIRUS TYPE	VIRAL CHARACTERISTICS	VIRUS	DISEASE
Poxviruses	Large, brick shape with envelope, dsDNA	Variola Vaccinia	Smallpox Cowpox
Polyoma-papilloma	dsDNA, polyhedral	Papillomavirus Polyomavirus	Warts Some tumors, some cancer
Herpesvirus	Polyhedral with envelope, dsDNA	Herpes simplex I Herpes simplex II Herpes zoster Varicella	Cold sores or fever blisters Genital herpes Shingles Chickenpox
Adenovirus	dsDNA, icosahedral, with envelope		Respiratory infections, pneumonia, conjunctivitis, some tumors
Picornaviruses (the name means small RNA viruses)	ssRNA, tiny icosahedral, with envelope	Rhinovirus Poliovirus Hepatitis types A and B Coxsackievirus	Colds Poliomyelitis Hepatitis Respiratory infections, meningitis
Reoviruses	dsRNA, icosahedral with envelope	Enterovirus	Intestinal infections
Myxoviruses	RNA, helical with envelope	Orthomyxoviruses types A and B Myxovirus parotidis Paramyxovirus Rhabdovirus	Influenza Mumps Measles (rubeola) Rabies
Arbovirus	Arthropodborne RNA, cubic	Mosquitoborne type B Mosquitoborne types A and B Tickborne, coronavirus	Yellow fever Encephalitis (many types) Colorado tick fever
Retrovirus	dsRNA, helical with envelope	RNA tumor virus HTLV virus HIV	Tumors Leukemia AIDS

ds, double-stranded; ss, single-stranded.

(about 50–1,000) escape from the cell. Thus, the lytic cycle ends with lysis of the host cell. Lysis is caused by an enzyme that is coded for by a phage gene. At the appropriate time—after assembly—the appropriate viral gene is expressed, the enzyme is produced, and the bacterial cell wall is destroyed. With certain bacteriophages, a phage gene codes for an enzyme that interferes with cell wall synthesis, leading to weakness and, finally, collapse of the cell wall. The lytic cycle is summarized in Figure 4-9.

The other category of bacteriophages—*temperate phages* (also known as *lysogenic phages*)—do not immediately initiate the lytic cycle, but rather, their DNA remains integrated into the bacterial cell chromosome, generation after generation. Temperate bacteriophages are discussed further in Chapter 7.

Bacteriophages are involved in two of the four major ways in which bacteria acquire new genetic information. These processes—called lysogenic conversion and transduction—are discussed in Chapter 7.

Because bacteriophages destroy bacteria, there has been much speculation and experimentation through the years regarding their use to destroy bacterial pathogens and treat bacterial infections. The earliest research of this nature was conducted in the 1930s, but ended when antibiotics were discovered in the 1940s. However, since

Unlike virulent bacteriophages, temperate bacteriophages do not immediately initiate the lytic cycle. Their DNA can remain integrated into the host cell's chromosome for generation after generation.

FIGURE 4-6. Comparative sizes of virions, their nucleic acids, and bacteria. (From Davis BD, et al. Microbiology, 4th ed. Philadelphia: JB Lippincott, 1990.)

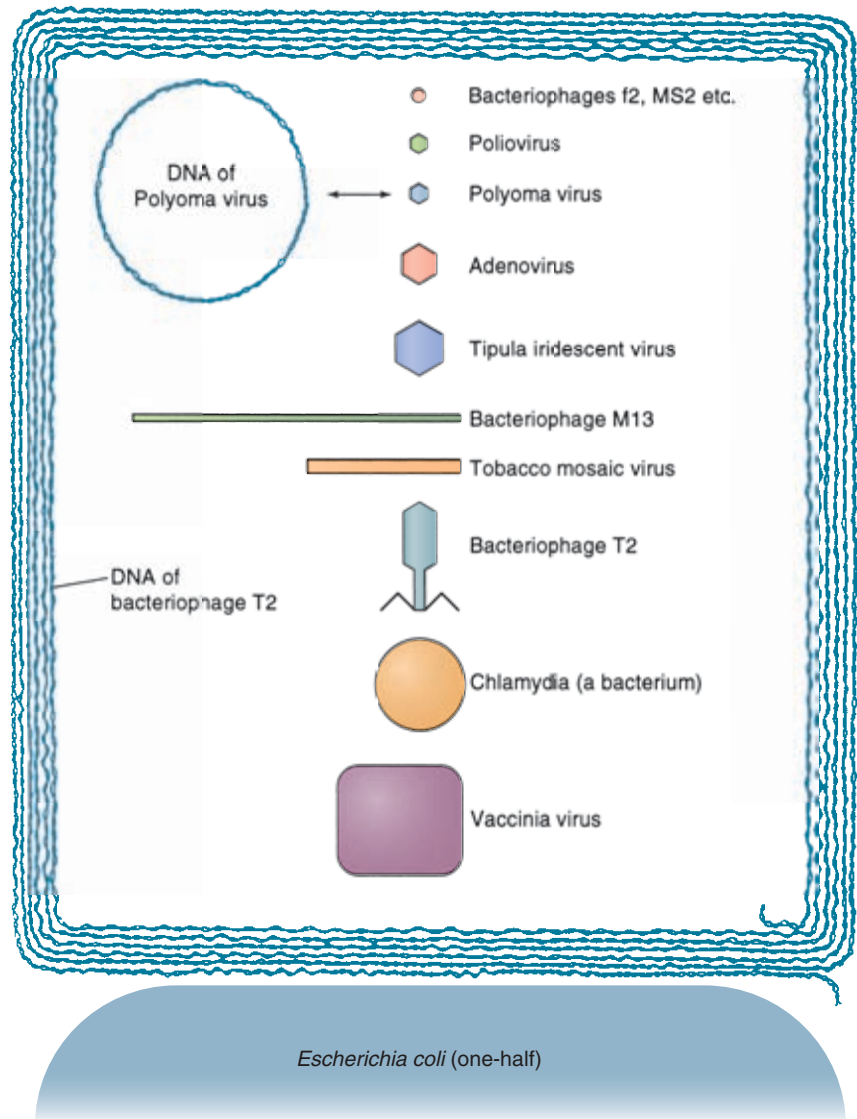


TABLE 4-3 Steps in the Multiplication of Bacteriophages (Lytic Cycle)

STEP	NAME OF STEP	WHAT OCCURS DURING THIS STEP
1	Attachment (adsorption)	The phage attaches to a protein or polysaccharide molecule (receptor) on the surface of the bacterial cell
2	Penetration	The phage injects its DNA into the bacterial cell; the capsid remains on the outer surface of the cell
3	Biosynthesis	Phage genes are expressed, resulting in the production of phage pieces or parts (i.e., phage DNA and phage proteins)
4	Assembly	The phage pieces or parts are assembled to create complete phages
5	Release	The complete phages escape from the bacterial cell by lysis of the cell

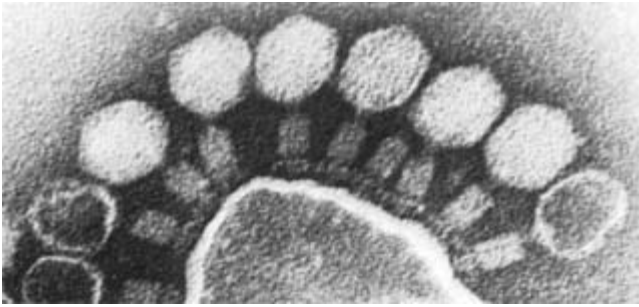


FIGURE 4-7. A partially lysed cell of a *Vibrio cholerae* bacterium, with many attached virions of phage CP-T1. (Courtesy of R.W. Taylor and J.E. Ogg, Colorado State University, Fort Collins, CO.)

the emergence of multidrug-resistant bacteria (“superbugs”), research into the use of bacteriophages to treat bacterial diseases has been renewed. Additionally, bacteriophage enzymes that destroy cell walls or prevent their synthesis are currently being studied for use as therapeutic agents. It is possible that, in the future, certain bacterial diseases will be treated using orally administered or injected pathogen-specific bacteriophages or bacteriophage enzymes.

Animal Viruses

Viruses that infect humans and animals are collectively referred to as *animal viruses*. Some animal viruses are DNA viruses; others are RNA viruses. Animal viruses may consist solely of nucleic acid surrounded by a protein coat (capsid), or they may be more complex. For example, they may be enveloped or they may contain enzymes that play a role in viral multiplication within host cells. The steps in the multiplication of animal viruses are shown in Table 4-4.

The first step in the multiplication of animal viruses is *attachment* (or adsorption) of the virus to the cell. Like bacteriophages, animal viruses can only attach to cells bearing

Like bacteriophages, animal viruses can only attach to and invade cells bearing appropriate surface receptors.

the appropriate protein or polysaccharide receptors on their surface. Did you ever wonder why certain viruses cause infections in dogs, but not in humans, or vice versa? Did you ever wonder why certain viruses cause respiratory infections, whereas others cause gastrointestinal infections? It all boils down to receptors. Viruses can only attach to and invade cells that bear a receptor that they can recognize.

The second step in the multiplication of animal viruses is *penetration*, but, unlike bacteriophages, the entire virion usually enters the host cell, sometimes because the cell phagocytizes the virus (Figs. 4-10, 4-11, and 4-12). This necessitates a third step that was not required for bacteriophages—*uncoating*—whereby the viral nucleic acid escapes from the capsid.

As with bacteriophages, from this point on, the viral nucleic acid “dictates” what occurs within the host cell. The fourth step is *biosynthesis*, whereby many viral pieces (viral nucleic acid and viral proteins) are produced. This step can be quite complicated, depending on what type of virus infected the cell (i.e., whether it was a single-stranded DNA virus, a double-stranded DNA virus, a single-stranded RNA virus, or a double-stranded RNA virus). Some animal viruses do not initiate biosynthesis right away, but rather, remain latent within the host cell for variable periods. Latent viral infections are discussed in more detail in a subsequent section.

The fifth step—*assembly*—involves fitting the virus pieces together to produce complete virions. After the virus particles are assembled, they must escape from the cell—a sixth step called *release*. How they escape from the cell depends on the type of virus that it is. Some animal viruses escape by destroying the host cell, leading to cell destruction and some of the symptoms associated with infection with that particular virus. Other viruses escape the cell by a process known as budding. Viruses that escape from the host cell cytoplasm by budding become surrounded with

Animal viruses escape from their host cells either by lysis of the cell or budding. Viruses that escape by budding become enveloped viruses.

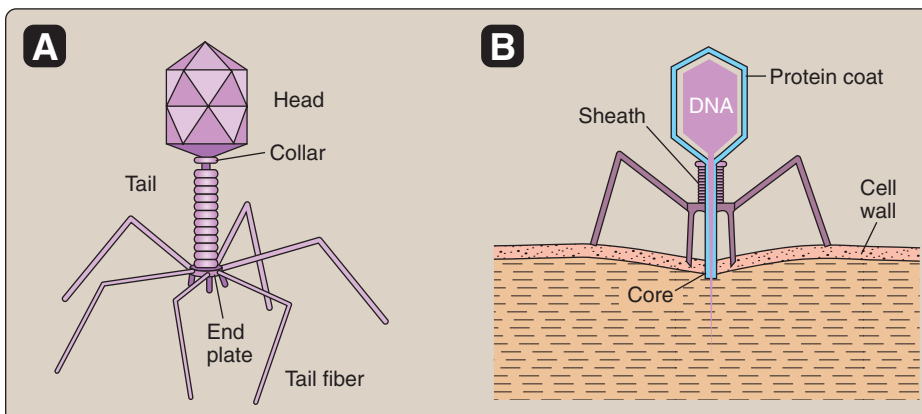


FIGURE 4-8. Bacteriophages.

(A) The bacteriophage T4 is an assembly of protein components. The head is a protein membrane with 20 facets, filled with DNA. It is attached to a tail consisting of a hollow core surrounded by a sheath and based on a spiked end plate to which six fibers are attached. (B) The sheath contracts, driving the core through the cell wall, and viral DNA enters the cell.

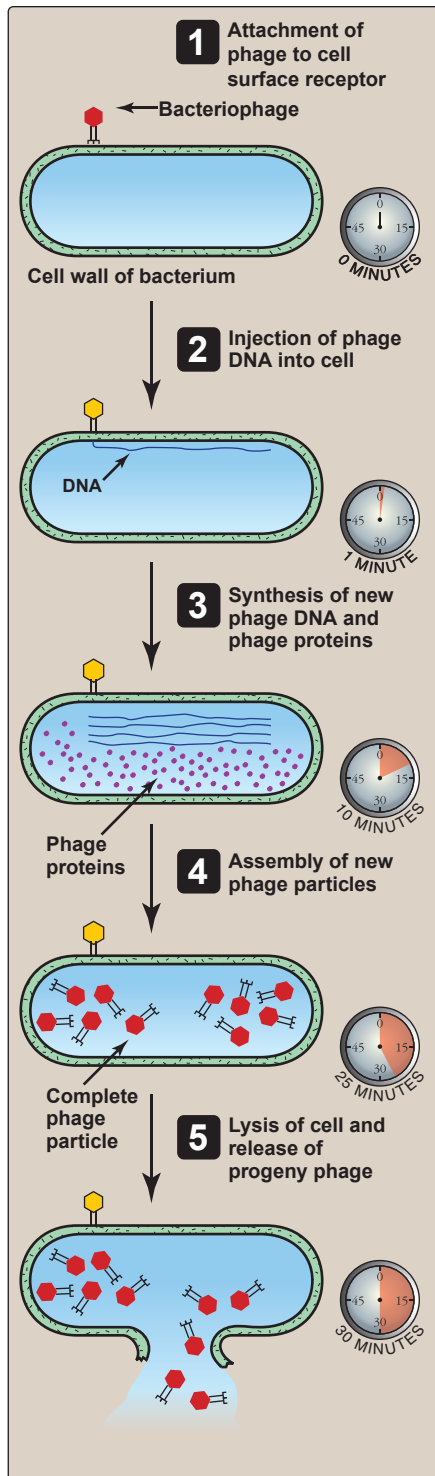


FIGURE 4-9. Summary of the lytic process.

(From Harvey RA et al. Lippincott's Illustrated Reviews: Microbiology, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

pieces of the cell membrane, thus becoming enveloped viruses. If it is an enveloped virus, you know that it escaped from its host cell by budding.

Remnants or collections of viruses, called *inclusion bodies*, are often seen in infected cells and are used as a

diagnostic tool to identify certain viral diseases. Inclusion bodies may be found in the cytoplasm (cytoplasmic inclusion bodies) or within the nucleus (intranuclear inclusion bodies), depending on the particular disease. In rabies, the cytoplasmic inclusion bodies in nerve cells are called Negri bodies. The inclusion bodies of AIDS and the Guarnieri bodies of smallpox are also cytoplasmic. Herpes and poliomyelitis viruses cause intranuclear inclusion bodies. In each case, inclusion bodies may represent aggregates or collections of viruses. Some important human viral diseases include AIDS, chickenpox, cold sores, the common cold, Ebola virus infections, genital herpes infections, German measles, Hantavirus pulmonary syndrome, infectious mononucleosis, influenza, measles, mumps, poliomyelitis, rabies, severe acute respiratory syndrome (SARS), and viral encephalitis. In addition, all human warts are caused by viruses.

Latent Virus Infections

Herpes virus infections, such as cold sores (fever blisters), are good examples of latent virus infections. Although the infected person is always harboring the virus in nerve cells, the cold sores come and go. A fever, stress, or excessive sunlight can trigger the viral genes to take over the cells and produce more viruses; in the process, cells are destroyed and a cold sore develops. Latent viral infections are usually limited by the defense systems of the human body—phagocytes and antiviral proteins called interferons that are produced by virus-infected cells (discussed in Chapter 15). Shingles, a painful nerve disease that is also caused by a herpesvirus, is another example of a latent viral infection. After a chickenpox infection, the virus can remain latent in the human body for many years. Then, when the body's immune defenses become weakened by old age or disease, the latent chickenpox virus resurfaces to cause shingles.

Antiviral Agents

Antibiotics function by inhibiting certain metabolic activities within cellular pathogens, and viruses are not cells. However, for certain patients with colds and influenza, antibiotics may be prescribed in an attempt to prevent secondary bacterial infections that might follow the virus infection. In recent years, a relatively small number

It is very important for healthcare professionals to understand that antibiotics are not effective against viral infections.

Drugs used to treat viral infections are called antiviral agents.

of chemicals—called antiviral agents—have been developed to interfere with virus-specific enzymes and virus production by either disrupting critical phases in viral cycles or inhibiting the synthesis of viral DNA, RNA, or proteins. Antiviral agents are discussed further in Chapter 9.

TABLE 4-4 Steps in the Multiplication of Animal Viruses

STEP	NAME OF STEP	WHAT OCCURS DURING THIS STEP
1	Attachment (adsorption)	The virus attaches to a protein or polysaccharide molecule (receptor) on the surface of a host cell
2	Penetration	The entire virus enters the host cell, in some cases because it was phagocytized by the cell
3	Uncoating	The viral nucleic acid escapes from the capsid
4	Biosynthesis	Viral genes are expressed, resulting in the production of pieces or parts of viruses (i.e., viral DNA and viral proteins)
5	Assembly	The viral pieces or parts are assembled to create complete virions
6	Release	The complete virions escape from the host cell by lysis or budding

Oncogenic Viruses

Viruses that cause cancer are called *oncogenic viruses* or *oncoviruses*. The first evidence that viruses cause cancers came from experiments with chickens. Subsequently, viruses were shown to be the cause of various types of cancers in rodents, frogs, and cats.

Viruses that cause cancer are known as oncogenic viruses or oncoviruses.

Although the causes of many (perhaps most) types of human cancers remain unknown, it is known that *some* human cancers are caused by viruses. Epstein-Barr virus (a type of herpesvirus) causes infectious mononucleosis (not a type of cancer), but also causes three types of human cancers: nasopharyngeal carcinoma, Burkitt lymphoma, and B-cell lymphoma. Kaposi sarcoma, a type of cancer common in AIDS patients, is caused by human

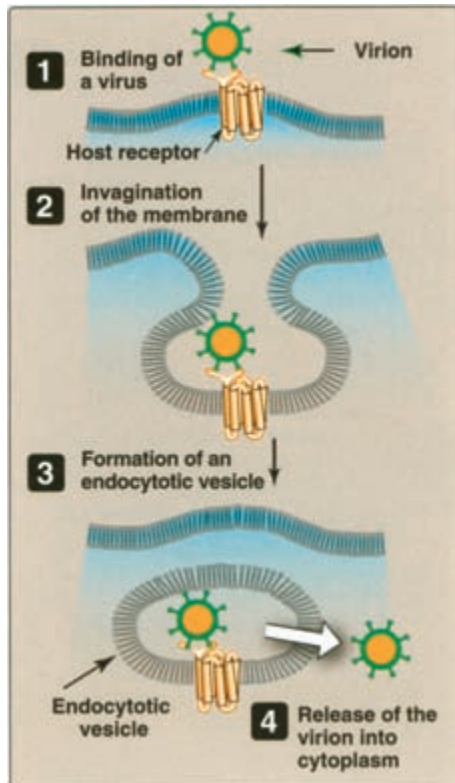


FIGURE 4-10. Penetration of a host cell by a nonenveloped virus via endocytosis. (From Harvey RA et al. Lippincott's Illustrated Reviews: Microbiology, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

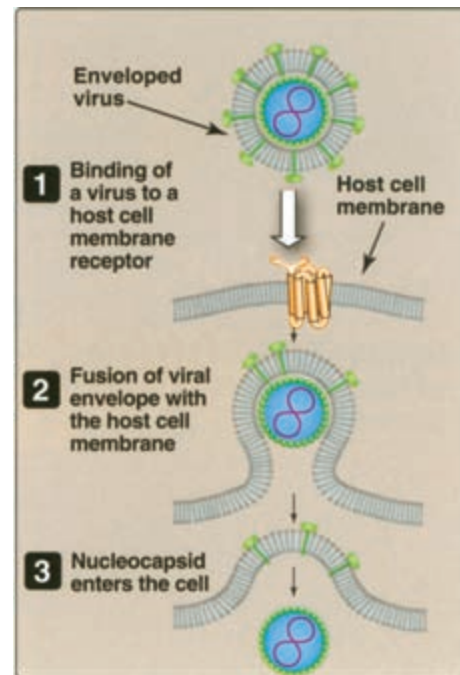


FIGURE 4-11. Penetration of a host cell by an enveloped virus. (From Harvey RA et al. Lippincott's Illustrated Reviews: Microbiology, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

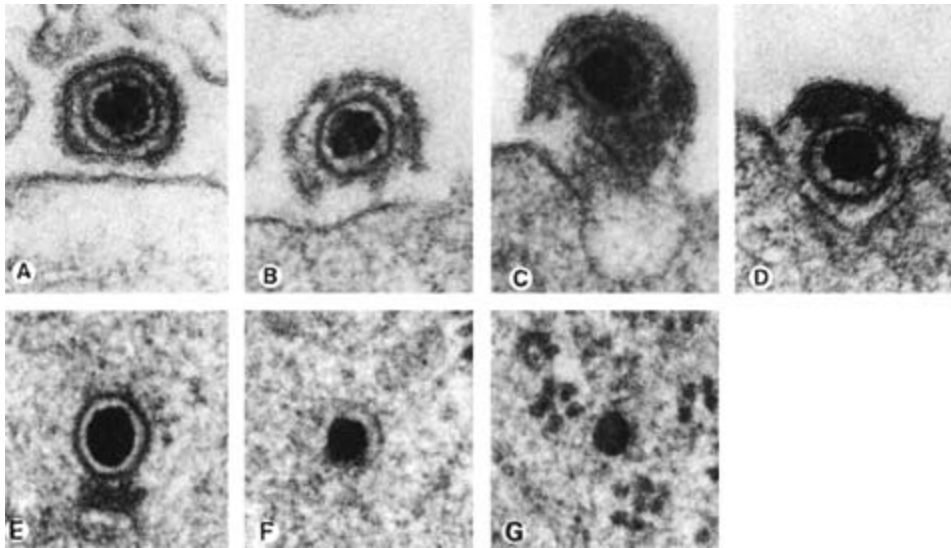


FIGURE 4-12. Infection of host cells by Herpes simplex virus. Adsorption (A), penetration (B–D), and uncoating and digestion of the capsid (E–G) of herpes simplex on HeLa cells, as deduced from electron micrographs of infected cell sections. Penetration involves local digestion of the viral and cellular membranes (B, C), resulting in fusion of the two membranes and release of the nucleocapsid into the cytoplasmic matrix (D). The naked nucleocapsid is intact in E, is partially digested in F, and has disappeared in G, leaving a core containing DNA and protein. (From Morgan C, et al. *J Virol* 1968;2:507.)

herpesvirus 8. Associations between hepatitis B and C viruses and hepatocellular (liver) carcinoma have been established. Human papillomaviruses (HPV; wart viruses) can cause different types of cancer, including cancers of the cervix and other parts of the genital tract. A retrovirus that is closely related to human immunodeficiency virus (HIV; the cause of acquired immunodeficiency syndrome [AIDS]), called human T-lymphotrophic virus type 1 (HTLV-1), causes a rare type of adult T-cell leukemia. All of the mentioned oncogenic viruses, except HTLV-1, are DNA viruses. HTLV-1 is an RNA virus.

Human Immunodeficiency Virus

Human immunodeficiency virus, the cause of AIDS, is an enveloped, single-stranded RNA virus^a (Fig. 4-13). It is a member of a genus of viruses called lentiviruses, in a family of viruses called Retroviridae (retroviruses). HIV is able to attach to and invade cells bearing receptors that the virus recognizes. The most important of these receptors is designated CD4, and cells possessing that receptor are called CD4⁺ cells. The most important of the CD4⁺ cells is the helper T cell (discussed in Chapter 16); HIV infections destroy these important cells of the immune system. Macrophages also possess CD4 receptors and can, thus,

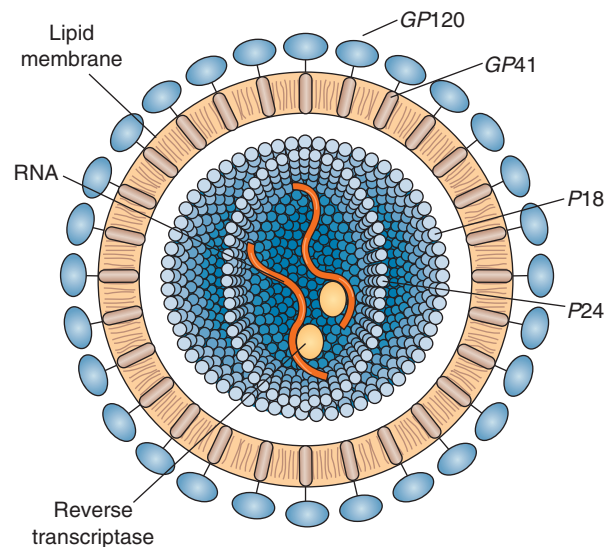


FIGURE 4-13. Human immunodeficiency virus (HIV). HIV is an enveloped virus, containing two identical single-stranded RNA molecules. Each of its 72 surface knobs contains a glycoprotein (designated gp120) capable of binding to a CD4 receptor on the surface of certain host cells (e.g., T-helper cells). The “stalk” that supports the knob is a transmembrane glycoprotein (designated gp41), which may also play a role in attachment to host cells. Reverse transcriptase is an RNA-dependent DNA polymerase. (From Porth CM. *Pathophysiology: Concepts of Altered Health States*, 5th ed. Philadelphia: Lippincott Williams & Wilkins, 1998.)

^a The HIV virion contains two single-stranded RNA molecules.

be invaded by HIV. In addition, HIV is able to invade certain cells that do not possess CD4 receptors, but do possess other receptors that HIV is able to recognize.

Mimivirus

An extremely large double-stranded DNA virus, called Mimivirus, has been recovered from amoebas. The virus was given the name Mimivirus because it “mimics” bacteria. It is so large that it can be observed using a standard compound light microscope. The Mimivirus particle has a 7 nm thick capsid with a diameter of 750 nm. An array of 80- to 125-nm long closely packed fibers project outward from the capsid surface (Fig. 4-14). Within the capsid, its DNA is surrounded by two 4-nm thick lipid membranes. Its genome is at least 10 times larger than that of the large viruses in the smallpox family and larger than the genome of some of the smallest bacteria. Some of its genes code for functions which were previously thought to be the exclusive province of cellular organisms, such as the translation of proteins and DNA repair

enzymes. Mimivirus contains several genes for sugar, lipid, and amino acid metabolism. And, unlike most DNA viruses, Mimivirus contains some RNA molecules. A limited number of reports suggest that Mimivirus may be the cause of some cases of human pneumonia.

Plant Viruses

More than 1,000 different viruses cause plant diseases, including diseases of citrus trees, cocoa trees, rice, barley, tobacco, turnips, cauliflower, potatoes, tomatoes, and many other fruits, vegetables, trees, and grains. These diseases result in huge economic losses, estimated to be in excess of \$70 billion per year worldwide. Plant viruses are usually transmitted via insects (e.g., aphids, leaf hoppers, whiteflies); mites; nematodes (round worms); infected seeds, cuttings, and tubers; and contaminated tools (e.g., hoes, clippers, and saws).

Viroids and Prions

Although viruses are extremely small nonliving infectious agents, viroids and prions are even smaller and less complex infectious agents. *Viroids* consist of short, naked fragments of single-stranded RNA (about 300–400 nucleotides in length) that can interfere with the metabolism of plant cells and stunt the growth of plants, sometimes killing the plants in the process. They are transmitted between plants in the same manner as viruses. Plant diseases thought or known to be caused by viroids include potato spindle tuber (producing small, cracked, spindle-shaped potatoes), citrus exocortis (stunting of citrus trees), and diseases of chrysanthemums, coconut palms, and tomatoes. Thus far, no animal diseases have been discovered that are caused by viroids.

Prions (pronounced “pre-ions”) are small infectious proteins that apparently cause fatal neurological diseases in animals, such as scrapie (pronounced “scrape-ee”) in sheep and goats; bovine spongiform encephalopathy (BSE; “mad cow disease”; see “Insight: Microbes in the News: ‘Mad Cow Disease’” on the CD-ROM); and kuru, Creutzfeldt-Jakob (C-J) disease, Gerstmann-Sträussler-Scheinker (GSS) disease, and fatal familial insomnia in humans. Similar diseases in mink, mule deer, Western white-tailed deer, elk,

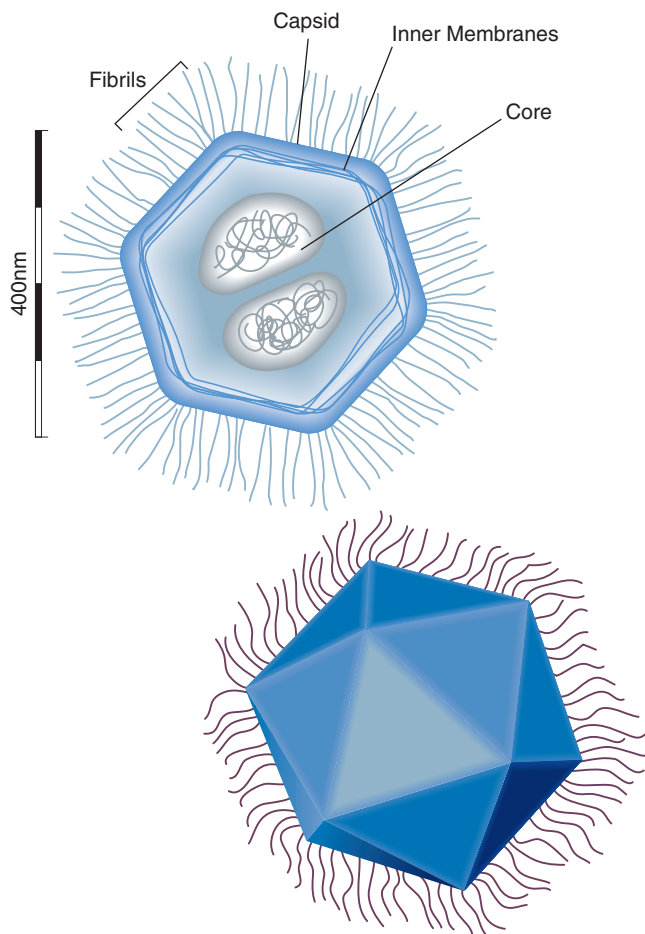


FIGURE 4-14. Mimivirus structure. The Mimivirus virion consists of a double-stranded DNA core, surrounded by two lipid membranes and a protein capsid. Numerous fibrils extend outward from the capsid surface. (Courtesy of Xanthine at <http://en.wikipedia.org>.)



STUDY AID

Beware of Similar Sounding Terms

A **virion** is a complete viral particle (i.e., one that has all its parts, including nucleic acid and a capsid). A **viroid** is an infectious RNA molecule.

and cats may also be caused by prions. The name “scrapie” comes from the observation that infected animals scrape themselves against fence posts and other objects in an effort to relieve the intense pruritus (itching) associated with the disease. The disease in deer and elk is called “chronic wasting disease,” in reference to the irreversible weight loss that the animals experience.

Kuru is a disease that was once common among natives in Papua, New Guinea, where women and children ate human brains as part of a traditional burial custom (ritualistic cannibalism). If the brain of the deceased person contained prions, then persons who ate that brain developed kuru. Kuru, C-J disease, and GSS disease involve loss of coordination and dementia. Dementia, a general mental deterioration, is characterized by disorientation and impaired memory, judgment, and intellect. In fatal familial insomnia, insomnia and dementia follow difficulty sleeping. All these diseases are fatal spongiform encephalopathies, in which the brain becomes riddled with holes (spongiform).

Scientists have been investigating the link between “mad cow disease” and a form of C-J disease (called variant CJD or vCJD) in humans. As of December 2008, 207 cases of vCJD had been diagnosed worldwide, including 164 in the United Kingdom; these cases probably resulted from eating prion-infected beef. The cattle may have acquired the disease through ingestion of cattle feed that contained ground-up parts of prion-infected sheep.

The 1997 Nobel Prize for Physiology or Medicine was awarded to Stanley B. Prusiner, the scientist who coined the term prion and studied the role of these proteinaceous infectious particles in disease. Of all pathogens, prions are believed to be the most resistant to disinfectants. The mechanism by which prions cause disease remains a mystery, although it is thought that prions convert normal protein molecules into nonfunctional ones by causing the normal molecules to change their shape. Many scientists remain unconvinced that proteins alone can cause disease.

THE DOMAIN BACTERIA

Characteristics

Recall from Chapter 3 that there are two domains of prokaryotic organisms: Domain *Bacteria* and Domain *Archaea*. The bacteriologist’s most important reference (sometimes referred to as the bacteriologist’s “bible”) is a five-volume set of books entitled *Bergey’s Manual of Systematic Bacteriology* (*Bergey’s Manual* for short), which is currently being rewritten. (An outline of these volumes can be found on CD-ROM Appendix 2: “Phyla and Medically Significant Genera Within the Domain *Bacteria*.”) When all five volumes have been completed, they will contain descriptions of more than 5,000 validly named species of bacteria. Some authorities believe that this number represents only from less than 1% to a few percent of the total number of bacteria that exist in nature.

According to *Bergey’s Manual*, the Domain *Bacteria* contains 23 phyla, 32 classes, 5 subclasses, 77 orders, 14 suborders, 182 families, 871 genera, and 5,007 species. Organisms in this domain are broadly divided into three phenotypic categories (i.e., categories based on their physical characteristics): (a) those that are Gram-negative and have a cell wall, (b) those that are Gram-positive and have a cell wall, and (c) those that lack a cell wall. (The terms Gram-positive and Gram-negative are explained in a subsequent section of this chapter.) Using computers, microbiologists have established numerical taxonomy systems that not only help to identify bacteria by their physical characteristics, but also can help establish how closely related these organisms are by comparing the composition of their genetic material and other cellular characteristics. (Note: as previously mentioned, throughout this book, the term “to identify an organism” means to learn the organism’s species name [i.e., to speciate it].)

Many characteristics of bacteria are examined to provide data for identification and classification. These characteristics include cell shape and morphological arrangement, staining reactions, motility, colony morphology, atmospheric requirements, nutritional requirements, biochemical and metabolic activities, specific enzymes that the organism produces, pathogenicity (the ability to cause disease), and genetic composition.

Cell Morphology

With the compound light microscope, the size, shape, and morphologic arrangement of various bacteria are easily observed. Bacteria vary greatly in size, usually ranging from spheres measuring about 0.2 μm in diameter to 10.0- μm -long spiral-shaped bacteria, to even longer filamentous bacteria. As previously mentioned, the average coccus is about 1 μm in diameter, and the average bacillus is about 1 μm wide \times 3 μm long. Some unusually large bacteria and unusually small bacteria have also been discovered (discussed later).

There are three basic shapes of bacteria (Fig. 4-15): (a) round or spherical bacteria—the *cocci* (sing., *coccus*); (b) rectangular or rod-shaped bacteria—the *bacilli* (sing., *bacillus*); and (c) curved and spiral-shaped bacteria (sometimes referred to as spirilla).

Recall from Chapter 3 that bacteria divide by binary fission—one cell splits in half to become two daughter cells. The time it takes for one cell to

A bacterium’s Gram reaction (Gram-positive or Gram-negative), basic cell shape, and morphological arrangement of the cells are very important clues to the organism’s identification.

The three general shapes of bacteria are round (cocci), rod-shaped (bacilli), and spiral-shaped.

Bacteria reproduce by binary fission. The time it takes for one bacterial cell to split into two cells is referred to as that organism’s generation time.

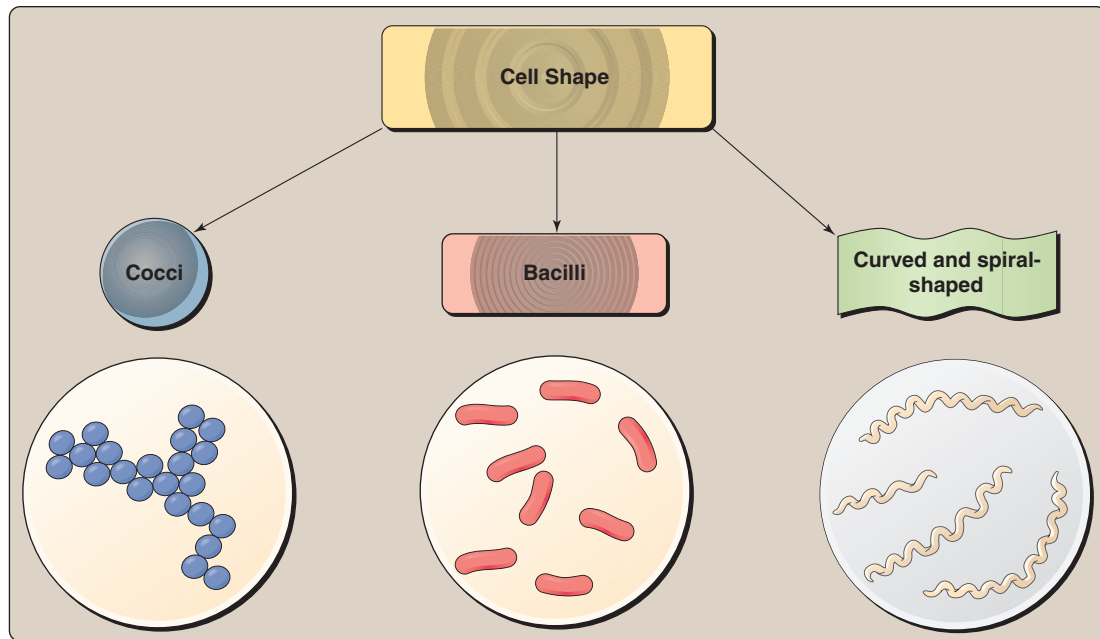


FIGURE 4-15. Categories of bacteria based on the shape of their cells.

split into two cells is referred to that organism's generation time. After binary fission, the daughter cells may separate completely from each other or may remain connected, forming various morphologic arrangements.

Cocci may be seen singly or in pairs (*diplococci*), chains (*streptococci*), clusters (*staphylococci*), packets of four (*tetrads*), or packets of eight (*octads*), depending on the particular species and the manner in which the cells divide (Figs. 4-16 and 4-17). Examples of medically important cocci include *Enterococcus* spp., *Neisseria* spp., *Staphylococcus* spp., and *Streptococcus* spp.

Pairs of cocci are known as diplococci.
Chains of cocci are known as streptococci.
Clusters of cocci are known as staphylococci.

Bacilli (often referred to as rods) may be short or long, thick or thin, and pointed or with curved or blunt ends. They may occur singly, in pairs (*diplobacilli*), in chains (*streptobacilli*), in long filaments, or branched. Some rods are quite short, resembling elongated cocci; they are called *coccobacilli*. *Listeria monocytogenes* and *Haemophilus influenzae* are examples of coccobacilli. Some bacilli stack up next to each other, side by side in a palisade arrangement, which is characteristic of *Corynebacterium diphtheriae* (the cause of diphtheria) and organisms that resemble it in appearance (called *diphtheroids*). Examples of medically important bacilli include members of the family *Enterobacteriaceae* (e.g., *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Salmonella*, and *Shigella* spp.), *Pseudomonas aeruginosa*, *Bacillus* spp., and *Clostridium* spp.

Curved and spiral-shaped bacilli are placed into a third morphologic grouping. For example, *Vibrio* spp., such as *V. cholerae* (the cause of cholera) and *V. parahaemolyticus* (a cause of diarrhea), are curved (comma-shaped) bacilli. Curved bacteria usually occur singly, but some species may form pairs. A pair of curved bacilli



STUDY AID

Bacterial Names Sometimes Provide a Clue to Their Shape

If “coccus” appears in the name of a bacterium, you automatically know the shape of the organism—spherical. Examples include genera such as *Enterococcus*, *Peptococcus*, *Peptostreptococcus*, *Staphylococcus*, and *Streptococcus*. However, not all cocci have “coccus” in their names (e.g., *Neisseria* spp.). If “bacillus” appears in the name of a bacterium, you automatically know the shape of the organism—rod-shaped or rectangular. Examples include genera such as *Actinobacillus*, *Bacillus*, *Lactobacillus*, and *Streptobacillus*. However, not all bacilli have “bacillus” in their names (e.g., *E. coli*).



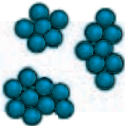
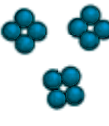



STUDY AID

Beware the Word “Bacillus”

Whenever you see the word *Bacillus*, capitalized and underlined or italicized, it is a particular genus of rod-shaped bacteria. However, if you see the word *bacillus*, and it is not capitalized, underlined, or italicized, it refers to any rod-shaped bacterium.

FIGURE 4-16. Morphologic arrangements of cocci and examples of bacteria having these arrangements.

Arrangement	Description	Appearance	Example	Disease
Diplococci	Cocci in pairs		<i>Neisseria gonorrhoeae</i>	Gonorrhea
Streptococci	Cocci in chains		<i>Streptococcus pyogenes</i>	Strep throat
Staphylococci	Cocci in clusters		<i>Staphylococcus aureus</i>	Boils
Tetrad	A packet of 4 cocci		<i>Micrococcus luteus</i>	Rarely pathogenic
Octad	A packet of 8 cocci		<i>Sarcina ventriculi</i>	Rarely pathogenic

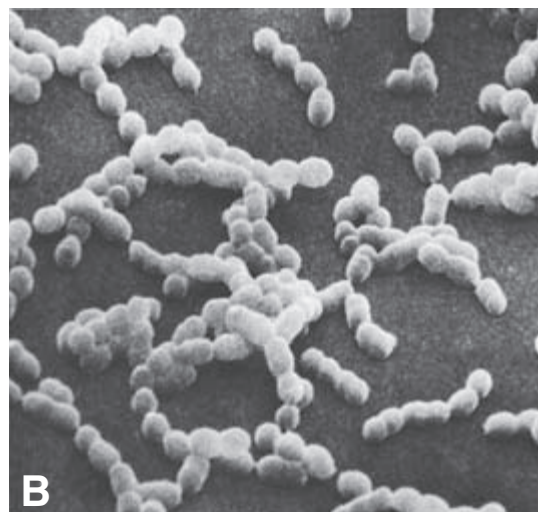
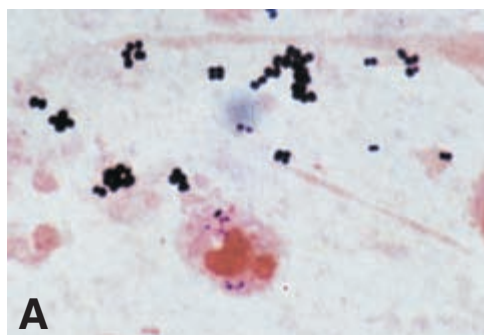


FIGURE 4-17. Morphologic arrangements of cocci. (A) Photomicrograph of Gram-stained *Staphylococcus aureus* cells illustrating Gram-positive (blue) cocci in grapelike clusters. A pink-stained white blood cell can also be seen in the lower portion of the photomicrograph. (B) Scanning electron micrograph of *Streptococcus mutans* illustrating cocci in chains. [A] From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006. [B] From Volk WA, et al. Essentials of Medical Microbiology, 5th ed. Philadelphia: Lippincott-Raven, 1996.)

resembles a bird and is described as having a gull-wing morphology. *Campylobacter* spp. (a common cause of diarrhea) have a gull-wing morphology. Spiral-shaped bacteria are referred to as spirochetes. Different species of spirochetes vary in size, length, rigidity, and the number and amplitude of their coils. Some are tightly coiled, such as *Treponema pallidum*, the cause of syphilis, with a flexible cell wall that enables them to move readily through tissues (Fig. 4-18). Its morphology and characteristic motility—spinning around its long axis—make *T. pallidum* easy to recognize in wet preparations of clinical specimens obtained from patients with primary syphilis. *Borrelia* spp., the causative agents of Lyme disease and relapsing fever, are examples of less tightly coiled spirochetes (Fig. 4-19).

Some bacteria may lose their characteristic shape because adverse growth conditions (e.g., the presence of certain antibiotics) prevent the production of normal cell walls. They are referred to as cell wall-deficient (CWD) bacteria. Some CWD bacteria revert to their original shape when placed in favorable growth conditions, whereas others do not. Bacteria in the genus *Mycoplasma* do not have cell walls; thus, when examined microscopically, they appear in various shapes. Bacteria that exist in a variety of shapes are described as being *pleomorphic*; the ability to exist in a variety of shapes is known as *pleomorphism*. Because they have no cell walls, mycoplasmas are resistant to antibiotics that inhibit cell wall synthesis.

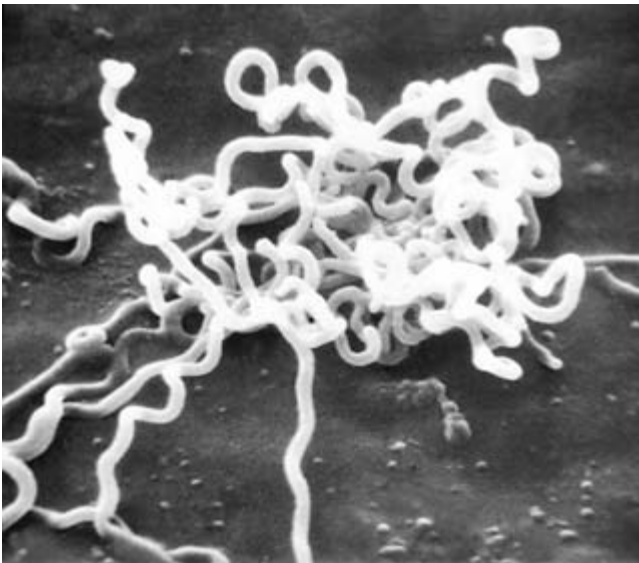


FIGURE 4-18. Scanning electron micrograph of *Treponema pallidum*, the bacterium that causes syphilis. (Courtesy of Dr. David Cox and the Centers for Disease Control and Prevention.)

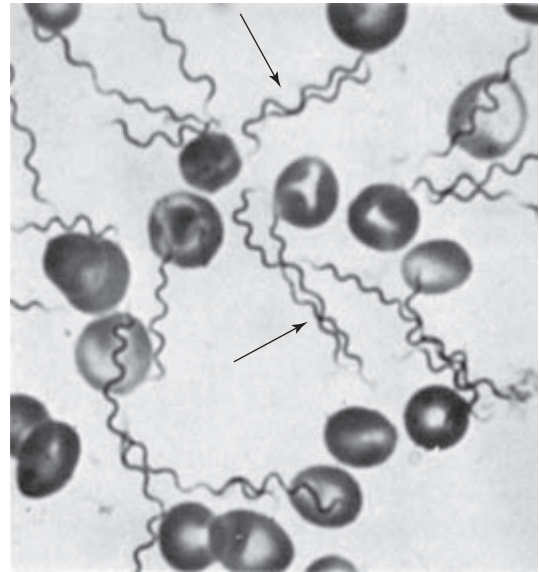


FIGURE 4-19. Spiral-shaped *Borrelia hermsii* (arrows), a cause of relapsing fever, in a stained blood smear. (From Volk WA, et al. *Essentials of Medical Microbiology*, 5th ed. Philadelphia: Lippincott-Raven, 1996.)

Staining Procedures

As they exist in nature, most bacteria are colorless, transparent, and difficult to see. Therefore, various staining methods have been devised to enable scientists to examine bacteria. In preparation for staining, the bacteria are smeared onto a glass microscope slide (resulting in what is known as a “smear”), air-dried, and then “fixed.” (Methods for preparing and fixing smears are further described in CD-ROM Appendix 5: “Clinical Microbiology Laboratory Procedures.”) The two most common methods of fixation are heat fixation and methanol fixation. Heat fixation is usually accomplished by passing the smear through a Bunsen burner flame. If not performed properly, excess heat can distort the morphology of the cells. Methanol fixation, which is accomplished by flooding the smear with absolute methanol for 30 seconds, is a more satisfactory fixation technique. In general, fixation serves three purposes:

1. It kills the organisms.
2. It preserves their morphology (shape).
3. It anchors the smear to the slide.

Specific stains and staining techniques are used to observe bacterial cell morphology (e.g., size, shape, morphologic arrangement, composition of cell wall, capsules, flagella, endospores).

A *simple stain* is sufficient to determine bacterial shape and morphologic arrangement (e.g., pairs, chains, clusters). For this method, shown in Figure 4-20, a dye (such

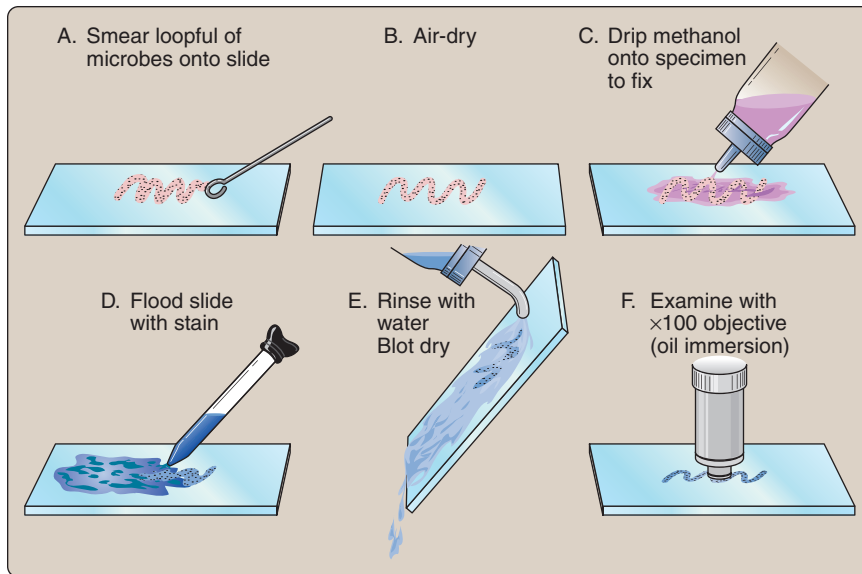


FIGURE 4-20. Simple bacterial staining technique. (A) With a flamed loop, smear a loopful of bacteria suspended in broth or water onto a slide. (B) Allow slide to air-dry. (C) Fix the smear with absolute (100%) methanol. (D) Flood the slide with the stain. (E) Rinse with water and blot dry with bibulous paper or paper towel. (F) Examine the slide with the $\times 100$ microscope objective, using a drop of immersion oil directly on the smear.

as methylene blue) is applied to the fixed smear, rinsed, dried, and examined using the oil immersion lens of the microscope. The procedures used to observe bacterial capsules, spores, and flagella are collectively referred to as *structural staining procedures*.

In 1883, Dr. Hans Christian Gram developed a staining technique that bears his name—the Gram stain or Gram staining procedure. The Gram stain has become the most important staining procedure in the bacteriology laboratory, because it differentiates between “Gram-positive” and “Gram-negative” bacteria (these terms will be explained shortly). The organism’s Gram reaction serves as an extremely important “clue” when

attempting to learn the identity (species) of a particular bacterium. The steps in the Gram staining procedure are described in CD-ROM Appendix 5: “Clinical Microbiology Laboratory Procedures” and illustrated in Fig. 4-21.

The color of the bacteria at the end of the Gram staining procedure depends on the chemical composition of their cell wall (Table 4-5). If the bacteria were not decolorized during the decolorization step, they will be blue to purple at the conclusion of the Gram staining procedure; such bacteria are said to be “Gram-positive.” The thick layer of peptidoglycan in the cell walls of Gram-positive bacteria makes it difficult to remove the crystal violet–iodine complex during the decolorization step. Figures 4-22 through 4-26 depict various Gram-positive bacteria.

If, on the other hand, the crystal violet was removed from the cells during the decolorization step, and the cells were subsequently stained by the safranin (a red dye), they will be pink to red at the conclusion of the Gram staining procedure; such bacteria are said to be “Gram-negative.” The thin layer of peptidoglycan in the cell walls of Gram-negative bacteria makes it easier to remove the crystal violet–iodine complex during decolorization. In addition, the decolorizer dissolves the lipid in the cell walls of Gram-negative bacteria; this destroys the integrity of the cell wall and makes it much easier to remove the crystal violet–iodine complex. Figures 4-27 and 4-28 depict various Gram-negative bacteria.



HISTORICAL NOTE

The Origin of the Gram Stain

While working in a laboratory in the morgue of a Berlin hospital in the 1880s, a Danish physician named **Hans Christian Gram** developed what was to become the most important of all bacterial staining procedures. He was developing a staining technique that would enable him to see bacteria in the lung tissues of patients who had died of pneumonia. The procedure he developed—now called the *Gram stain*—demonstrated that two general categories of bacteria cause pneumonia: some of them stained blue and some of them stained red. The blue ones came to be known as Gram-positive bacteria, and the red ones came to be known as Gram-negative bacteria. It was not until 1963 that the mechanism of Gram differentiation was explained by M.R.J. Salton.

If a bacterium is blue to purple at the end of the Gram staining procedure, it is said to be Gram-positive. If, on the other hand, it ends up being pink to red, it is said to be Gram-negative.

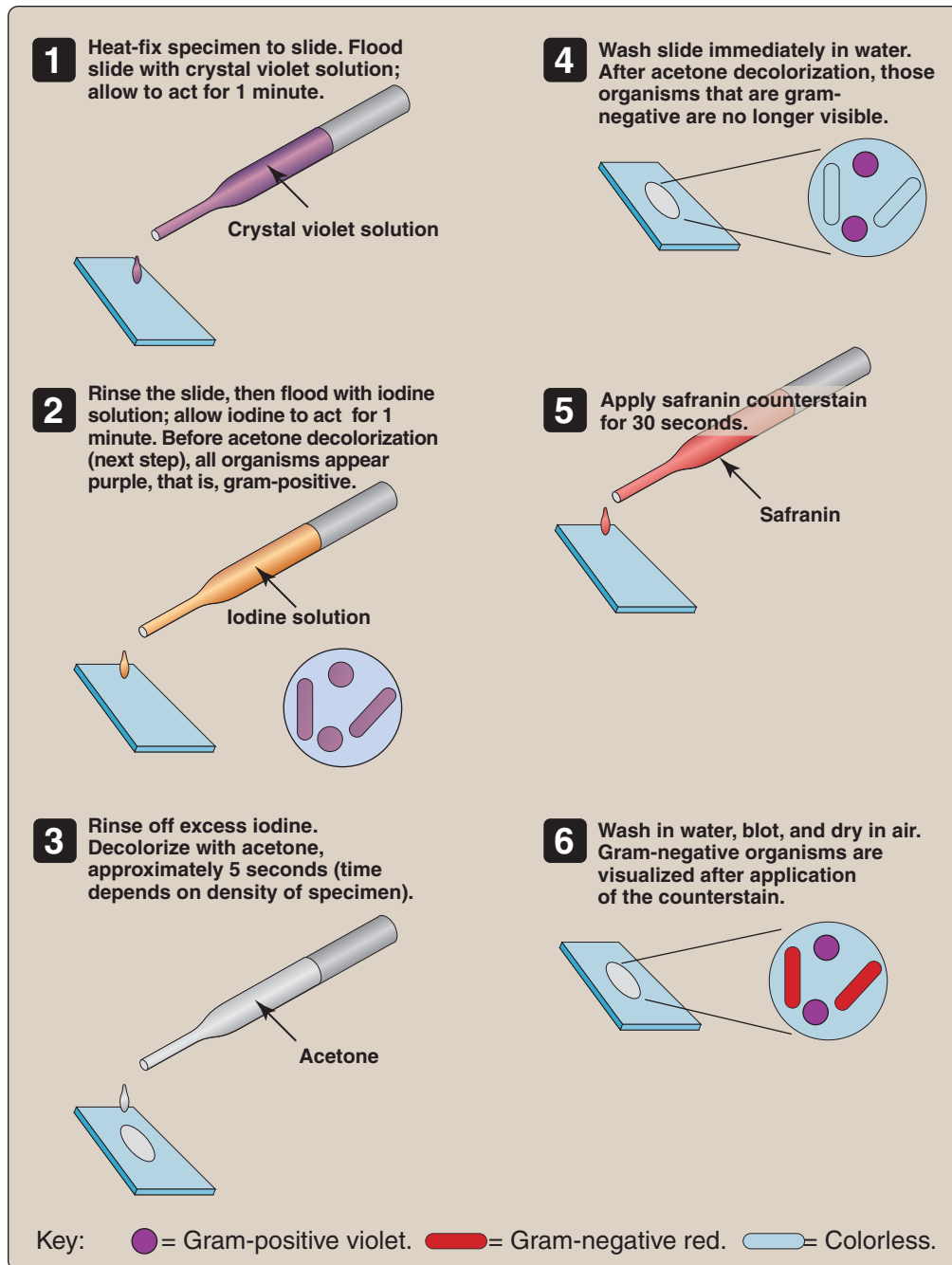


FIGURE 4-21. Steps in the Gram staining technique. (From Harvey RA et al. Lippincott's Illustrated Reviews: Microbiology, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

TABLE 4-5

Differences between Gram-Positive and Gram-Negative Bacteria

	GRAM-POSITIVE BACTERIA	GRAM-NEGATIVE BACTERIA
Color at the end of the Gram staining procedure	Blue-to-purple	Pink-to-red
Peptidoglycan in cell walls	Thick layer	Thin layer
Teichoic acids and lipoteichoic acids in cell walls	Present	Absent
Lipopolysaccharide in cell walls	Absent	Present



FIGURE 4-22. Chains of Gram-positive streptococci in a Gram-stained smear from a broth culture. (From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.)

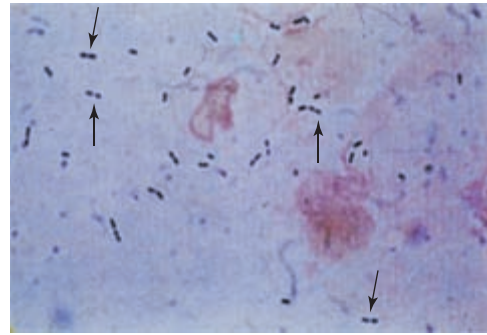


FIGURE 4-23. Gram-positive *Streptococcus pneumoniae* in a Gram-stained smear of a blood culture. Note the pairs of cocci, known as diplococci (arrows). (From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.)

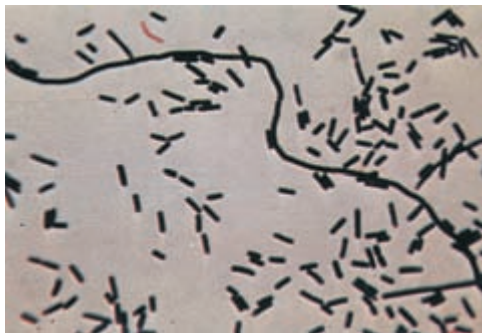


FIGURE 4-24. Gram-positive bacilli (*Clostridium perfringens*) in a Gram-stained smear prepared from a broth culture. Individual bacilli and chains of bacilli (streptobacilli) can be seen. (From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.)

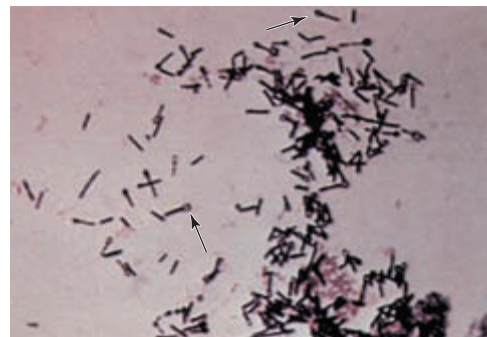


FIGURE 4-25. Gram-positive bacilli (*Clostridium tetani*) in a Gram-stained smear from a broth culture. Terminal spores can be seen on some of the cells (arrows). (From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.)

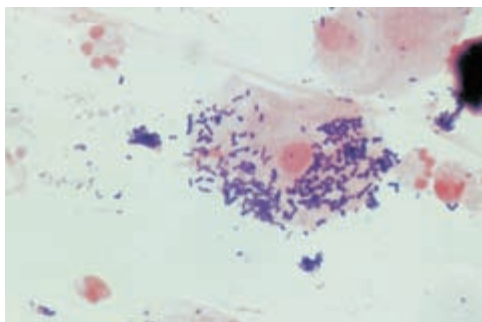


FIGURE 4-26. Many Gram-positive bacteria can be seen on the surface of a pink-stained epithelial cell in this Gram-stained sputum specimen. Several smaller pink-staining polymorphonuclear leukocytes can also be seen. (From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.)

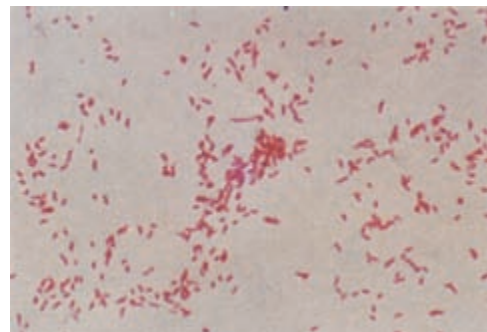


FIGURE 4-27. Gram-negative bacilli in a Gram-stained smear prepared from a bacterial colony. Individual bacilli and a few short chains of bacilli can be seen. (From Koneman E, et al. Color Atlas and Textbook of Diagnostic Microbiology, 5th ed. Philadelphia: Lippincott Williams & Wilkins, 1997.)

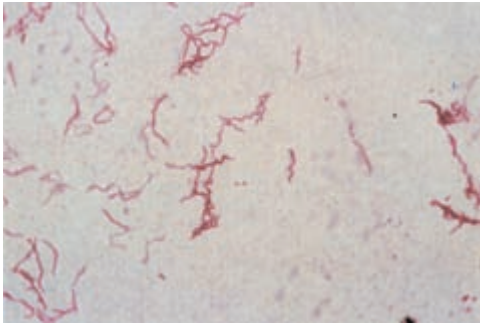


FIGURE 4-28. Loosely coiled Gram-negative spirochetes. *Borrelia burgdorferi* is the etiologic agent (cause) of Lyme disease. (From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.)

Figure 4-29 illustrates the various shapes of bacteria that may be observed in a Gram-stained clinical specimen. Some strains of bacteria are neither consistently blue to purple nor pink to red after Gram staining; they are referred to as Gram-variable bacteria. Examples of Gram-variable bacteria are members of the genus *Mycobacterium*, such as *M. tuberculosis* and *M. leprae*. Refer to Table 4-6 and Figures 4-22 through 4-28 for the staining characteristics of certain pathogens.

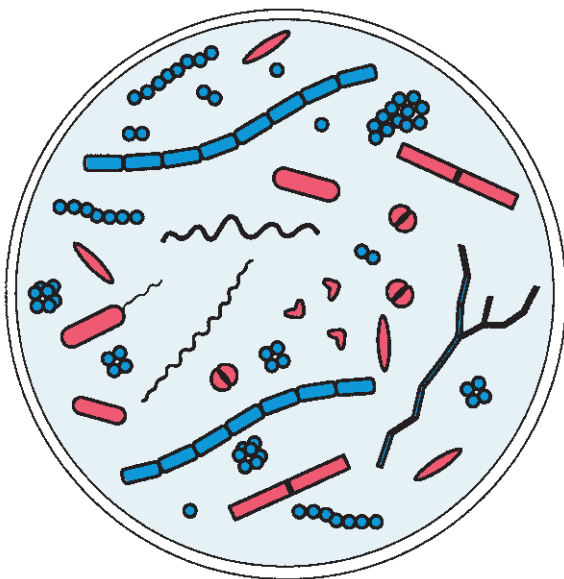


FIGURE 4-29. Various forms of bacteria that might be observed in Gram-stained smears. Shown here are single cocci, diplococci, tetrads, octads, streptococci, staphylococci, single bacilli, diplobacilli, streptobacilli, branching bacilli, loosely coiled spirochetes, and tightly coiled spirochetes. (See text for explanation of terms.)

Mycobacterium species are more often identified using a staining procedure called the **acid-fast stain**. In this procedure, carbol fuchsin (a bright red dye) is first driven into the bacterial cell using heat (usu-

ally by flooding the smear with carbol fuchsin, and then holding a Bunsen burner flame under the slide until steaming of the carbol fuchsin occurs). The heat is necessary because the cell walls of mycobacteria contain waxes, which prevent the stain from penetrating the cells. The heat softens the waxes, enabling the stain to penetrate. A decolorizing agent (a mixture of acid and alcohol) is then used in an attempt to remove the red color from the cells. Because mycobacteria are not decolorized by the acid-alcohol mixture (again owing to the waxes in their cell walls), they are said to be acid-fast. Most other bacteria are decolorized by the acid-alcohol treatment; they are said to be non-acid-fast. The acid-fast stain is especially useful in the tuberculosis laboratory ("TB lab") where the acid-fast mycobacteria are readily seen as red bacilli (referred to as acid-fast bacilli or AFB) against a blue or green background in a sputum specimen from a tuberculosis patient. Figures 4-30 and 4-31 depict the appearance of mycobacteria after the acid-fast staining procedure. The acid-fast staining procedure was developed in 1882 by Paul Ehrlich—a German chemist.

The Gram and acid-fast staining procedures are referred to as *differential staining procedures* because they enable microbiologists to differentiate one group of bacteria from another (i.e., Gram-positive bacteria from Gram-negative bacteria, and acid-fast bacteria from non-acid-fast bacteria). Table 4-7 summarizes the various types of bacterial staining procedures.



STUDY AID

A Method of Remembering a Particular Bacterium's Gram Reaction

A former student used this method to remember the Gram reaction of a particular bacterium. In her notebook, she drew two large circles. She lightly shaded in one circle, using a blue colored pencil. The other circle was lightly shaded red. Within the blue circle, she wrote the names of bacteria studied in the course that were Gram-positive. Within the red circle, she wrote the names of bacteria that were Gram-negative. She then studied the two circles. Later, whenever she encountered the name of a particular bacterium, she would remember which circle it was in. If it was in the blue circle, then the bacterium was Gram-positive. If it was in the red circle, the bacterium was Gram-negative. Clever!

TABLE 4-6 Characteristics of Some Important Pathogenic Bacteria

STAINING REACTION	MORPHOLOGY	BACTERIUM	DISEASE(S)
Gram-positive	Cocci in clusters	<i>Staphylococcus aureus</i>	Wound infections, boils, pneumonia, septicemia, food poisoning
	Cocci in chains	<i>Streptococcus pyogenes</i>	Strep throat, scarlet fever, necrotizing fasciitis, septicemia
	Diplococci	<i>Streptococcus pneumoniae</i>	Pneumonia, meningitis, ear and sinus infections
	Bacillus	<i>Corynebacterium diphtheriae</i>	Diphtheria
	Spore-forming bacillus	<i>Bacillus anthracis</i> <i>Clostridium botulinum</i> <i>Clostridium perfringens</i> <i>Clostridium tetani</i>	Anthrax Botulism Wound infections, gas gangrene, food poisoning tetanus
Gram-negative	Diplococci	<i>Neisseria gonorrhoeae</i>	gonorrhea
		<i>Neisseria meningitidis</i>	Meningitis, respiratory infections
	Bacillus	<i>Bordetella pertussis</i>	Whooping cough (pertussis)
		<i>Brucella abortus</i>	Brucellosis
		<i>Chlamydia trachomatis</i>	Genital infections, trachoma
		<i>Escherichia coli</i>	Urinary tract infections, septicemia
		<i>Francisella tularensis</i>	Tularemia
		<i>Haemophilus ducreyi</i>	Chancroid
		<i>Haemophilus influenzae</i>	Meningitis; respiratory, ear and sinus infections
		<i>Klebsiella pneumoniae</i>	Urinary tract and respiratory infections
		<i>Proteus vulgaris</i>	Urinary tract infections
		<i>Pseudomonas aeruginosa</i>	Respiratory, urinary, and wound infections
		<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever
		<i>Salmonella typhi</i>	Typhoid fever
		<i>Salmonella</i> spp.	Gastroenteritis
		<i>Shigella</i> spp.	Gastroenteritis
		<i>Yersinia pestis</i>	Plague
	Curved bacillus Spirochete	<i>Vibrio cholerae</i>	Cholera
		<i>Treponema pallidum</i>	Syphilis
Acid-fast, Gram-variable	Branching bacilli	<i>Mycobacterium leprae</i>	Leprosy (Hansen disease)
		<i>Mycobacterium tuberculosis</i>	Tuberculosis

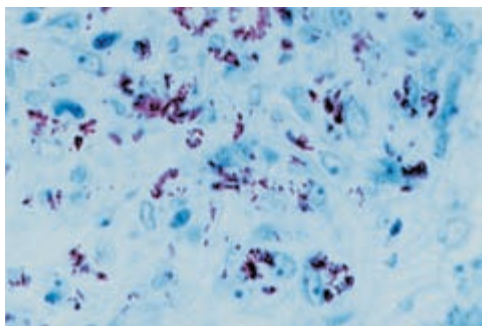


FIGURE 4-30. Many red acid-fast mycobacteria can be seen in this acid-fast stained liver biopsy specimen. (From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.)

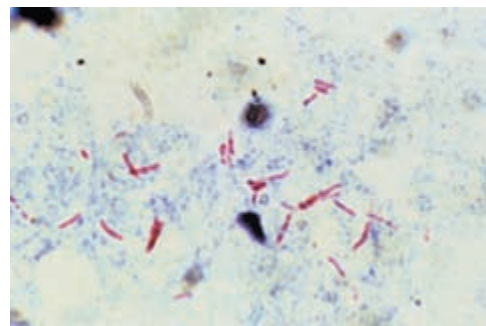


FIGURE 4-31. Many red acid-fast bacilli (*Mycobacterium tuberculosis*) can be seen in this acid-fast stained concentrate from a digested sputum specimen. (From Koneman, E, et al. Color Atlas and Textbook of Diagnostic Microbiology, 5th ed. Philadelphia: Lippincott Williams & Wilkins, 1997.)

TABLE 4-7 Types of Bacterial Staining Procedures

CATEGORY	EXAMPLE(S)	PURPOSE
Simple staining procedure	Staining with methylene blue	Merely to stain the cells so that their size, shape, and morphologic arrangement can be determined
Structural staining procedures	Capsule stains	To determine whether the organism is encapsulated
	Flagella stains	To determine whether the organism possesses flagella and, if so, their number and location on the cell
	Endospore stains	To determine whether the organism is a spore-former and, if so, to determine whether the spores are terminal or subterminal spores
Differential staining procedures	Gram stain	To differentiate between Gram-positive and Gram-negative bacteria
	Acid-fast stain	To differentiate between acid-fast and non-acid-fast bacteria

Motility

If a bacterium is able to “swim,” it is said to be motile. Bacteria unable to swim are said to be nonmotile. Bacterial motility is most often associated with the presence of flagella or axial filaments, although some bacteria exhibit a type of gliding motility on secreted slime. Bacteria never possess cilia. Most spiral-shaped bacteria and about one half of the bacilli are motile by means of flagella, but cocci are generally nonmotile. A flagella stain can be used to demonstrate the presence, number, and location of flagella on bacterial cells. Various terms (e.g., monotrichous, amphitrichous, lophotrichous, peritrichous) are used to describe the number and location of flagella on bacterial cells (see Chapter 3).

Motility can be demonstrated by stabbing the bacteria into a tube of semisolid agar or by using the hanging-drop technique. Growth (multiplication) of bacteria in semisolid agar produces turbidity (cloudiness). Nonmotile organisms will grow only along the stab line (thus, turbidity will be seen only along the stab line), but motile organisms will spread away from the stab line (thus, producing turbidity throughout the medium; see Fig. 4-32). In the hanging-drop method (Fig. 4-33), a drop of a bacterial suspension is placed onto a glass coverslip. The coverslip is then inverted over a depression slide. When the preparation is examined microscopically, motile bacteria within the “hanging drop” will be seen darting around in every direction.

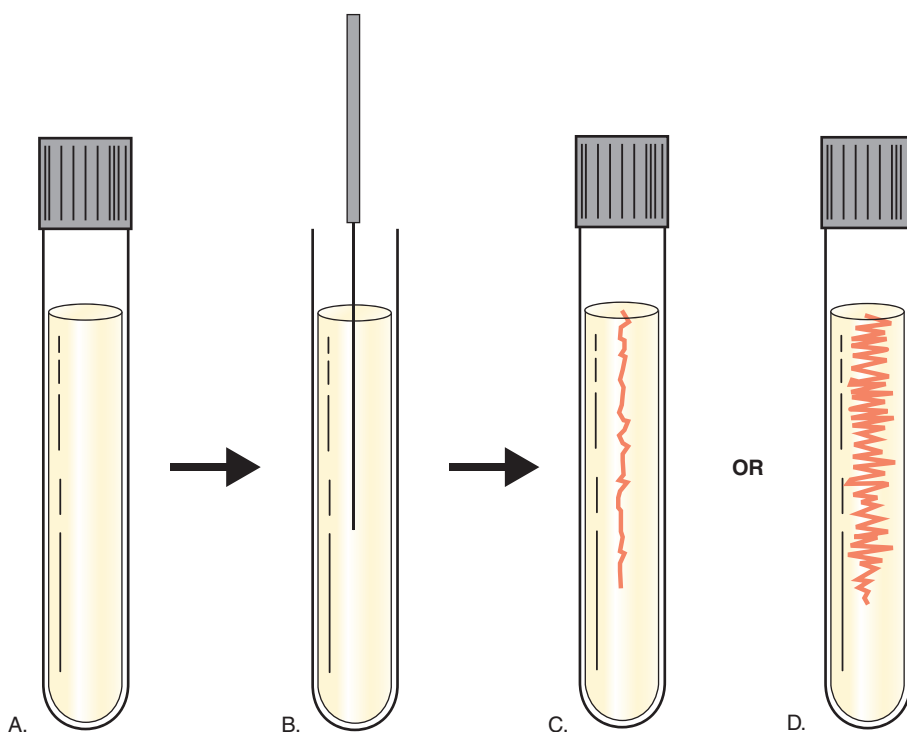


FIGURE 4-32. Semisolid agar method for determining motility. (A) Uninoculated tube of semisolid agar. (B) Same tube being inoculated by stabbing the inoculating wire into the medium. (C) Pattern of growth of a nonmotile organism, after incubation. (D) Pattern of growth of a motile organism, after incubation.

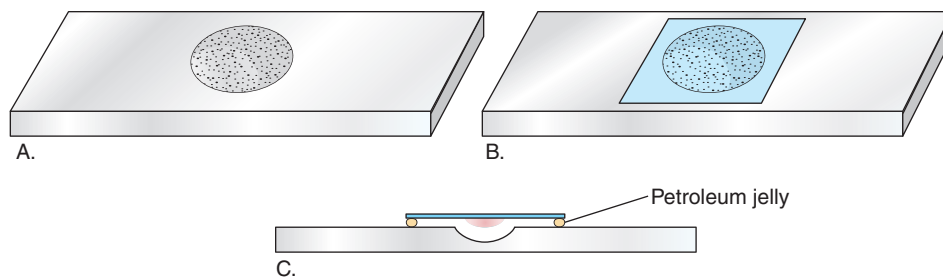


FIGURE 4-33. Hanging-drop preparation for study of living bacteria. (A) Depression slide. (B) Depression slide with coverglass over the depression area. (C) Side view of hanging-drop preparation showing the drop of liquid culture medium hanging from the center of the coverglass above the depression.

Colony Morphology

A single bacterial cell that lands on the surface of a solid culture medium cannot be seen, but after it divides over and over again, it produces a mound or pile of bacteria, known as a bacterial colony (Fig. 4-34). A colony contains millions of organisms. The colony morphology (appearance of the colonies) of bacteria varies from one species to another. Colony morphology includes the size, color, overall shape, elevation, and the appearance of the edge or margin of the colony. As is true for cell morphology and staining characteristics, colony features serve as important “clues” in the identification of bacteria. Size of colonies is determined by the organism’s rate of growth (generation time), and is an important characteristic of a particular bacterial species. Colony morphology also includes the results of enzymatic

A mound or pile of bacteria on a solid culture medium is known as a bacterial colony.

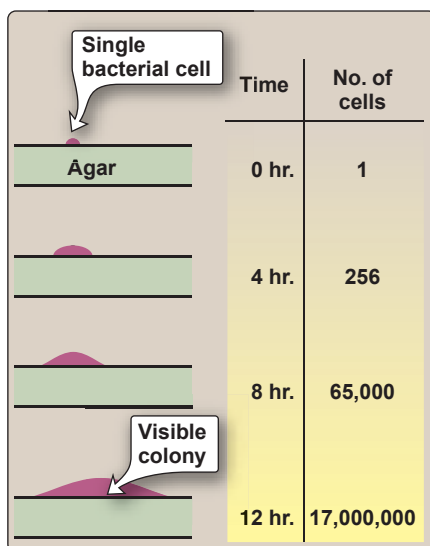


FIGURE 4-34. Formation of a bacterial colony on solid growth medium. In this illustration, the generation time is assumed to be 30 minutes. (From Harvey RA, et al. Lippincott’s Illustrated Reviews: Microbiology, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.)

activity on various types of culture media, such as those shown in Figures 8-3 through 8-5 in Chapter 8.

Atmospheric Requirements

In the microbiology laboratory, it is useful to classify bacteria on the basis of their relationship to oxygen (O_2) and carbon dioxide (CO_2). With respect to oxygen, a bacterial isolate can be classified into one of five major groups: obligate aerobes, microaerophilic aerobes (microaerophiles), facultative anaerobes, aerotolerant anaerobes, and obligate anaerobes (Fig. 4-35). In a liquid medium such as thioglycollate broth, the region of the medium in which the organism grows depends on the oxygen needs of that particular species.

To grow and multiply, *obligate aerobes* require an atmosphere containing molecular oxygen in concentrations comparable to that found in room air (i.e., 20%–21% O_2). *Mycobacteria* and certain fungi are examples of microorganisms that are obligate aerobes. *Microaerophiles* (microaerophilic aerobes) also require oxygen for

Obligate aerobes and microaerophiles require oxygen. Obligate aerobes require an atmosphere containing about 20% to 21% oxygen, whereas microaerophiles require reduced oxygen concentrations (usually around 5% oxygen).

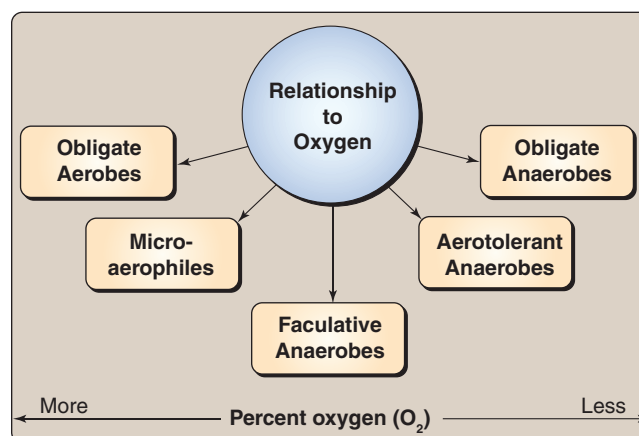


FIGURE 4-35. Categories of bacteria based on their relationship to oxygen.

multiplication, but in concentrations lower than that found in room air. *Neisseria gonorrhoeae* (the causative agent of gonorrhea) and *Campylobacter* spp. (which are major causes of bacterial diarrhea) are examples of microaerophilic bacteria that prefer an atmosphere containing about 5% oxygen.

Anaerobes can be defined as organisms that do not require oxygen for life and reproduction. However, they vary in their sensitivity to oxygen. The terms obligate anaerobe, aerotolerant anaerobe, and facultative anaerobe are used to describe the organism's relationship to molecular oxygen. An *obligate anaerobe* is an anaerobe that can only grow in an anaerobic environment (i.e., an environment containing no oxygen) (see "Insight: Life in the Absence of Oxygen" on the CD-ROM).

An *aerotolerant anaerobe* does not require oxygen, grows better in the absence of oxygen, but can survive in atmospheres containing molecular oxygen (such as air and a CO₂ incubator). The concentration of oxygen that an aerotolerant anaerobe can tolerate varies from one species to another. *Facultative anaerobes* are capable of surviving in either the presence or absence of oxygen; anywhere from 0% O₂ to 20% to 21% O₂. Many of the bacteria routinely isolated from clinical specimens are facultative anaerobes (e.g., members of the family *Enterobacteriaceae*, most streptococci, most staphylococci).

Room air contains less than 1% CO₂. Some bacteria, referred to as *capnophiles* (capnophilic organisms), grow better in the laboratory in the presence of increased concentrations of CO₂. Some anaerobes (e.g., *Bacteroides* and *Fusobacterium* species) are capnophiles, as are some aerobes (e.g., certain *Neisseria*, *Campylobacter*, and *Haemophilus* species). In the clinical microbiology laboratory, CO₂ incubators are routinely calibrated to contain between 5% and 10% CO₂.

Nutritional Requirements

All bacteria need some form of the elements carbon, hydrogen, oxygen, sulfur, phosphorus, and nitrogen for growth. Special elements, such as potassium, calcium, iron, manganese, magnesium, cobalt, copper, zinc, and uranium, are required by some bacteria. Certain microbes have specific vitamin requirements and some need organic substances secreted by other living microorganisms during their growth. Organisms with especially demanding nutritional requirements are said to be fastidious; think of them as being "fussy." Special enriched media must be used to grow fastidious organisms in the laboratory. The nutritional needs of a particular organism are usually characteristic for that species of bacteria and sometimes serve as important clues when attempting

to identify the organism. Nutritional requirements are discussed further in Chapters 7 and 8.

Biochemical and Metabolic Activities

As bacteria grow, they produce many waste products and secretions, some of which are enzymes that enable them to invade their host and cause disease. The pathogenic strains of many bacteria, such as staphylococci and streptococci, can be tentatively identified by the enzymes they secrete. Also, in particular environments, some bacteria are characterized by the production of certain gases, such as carbon dioxide, hydrogen sulfide, oxygen, or methane. To aid in the identification of certain types of bacteria in the laboratory, they are inoculated into various substrates (e.g., carbohydrates and amino acids) to determine whether they possess the enzymes necessary to break down those substrates. Learning whether a particular organism is able to break down a certain substrate serves as a clue to the identity of that organism. Different types of culture media are also used in the laboratory to learn information about an organism's metabolic activities (to be discussed in Chapter 8).

Pathogenicity

The characteristics that enable bacteria to cause disease are discussed in Chapter 14. Many pathogens are able to cause disease because they possess capsules, pili, or endotoxins (biochemical components of the cell walls of Gram-negative bacteria), or because they secrete exotoxins and exoenzymes that damage cells and tissues. Frequently, pathogenicity (the ability to cause disease) is tested by injecting the organism into mice or cell cultures. Some common pathogenic bacteria are listed in Table 4-6.

Genetic Composition

Most modern laboratories are moving toward the identification of bacteria using some type of test procedure that analyzes the organism's deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). These test procedures are collectively referred to as molecular diagnostic procedures. The composition of the genetic material (DNA) of an organism is unique to each species. DNA probes make it possible to identify an isolate without relying on phenotypic characteristics. A DNA probe is a single-stranded DNA sequence that can be used to identify an organism by hybridizing with a unique complementary sequence on the DNA or rRNA of that organism. Also, through the use of 16S rRNA sequencing (see Chapter 3), a researcher can determine the degree of relatedness between two different bacteria.

Unique Bacteria

Rickettsias, chlamydias, and mycoplasmas are bacteria, but they do not possess all the attributes of typical bacterial cells. Thus, they are often referred to as "unique" or "rudimentary" bacteria. Because they are so small and difficult to isolate, they were formerly thought to be viruses.

Rickettsias, Chlamydias, and Closely Related Bacteria

Rickettsias and chlamydias are bacteria with a Gram-negative-type cell wall. They are obligate intracellular pathogens that cause diseases in humans and other animals. As the name implies, an obligate intracellular pathogen is a pathogen that must live within a host cell. To grow such organisms in the laboratory, they must be inoculated into embryonated chicken eggs, laboratory animals, or cell cultures. They will not grow on artificial (synthetic) culture media.

The genus *Rickettsia* was named for Howard T. Ricketts, a U.S. pathologist; these organisms have no connection to the disease called rickets, which is the result of vitamin D deficiency. Because they appear to have leaky cell membranes, most rickettsias must live inside another cell to retain all necessary cellular substances (Fig. 4-36). All diseases caused by *Rickettsia* species are arthropod-borne, meaning that they are transmitted by arthropod *vectors* (carriers); see Table 4-8.

Arthropods such as lice, fleas, and ticks transmit the rickettsias from one host to another by their bites or waste products. Diseases caused by *Rickettsia* spp. include typhus and typhuslike diseases (e.g., Rocky Mountain spotted fever). All these diseases involve production of a rash. Medically important bacteria that are closely related to rickettsias include *Coxiella burnetii*, *Bartonella quintana* (formerly *Rochalimaea quintana*), *Ehrlichia* spp., and *Anaplasma* spp. *C. burnetii* (the cause of Q fever) is

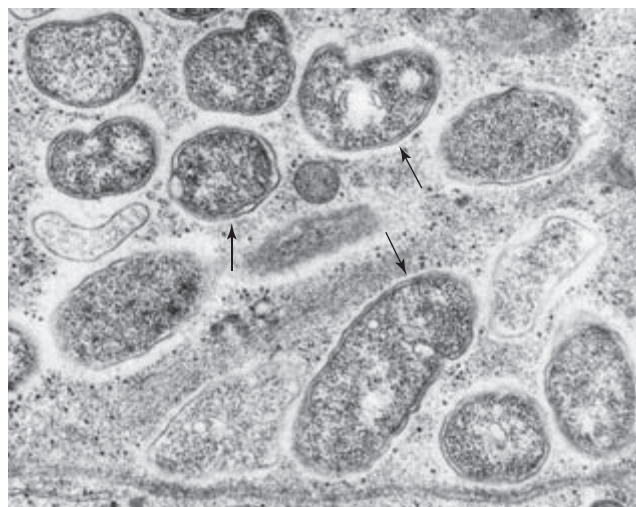


FIGURE 4-36. *Rickettsia prowazekii* (arrows), the cause of epidemic louseborne typhus, in experimentally infected tick tissue. (From Volk WA, et al. *Essentials of Medical Microbiology*, 5th ed. Philadelphia: Lippincott-Raven, 1996.)

transmitted primarily by aerosols, but can be transmitted to animals by ticks. *B. quintana* is associated with trench fever (a louseborne disease), cat scratch disease, bacteremia, and endocarditis. *Ehrlichia* and *Anaplasma* spp. cause human tickborne diseases such as human monocytic ehrlichiosis (HME) and human granulocytic

TABLE 4-8 Human Diseases Caused by Unique Bacteria

GENUS	SPECIES	HUMAN DISEASE(S)
<i>Rickettsia</i>	<i>R. akari</i>	Rickettsialpox (a miteborne disease)
	<i>R. prowazekii</i>	Epidemic typhus (a louseborne disease)
	<i>R. rickettsii</i>	Rocky Mountain spotted fever (a tickborne disease)
	<i>R. typhi</i>	Endemic or murine typhus (a fleaborne disease)
<i>Ehrlichia</i> spp.	<i>E. chaffeensis</i>	Human monocytic ehrlichiosis
<i>Anaplasma</i> spp.	<i>Anaplasma phagocytophilum</i>	Human granulocytic ehrlichiosis
<i>Chlamydia</i> (and <i>Chlamydia</i> -like bacteria)	<i>Chlamydomphila pneumoniae</i>	Pneumonia
	<i>Chlamydomphila psittaci</i>	Psittacosis (a respiratory disease; a zoonosis; sometimes called "parrot fever")
	<i>Chlamydia trachomatis</i>	Different serotypes cause different diseases, including trachoma (an eye disease) inclusion conjunctivitis (an eye disease), nongonococcal urethritis (NGU; a sexually transmitted disease), lymphogranuloma venereum (LGV; a sexually transmitted disease)
<i>Mycoplasma</i>	<i>M. pneumoniae</i>	Atypical pneumonia
	<i>M. genitalium</i>	Nongonococcal urethritis (NGU)
<i>Orientia</i>	<i>O. tsutsugamushi</i>	Scrub typhus (a miteborne disease)
<i>Ureaplasma</i>	<i>U. urealyticum</i>	Nongonococcal urethritis (NGU)

ehrlichiosis (HGE). *Ehrlichia* and *Anaplasma* spp. are intraleukocytic pathogens, meaning that they live within certain types of white blood cells.

The term “chlamydias” refers to *Chlamydia* spp. and closely related organisms (such as *Chlamydophila* spp.). Chlamydias are referred to as “energy parasites.” Although they can produce adenosine triphosphate (ATP) molecules, they preferentially use ATP molecules produced by their host cells. ATP molecules are the major energy-storing or energy-carrying molecules of cells (see Chapter 7). Chlamydias are obligate intracellular pathogens that are transferred by inhalation of aerosols or by direct contact between hosts—not by arthropods. Medically important chlamydias include *Chlamydia trachomatis*, *Chlamydophila pneumoniae*, and *Chlamydophila psittaci*. Different serotypes of *C. trachomatis* cause different diseases, including trachoma (the leading cause of blindness in the world), inclusion conjunctivitis (another type of eye disease), and nongonococcal urethritis (NGU; a term given to urethritis that is not caused by *Neisseria gonorrhoeae*). *C. pneumoniae* causes a type of pneumonia, and *C. psittaci* causes a respiratory disease called psittacosis. Chlamydial diseases are listed in Table 4-8.

Mycoplasmas

Mycoplasmas are the smallest of the cellular microbes (Fig. 4-37). Because they lack cell walls, they assume many shapes, from coccoid to filamentous; thus, they appear pleomorphic when examined microscopically. Sometimes they are confused with cell wall-deficient (CWD) forms of bacteria, described earlier; however, even in the most favorable growth media, mycoplasmas are not able to produce cell walls,

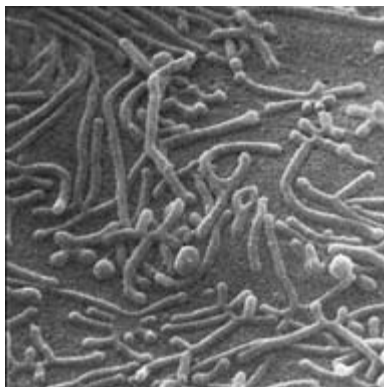


FIGURE 4-37. Scanning electron micrograph of *Mycoplasma pneumoniae*. (From Strohl WA, et al. Lippincott's Illustrated Reviews: Microbiology. Philadelphia: Lippincott Williams & Wilkins, 2001.)



STUDY AID

“Strains” versus “Serotypes”

Within a given species, there are usually different *strains*. For example, there are many different strains of *E. coli*. If the *E. coli* that has been isolated from Patient X is producing an enzyme that is not being produced by the *E. coli* from Patient Z, the two *E. coli* isolates are considered to be different strains. Or, if one isolate of *E. coli* is resistant to ampicillin (an antibiotic), and the other *E. coli* isolate is susceptible to ampicillin, then these isolates are considered to be different strains of *E. coli*. Also, there are usually different *serotypes* (sometimes called serovars) within a given species. Serotypes of an organism differ from each other as a result of differences in their surface molecules (surface antigens). Sometimes, as is true for *C. trachomatis* and *E. coli*, different serotypes of a given species cause different diseases.

which is not true for CWD. Mycoplasmas were formerly called pleuropneumonia-like organisms (PPLO), first isolated from cattle with lung infections. They may be free-living or parasitic and are pathogenic to many animals and some plants. In humans, pathogenic mycoplasmas cause primary atypical pneumonia and genitourinary infections; some species can grow intracellularly. Because they have no cell wall, they are resistant to treatment with penicillin and other antibiotics that work by inhibiting cell wall synthesis. Mycoplasmas can be cultured on artificial media in the laboratory, where they produce tiny colonies (called “fried egg colonies”) that resemble sunny-side-up fried eggs in appearance. The absence of a cell wall prevents mycoplasmas from staining with the Gram stain procedure. Diseases caused by mycoplasmas and a closely related organism (*Ureaplasma urealyticum*) are shown in Table 4-8.

Especially Large and Especially Small Bacteria

The size of a typical coccus (e.g., a *Staphylococcus aureus* cell) is 1 μm in diameter. A typical bacillus (e.g., an *E. coli* cell) is about 1.0 μm wide \times 3.0 μm long, although some bacilli are long thin filaments—up to about 12 μm in length or even longer—but still only about 1 μm wide. Thus, most bacteria are microscopic, requiring the use of a microscope to be seen.

Perhaps the largest of all bacteria—large enough to be seen with the unaided human eye—is *Thiomargarita namibiensis*, a colorless, marine, sulfide-oxidizing bacterium. Single spherical cells of *T. namibiensis* are 100 to 300 μm , but may be as large as 750 μm (0.75 mm). In terms of size, comparing a *T. namibiensis* cell to an *E. coli* cell would be like comparing a blue whale to a newly born mouse. Other marine sulfide-oxidizing bacteria in the genera *Beggiatoa* and *Thioploca* are also especially



STUDY AID

Beware of Similar Sounding Names

Do not confuse *Mycoplasma* with *Mycobacterium*. Each is a genus of bacteria. The unique thing about *Mycoplasma* spp. is that they lack cell walls. The unique thing about *Mycobacterium* spp. is that they are acid-fast.

large, having diameters from 10 μm to more than 100 μm . Although *Beggiatoa* and *Thioploca* form filaments, *Thiomargarita* cells do not.

Another enormous bacterium, named *Epulopiscium fishelsonii*, has been isolated from the intestines of the reef surgeonfish; this bacillus is about 80 μm wide \times 600 μm (0.6 mm) long. *Epulopiscium* cells are about five times longer than eucaryotic *Paramecium* cells.^b The volume of an *Epulopiscium* cell is about a million times greater than the volume of a typical bacterial cell. Spore-forming bacteria called metabacteria, found in the intestines of herbivorous rodents, are closely related to *Epulopiscium*, but they reach lengths of only 20 to 30 μm . Although shorter than *Epulopiscium*, metabacteria are much longer than most bacteria.

At the other end of the spectrum, there are especially tiny bacteria called *nanobacteria*. Their sizes are expressed in nanometers because these bacteria are less than 1 μm in diameter; hence the name, nanobacteria. In some cases, they are as small as 20 nm in diameter. Nanobacteria have been found in soil, minerals, ocean water, human and animal blood, human dental calculus (plaque), arterial plaque, and even rocks (meteorites) of extraterrestrial origin. The existence of nanobacteria is controversial, however. Some scientists believe that these tiny structures were formed by geological, rather than biological, processes. They feel that nanobacteria are smaller than the minimum possible size for a living cell.

Photosynthetic Bacteria

Photosynthetic bacteria include purple bacteria, green bacteria, and cyanobacteria (erroneously referred to in the past as blue-green algae). Although all three groups use light as an energy source, they do not all carry out photosynthesis in the same way.

For example, purple and green bacteria (which, in some cases, are not actually those colors) do not produce oxygen, whereas cyanobacteria do. Photosynthesis that produces oxygen is called *oxygenic photosynthesis*, whereas photosynthesis that does not produce oxygen is called *anoxygenic photosynthesis*.

In photosynthetic eucaryotes (algae and plants), photosynthesis takes place in plastids, which were discussed in Chapter 3. In cyanobacteria, photosynthesis takes place on intracellular membranes known as thylakoids. Thylakoids are attached to the cell membrane at various points and are thought to represent invaginations of the cell membrane. Attached to the thylakoids, in orderly rows, are numerous phycobilisomes—complex protein pigment aggregates where light harvesting occurs.

Many scientists believe that cyanobacteria were the first organisms capable of carrying out oxygenic photosynthesis and, thus, played a major part in the oxygenation of the atmosphere. Fossil records reveal that cyanobacteria were already in existence 3.3 to 3.5 billion years ago. Photosynthesis is discussed further in Chapter 7. Cyanobacteria vary widely in shape; some are cocci, some are bacilli, and others form long filaments.

When appropriate conditions exist, cyanobacteria in pond or lake water will overgrow, creating a water bloom—a “pond scum” that resembles a thick layer of bluish green (turquoise) oil paint. The conditions include a mild or no wind, a balmy water temperature (15°–30°C), a water pH of 6 to 9, and an abundance of the nutrients nitrogen and phosphorous in the water. Many cyanobacteria are able to convert nitrogen gas (N_2) from the air into ammonium ions (NH_4^+) in the soil or water; this process is known as *nitrogen fixation* (Chapter 10).

Some cyanobacteria produce toxins (poisons), such as neurotoxins (which affect the central nervous system), hepatotoxins (which affect the liver), and cytotoxins (which affect other types of cells).

These cyanotoxins can cause disease and even death in wildlife species and humans that consume contaminated water. Additional information about these toxins can be found in the CD-ROM Appendix 1, entitled “Microbial Intoxications.”

THE DOMAIN ARCHAEA

Prokaryotic organisms thus far described in this chapter are all members of the Domain *Bacteria*. Prokaryotic organisms in the Domain *Archaea* were discovered in 1977. Although they were once referred to as archaebacteria (or archaeobacteria), most scientists now feel that there are sufficient differences between archaea and bacteria to stop referring to archaea as bacteria. *Archae* means “ancient,” and the name *archaea* was originally assigned when it was thought that these prokaryotes evolved earlier than bacteria. Now, there is considerable debate as to whether bacteria or archaea came first. Genetically, even though they are prokaryotes, archaea are more closely related to eucaryotes than they are to bacteria; some possess genes otherwise found only in eucaryotes. Many scientists believe that bacteria and archaea diverged from a

^b *Paramecium* is a genus of freshwater protozoa.

TABLE 4-9

Examples of Extremophiles

Type of Extreme Environment	Name Given To These Types of Extremophiles
Extremely acidic	Acidophiles
Extremely alkaline	Alkaliphiles
Extremely hot	Thermophiles
Extremely cold	Psychrophiles
Extremely salty	Halophiles
Extremely high pressure	Piezophiles (formerly barophiles)

common ancestor relatively soon after life began on this planet. Later, the eucaryotes split off from the archaea.

According to *Bergey's Manual of Systematic Bacteriology*, the Domain *Archaea* contains 2 phyla, 8 classes, 12 orders, 21 families, 69 genera, and 217 species. Archaea vary widely in shape; some are cocci, some are bacilli, and others form long filaments. Many, but not all, archaea are “extremophiles,” in the sense that they live in extreme environments, such as extremely acidic, alkaline, hot, cold, or salty environments, or environments where there is extremely high pressure (Table 4-9).

Some live at the bottom of the ocean in and near thermal vents, where, in addition to heat and salinity, there is extreme pressure. Other archaea, called methanogens, produce methane, which is a flammable gas. Although virtually all archaea possess cell walls, their cell walls contain no peptidoglycan. In contrast, all bacterial cell walls contain peptidoglycan. The 16S rRNA sequences of archaea are quite different from the 16S rRNA sequences of bacteria. The 16S rRNA sequence data suggest that archaea are more closely related to eucaryotes than they are to bacteria. You will recall from Chapter 3 that differences in rRNA structure form the basis of the Three-Domain System of Classification.



ON THE CD-ROM

- Terms Introduced in This Chapter
- Review of Key Points
- Insight
 - Microbes in the News: “Mad Cow Disease”
 - Life in the Absence of Oxygen
- Increase Your Knowledge
- Critical Thinking
- Additional Self-Assessment Exercises

SELF-ASSESSMENT EXERCISES

After studying this chapter, answer the following multiple-choice questions.

1. Which one of the following steps occurs during the multiplication of animal viruses, but not during the multiplication of bacteriophages?
 - a. assembly
 - b. biosynthesis
 - c. penetration
 - d. uncoating
2. Which one of the following diseases or groups of diseases is not caused by prions?
 - a. certain plant diseases
 - b. chronic wasting disease of deer and elk
 - c. Creutzfeldt-Jacob disease of humans
 - d. “mad cow disease”
3. Most procaryotic cells reproduce by:
 - a. binary fission.
 - b. budding.
 - c. gamete production.
 - d. spore formation.
4. The group of bacteria that lack rigid cell walls and take on irregular shapes is:
 - a. chlamydias.
 - b. mycobacteria.
 - c. mycoplasmas.
 - d. rickettsias.
5. At the end of the Gram staining procedure, Gram-positive bacteria will be:
 - a. blue to purple.
 - b. green.
 - c. orange.
 - d. pink to red.
6. Which one of the following statements about rickettsias is false?
 - a. Diseases caused by rickettsias are arthropod-borne.
 - b. Ricketts is caused by a *Rickettsia* species.
 - c. *Rickettsia* species cause typhus and typhuslike diseases.
 - d. Rickettsias have leaky membranes.
7. Which one of the following statements about *Chlamydia* and *Chlamydophila* spp. is false?
 - a. They are obligate intracellular pathogens.
 - b. They are considered to be “energy parasites.”
 - c. The diseases they cause are all arthropod-borne.
 - d. They are considered to be Gram-negative bacteria.
8. Which one of the following statements about cyanobacteria is false?
 - a. Although cyanobacteria are photosynthetic, they do not produce oxygen as a result of photosynthesis.
 - b. At one time, cyanobacteria were called blue-green algae.
 - c. Some cyanobacteria are capable of nitrogen fixation.
 - d. Some cyanobacteria are important medically because they produce toxins.

9. Which one of the following statements about archaea is false?
- a. Archaea are more closely related to eucaryotes than they are to bacteria.
 - b. Both archaea and bacteria are procaryotic organisms.
 - c. Some archaea live in extremely hot environments.
 - d. The cell walls of archaea contain a thicker layer of peptidoglycan than the cell walls of bacteria.
10. An organism that does not require oxygen, grows better in the absence of oxygen, but can survive in atmospheres containing some molecular oxygen is known as a(n):
- a. aerotolerant anaerobe.
 - b. capnophile.
 - c. facultative anaerobe.
 - d. microaerophile.