The Microbial World

The Microbe is so very small You cannot make him out at all, But many sanguine people hope To see him down a microscope. Oh! Let us never, never doubt What nobody is sure about!

—Hilaire Belloc, More Beasts for Worse Children

Preview

In the introductory section the term *microbe* has been used extensively because this text is about microbes, particularly those relatively few that are pathogens. The term *microbe* was not defined or even adequately described, but the six groups of microbes were named—prions, viruses, bacteria, protozoans, unicellular algae, and fungi. (Worms, biologically known as helminths, are frequently included in microbiology texts even though they are not microbes because a number of species cause infections resembling microbial infections.)

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Photo courtesy of Janice Haney Carr/CDC.

Some Basic Biological Principles

Cell Theory

To further understand microbes, whether pathogens or not, it is necessary to review a few very basic concepts of biology, because all microbes are biological packages with certain unique characteristics. **Cells** are considered the basic unit of life, based on the observations of Robert Hooke in 1665. Hooke used the word *cella* in his examination of cork, which revealed tiny compartments that reminded him of the cells in which monks lived. His studies ultimately gave rise to the cell theory, a fundamental concept in biology, as postulated by Matthias Schleiden and Theodor Schwann (1838) and Rudolf Virchow (1858). The major points of the cell theory are as follows:

- 1. All organisms are composed of fundamental units called cells.
- **2.** All organisms are unicellular (single cells) or multicellular (more than one cell).
- **3.** All cells are fundamentally alike with regard to their structure and their metabolism.
- Cells arise only from previously existing cells ("life begets life").

"Life begets life" is a refutation of the doctrine of **spontaneous generation**, a concept that was disproved by the end of the nineteenth century. An understanding of the cell theory is the basis for an understanding of life, including microbial life. The cell theory does not apply to viruses and prions; they are described as *acellular*, *subcellular*, or as *biological agents*, terms that are used somewhat interchangeably. Nevertheless, as a matter of convenience license is sometimes taken, and they are described as microbes or microorganisms. Viruses are not considered by scientists as being "alive," but they come close; they are in that gray area between living and nonliving. Prions are even less biologically complex than viruses.

Metabolic Diversity

The term *life* is elusive and cannot be given an exact definition; at best, it can only be described. Nevertheless, several attributes are associated with living systems that, collectively, establish life. By one strategy or another all organisms exhibit these characteristics, summarized in TABLE 2.1. A major property of life is the ability to constantly satisfy the requirement for energy. It takes energy for every cell to stay alive, whether it is a single cell or a component of a multicellular organism; in the latter case each cell contributes to the total energy requirement of the organism. Your body constantly expends energy.

It takes energy to breathe even during sleep and for the heart to constantly push blood through an interconnected and tortuous maze of blood vessels. Because you don't fill up at the gas station, it's obvious that your energy is derived from the foods you eat. Through a complex series of biochemical reactions, the body metabolizes the **organic** compounds (proteins, fats, and carbohydrates) of your diet and releases the energy stored in their chemical bonds into a biologically available high-energy compound known as adenosine triphosphate (**ATP**); you live directly off of this and constantly replace it as you take in nutrients. Most organisms, including most microbes, are **heterotrophs**, meaning that they require organic compounds as an energy source;

AUTHOR'S NOTE (RIK)-

Even when you doze in class, it takes energy to keep from slithering out of your chair and onto the floor. I have seen this happen only once in all my years at the lecture podium.

TABLE 2.1 Characteristics of Life

Characteristic	Description		
Cellular organization	The cell is the basic unit of life; organisms are unicellular or multicellular.		
Energy production	Organisms require energy and a biochemical strategy to meet their energy requirement.		
Reproduction	Organisms have the capacity to reproduce by asexual or sexual methods and in doing so pass on genetic material (DNA) to their progeny.		
Irritability	Organisms respond to internal and external stimuli.		
Growth and development	Organisms grow and develop in each new generation; specialization and differentiation occur in multicellular organisms.		

Solar energy Photosynthetic autotrophs Autotrophs Chemosynthetic autotrophs Organic $(C_6H_{12}O_6 + O_2)$ Heterotrophs Inorganic $(C_2 + H_2O)$

humans are heterotrophs. Other microorganisms and plant life are **autotrophs** and do not require organic compounds, but they do require energy. Some are able to directly use the energy of the sun (**photosynthetic autotrophs**), and others derive energy from the metabolism of **inorganic** compounds (**chemosynthetic auto-trophs**). In so doing autotrophs produce organic compounds and oxygen (O₂). Hence, heterotrophs are dependent on autotrophs for energy (**FIGURE 2.1**).

FIGURE 2.1 A pathway map showing heterotroph dependency on autotrophs and the autotrophs' energy sources.

Requirement for Oxygen

In addition to metabolic diversity, organisms exhibit diversity in their O_2 requirement. The "higher" organisms that are more familiar to you are **aerobes**, meaning they require O_2 for their metabolic activities. Some bacteria are **anaerobes** and do not require oxygen; other anaerobes are actually killed by O_2 . **Facultative anaerobes** are bacteria that grow better in the presence of O_2 but can shift their metabolism, allowing them to grow in the absence of O_2 . Knowledge of the oxygen requirements

of pathogens is important in clinical microbiology. For example, specimens from infections caused by bacteria suspected of being anaerobes must be transported and cultured under anaerobic conditions (FIGURE 2.2).

Genetic Information

The genetic information for the structure and functioning of all cells is stored in molecules of **deoxyribonucleic acid** (**DNA**), a large and complex organic molecule. **Genes** are segments of the DNA molecule. Since the establishment of DNA as the hereditary material, the expression "life begets

life" can be expanded to explain the mechanism by which a particular life form gives rise to the same life form; that is, tomatoes produce tomatoes, humans produce humans, and *Escherichia coli* produces *Escherichia coli*. Each of these groups has its characteristics embedded in DNA that confer its identity. The DNA is transferred, by a variety of reproductive strategies, from parent to offspring.



FIGURE 2.2 Culturing anaerobic bacteria. Some bacteria cannot grow in the presence of oxygen. The GasPak tray is a means of culturing anaerobes. Courtesy and © Becton, Dickinson, and Company.

What Makes a Microbe?

With these basic biological principles in mind, the term *microbe* (or *microorganism*) can now be better described. The question to be considered is what makes a microbe a microbe? As will become apparent, this question is not easily answered. Your first response may be "they are all too small to be seen without a microscope" or are



FIGURE 2.3 Mold growing on a tomato. © Jones & Bartlett Learning.

microscopic. Wrong. At first thought this would appear to be true, but what about the algae and the fungi? Are fungi microscopic? No doubt you have seen molds (FIGURE 2.3) classified as fungi, growing on food left too long in the refrigerator or perhaps on a pair of old sneakers that you forgot about in the dank basement or hidden away in a dormitory closet. They are **macroscopic**; that is, they can be seen with the naked eye. Hence, "microscopic" is not a distinguishing microbial characteristic. To describe all microbes as being unicellular is also not correct because the fungi and many of the algal forms are macroscopic and clearly multicellular. (As pointed out later, some fungi, namely yeasts, are unicellular.) These organisms must be multicellular; if they were unicellular, that one cell would be enormous—a ridiculous idea!

There are exceptions to the rule that all bacteria are unicellular and microscopic. This might seem like an amazing fish story, but in 1985 a large cigar-shaped microorgan-

ism was found in the guts of the Red Sea brown surgeonfish. This organism was subsequently identified as a bacterium, approximately a million times larger in volume than *E. coli*, and was christened *Epulopiscium fishelsoni*. Twelve years later, in 1997, an even more monstrous bacterium was discovered in sediment samples residing off the coast of Namibia (BOX 2.1); the organism has the tongue-twisting name *Thiomargarita namibiensis* and to date is one for the *Guinness Book of World Records*. They are visible to the naked eye.

To give you some idea of size relationships, if an ordinary bacterium were the size of a baby mouse, *E. fishelsoni* would be equivalent to a lion and *T. namibiensis* would be the size of a blue whale, the world's largest animal. The blue whale measures up to 90 feet (29 meters) and weighs about 120 tons. How many cells might make up such an enormous creature? The number would be in the trillions. Each of these cells exhibits the same fundamental life characteristics as the single microbe. Microbes are sometimes described as "simple" because many consist of only a single cell or are less than a cell (viruses and prions). Consider, however, that this single cell must fulfill all the functions of life. On the other hand, in a multicellular organism (like the whale), although each cell fulfills all the criteria for life, there is a "sharing" of function because of specialization into a variety of cell types (for example, muscle cells, nerve cells, and blood cells). Perhaps that makes life easier. Hence, single-celled organisms, and even those multicellular organisms consisting of only a small number of cells without evidence of true specialization, are simple only in the sense of numbers and not in a physiological (functional) sense.

So, if microbes are not necessarily microscopic and/or unicellular, then what is a microbe? There really is no unifying principle or precise definition. The term

BOX 2.1

"Monster" Bacteria

If asked to describe bacteria, just about everyone would reply that they are too small to be seen without a microscope. However, in 1985 *Epulopiscium fishelsoni*, a giant bacterium that can be seen without a microscope, was discovered in the guts of surgeonfish in the warm waters of the Red Sea and off the coast of Australia. The organism can grow to about 500 micrometers, or about the size of the period at the end of this sentence. To give you some idea of size, one scientist projected that "if ordinary bacteria were mouse sized, *E. fishelsoni* would be equivalent to a lion." This organism is referred to as "epulos" and was originally thought to be protozoan-like. However, analysis of their DNA revealed that they are, in fact, bacteria.

In 1997 *Thiomargarita namibiensis* stole the prize for size from *Epulopiscium*. This "monster" bacterium, approximately the size of a fruit fly's head, was discovered in samples of sediment in the greenish ooze off the coast of Namibia in Africa. These spherical cells range from 100 to 750 micrometers in size. Dispersed throughout their cytoplasm are globules of sulfur. The bacteria tend to organize into strands of cells that glisten white from light reflected off their sulfur globules, which explains the name. *T. namibiensis* means Namibian sulfur pearl.

Both epulos and the sulfur pearl are anomalies in the bacterial world. The sizes of cells of all kinds, not only bacterial cells, are limited by the surface area of the membrane, because nutrients and waste are transported in and out of the cells by diffusion across the cell membrane. As cells increase in size, both volume and surface area increase, but surface area increases to a lesser degree than does volume. At some point the surface area becomes too limited to allow for sufficient diffusion between the cell and its environment.

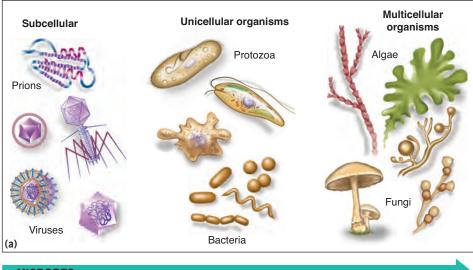
So how did *E. fishelsoni* and *T. namibiensis* manage to become so big? What are the physiological adaptations? In the case of epulos, microscopic examination reveals that the cell membrane, rather than being stretched smoothly around the cell, is convoluted (wrinkled), resulting in "hills and valleys," a phenomenon that greatly increases cell surface. (This adaptation is not unique to bacterial cells; the surface of the human brain is highly convoluted, resulting in a greater surface area, a factor that correlates with species intelligence.) The large size of *T. namibiensis* is attributed to the presence of a large fluid-filled sac occupying over 90% of the cell's interior. The sac is packed with nitrate that the cell uses in its metabolism to produce energy, making it less dependent on constant diffusion across the membrane to transport nutrients and waste.

microbe, or microorganism, is a term of convenience used to describe biological agents, in a collective sense, that in general are too small to be seen without the aid of a microscope. The term is also used for microbes that are cultured and identified using similar techniques. Based on what has been presented here, it is clear that these descriptions are not always true. Some biologists consider microbes to be organisms that are at less than the tissue level of organization. This statement requires some explanation and is based on what is referred to as "biological hierarchy," or levels of biological organization.

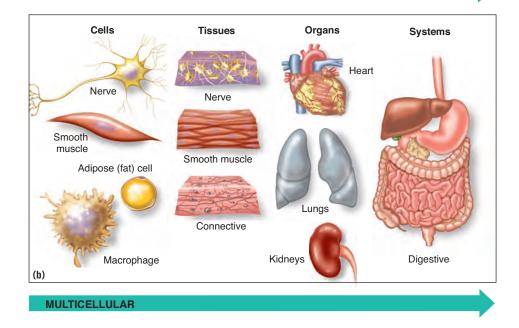
Recall that a cell is the fundamental unit of biological organization and that groups of cells establish multicellularity. Consider the human, or any other multicellular animal or plant, and it is obvious that in addition to an increase in cell numbers, the process of differentiation and specialization has taken place. For example, over 200 cell types make up the human, including red blood cells, five categories of white blood cells, epithelial cells, connective tissue cells, nerve cells, and muscle cells. All these cells, as stated in the cell theory, share common fundamental characteristics, but superimposed on their basic structure and function is a specialization of structure and function. Cells of the same type constitute the **tissue** level of organization, as exemplified by nerve tissue, blood tissue, and connective tissue. Tissues in turn constitute **organs**, structures composed of more than one tissue

AUTHOR'S NOTE (RIK)

Some years ago I attended the annual meeting of the American Society for Microbiology in Miami Beach, Florida and overheard two airport baggage handlers commenting that about twelve thousand microbiologists were expected to attend. One asked the other, "What's a microbiologist, anyway?" to which the other replied, "Beats me! I suppose it's a small biologist." Several miles from the airport was a huge billboard with the words "Orkin Pest Control welcomes microbiologists." It was a memorable meeting. FIGURE 2.4 Levels of biological organization. (a) Microbes. (b) Multicellular.







type; the heart, brain, stomach, and kidney are examples. Organs in turn constitute **organ systems**, a collection of organs that contribute to an overall function or functions. The digestive system, nervous system, respiratory system, excretory system, and reproductive system are examples familiar to you. This hierarchy is summarized as follows and is further illustrated in **FIGURE 2.4**: subcellular \rightarrow cells \rightarrow tissues \rightarrow organs \rightarrow organ systems.

All microbes are devoid of tissues. That is, they are all at the subcellular or cellular level of organization, although fungi and some algae hint at specialization and approach the tissue level of organization. Prions and viruses can be properly placed at the acellular or subcellular level, which, simply put, means that they are less than cells and are at the threshold of life.

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Procaryotic and Eucaryotic Cells

Biologists recognize the existence of two very distinct types of cells, referred to as **procaryotic** and **eucaryotic** cells (Greek, *pro*, before, + *karyon*, nut or kernel, + *eu*, true). Procaryotic cells have a simpler morphology than eucaryotic cells and are primarily distinguished by the fact that there is no membrane around the nucleus. There is a nuclear area rich in DNA that serves as the carrier of genetic information, as in all cells, but that DNA is not enclosed within a nuclear membrane. This DNA-rich area is referred to as a **nucleoid** rather than as a true nucleus. Further, in procaryotic cells there are no membrane-bound cellular structures (organelles) in contrast to the cellular anatomy of the eucaryotic cells.

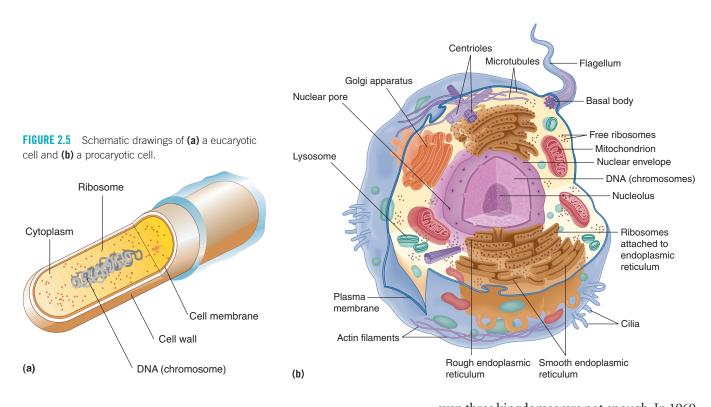
Procaryotic and eucaryotic cells are compared in TABLE 2.2 and in FIGURE 2.5. Bacteria are procaryotic microorganisms; protozoans, unicellular algae, fungi, and all other forms of life (except viruses and prions) are composed of eucaryotic cells.

Microbial Evolution and Diversity

Procaryotes date back 3.5 billion years and were the only life forms for 2.5 billion years; they are the ancestors of eucaryotes. Aristotle pondered the relationships among organisms, as do scientists today. In the eighteenth century the botanist Carolus Linnaeus classified all life forms as belonging to either the plant or the animal kingdom. (Students would be delighted if only this were the case today!) Microbes were largely ignored because little was known about them, but, because they had to be placed somewhere, they were considered plants, probably because those that had been observed possessed cell walls. Various schemes of classification have been proposed over the last few centuries, and taxonomy, the science of classification, became more and more complex. In 1866 Ernst Haeckel proposed a three-kingdom system—animals, plants, and a new kingdom, Protista, a collection to accommodate microbes. In the light of modern biology, it became apparent that

Characteristic	Procaryotes	Eucaryotes
Life form	Bacteria, Archaea	All microbial cells (with the exception of bacteria, viruses, and prions) and all other cells
Nucleus	DNA chromosome but not enveloped by membrane	Chromosome present and enveloped by membrane
Cell size	About 1–10 micrometers	Over 100 micrometers
Chromosomes	Single circular DNA (two chromosomes in a few)	Multiple paired chromosomes present in nucleus
Cell division	Asexual binary fission; no "true" sexual reproduction	Cell division by mitosis; sexual reproduction by meiosis
Internal compart- mentalization	No membrane-bound internal compartments	Organelles bound by membrane
Ribosomes	Smaller than eucaryotic cells and not membrane bound	Membrane bound and free

TABLE 2.2 Comparison of Procaryotic and Eucaryotic Cells



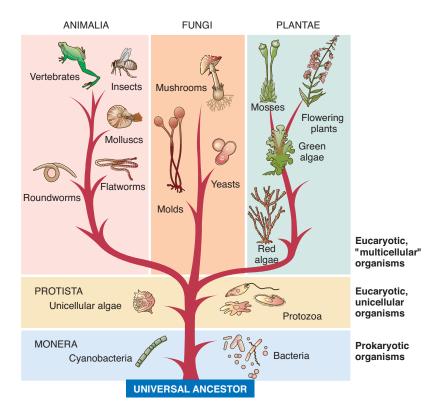


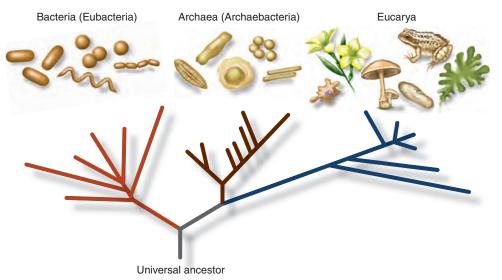
FIGURE 2.6 Whittaker's five-kingdom system.

even three kingdoms were not enough. In 1969 a five-kingdom system was proposed by Robert Whittaker and initially accepted by most biologists. This classification describes organisms as belonging to the kingdoms Monera, Protista, Fungi, Animalia, and Plantae (FIGURE 2.6). Recall that microbes consist of six groups accommodated in one of Whittaker's five kingdoms as follows: Bacteria are classified as Monera, protozoans and unicellular algae are classified as Protista, and fungi are classified as Fungi. Note that viruses and prions are not considered in this scheme of classification, because they are neither procaryotic nor eucaryotic cells but are subcellular.

The 1950s ushered in the tide of molecular biology, and its wake introduced new techniques. Biologist Carl Woese and his colleagues at the University of Illinois focused in on ribosomal ribonucleic acid (rRNA) as a "fingerprint" to identify shared characteristics of microbes and thus gain insight into their relatedness, which in turn would point to their evolutionary history.

In 1990 Woese, along with Otto Kandler and Mark L. Wheelis, proposed a novel

scheme of classification based on Woese' analysis. The Woese system assigns all organisms to one of three domains or "superkingdoms"-the Bacteria, Archaea (formerly Archaebacteria), and Eucarya (FIGURE 2.7), all of which arose from a single ancestral line. (All of Whittaker's five traditional kingdoms can be reassigned among the three domains.) The Bacteria and the Eucarya first diverged from an ancestral stock, followed by the divergence of the Archaea from the Eucarya line. The domains



differ remarkably from one another in their chemical composition and in other characteristics, as summarized in TABLE 2.3. The term *bacteria* as commonly used includes both the bacteria and the archaea.

FIGURE 2.7 Woese's three-domain system.

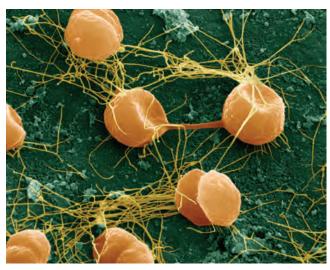
It should be apparent that classification, particularly at the level of microorganisms, is not cast in concrete but is constantly under revision as new information becomes available. It is a credit to the scientific process that reevaluation is the name of the game. Admittedly, it is confusing, but to quote William Shakespeare (who probably never even took a course in biology), "What's in a name? That which we call a rose by any other name would smell as sweet." So you need not sweat it too much! No matter what the classification, bacteria were the first forms of life on Earth. Fossilized bacteria have been discovered in **stromatolites**, stratified rocks dating back 3.5 billion to 3.8 billion years, a long time ago in the history of the estimated 4.6 billion-year-old planet Earth. When life arose, the Earth's ancient atmosphere contained little or no free oxygen but consisted principally of carbon dioxide and nitrogen with smaller

TABLE 2.3 Comparisons of Bacteria, Archaea, and Eucarya^a

Domain	Membrane- bound Nucleus	Cell Wall	Antibiotic Susceptibility	Characteristic
Bacteria	No	Present	Yes	Large number of bacterial species
Archaea	No	Present	No	"Extreme" bacteria growing in high- salt environment and at extreme temperatures
Eucarya	No	Variable	No (some exceptions in fungi)	Algae (most), fungi, protozoans, "higher" animals and plants

^aMajor differences are present in the biochemistry of cell walls, cell membranes, genetic material, and structures in the cytoplasm.

FIGURE 2.8 (a) *Pyrocoecus furiosus*, a highly heat-resistant bacterium. © Eye of Science/Photo Researchers, Inc. (b) Psychrophilic *Methanococcoides burtonii* discovered in 1992 in Ace Lake, Antartica, can survive in temperatures as low as -2.5°C. © Dr. M. Rohde, GBF/Photo Researchers, Inc.



amounts of gases, including hydrogen (H₂), hydrogen sulfide (H₂S), and carbon monoxide (CO). This ancient atmosphere, devoid of O_2 , would not have supported life as we know it. Only microbes that were able to meet their energy requirements with non-oxygen-requiring chemical reactions populated the primordial environment. The early microbes were photosynthetic and used water and carbon dioxide (CO₂) in photosynthetic reactions, resulting in the production of O_2 and **carbohydrates**. This process was responsible for the generation of O_2 in the Earth's atmosphere approximately two billion years ago.

Since their origin on Earth billions of years ago, bacteria have exhibited remarkable diversity and have filled every known ecological niche. Yet, according to some estimates, fewer than 2% of microbes have been identified and even fewer have been cultured and studied. Perhaps you can recall the old Mother Goose nursing rhyme "Peas Porridge Hot" which states "some like it hot and some like it cold." (Ask your instructor to sing it while you fall asleep in class!) It's like the diversity in bacteria. Bacteria belonging to the domain *Archaea* continue to be found in environments once considered too extreme or too harsh for life at any level. In

> most cases these organisms, extremophiles, cannot be grown by existing culture techniques; evidence of their presence has been obtained by molecular biology techniques that allow scientists to examine minute amounts of their deposited ribonucleic acid (RNA). Some like it hot and are called hyperthermophiles ("heat lovers"). Some hyperthermophiles have been identified in the hot springs in Yellowstone Park where the temperatures exceed 70°C. Some microbes do best at temperatures even higher, above 100°C. Thermus aquaticus was isolated from hot springs in the 1960s. It produced an enzyme (Taq polymerase), which is essential to a very important technique (polymerase chain reaction; PCR) in molecular biology allowing for rapid DNA synthesis and sequencing. Pyrococcus furiosus lives in boiling water bubbling from undersea hot vents and freezes to death in temperatures below 70°C (FIGURE 2.8a). Some extremophiles, the psychrophiles, like it cold. Psychrophiles have growth temperatures lower than -20°C and are happy in Arctic and Antarctic environments (Figure 2.8b). Finally, if you like tongue-twisters, try your tongue around Psychromonas ingrahamii and Colwellia psychrerythraea, both at home and living comfortably in frigid waters. Some bacteria are extreme halophiles ("salt lovers") (FIGURE 2.9), and some produce methane gas in their metabolism. These bizarre examples indicate that many of the archaea live at the extremes of life zones (BOX 2.2). Archaea have not been implicated as disease producers and are not further considered in this text.

> In 2010, NASA scientists reported the discovery of bacteria that could substitute arsenic for phosphorous, a necessary component of DNA. If correct, the finding

(b)

(a)

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FIGURE 2.9 The Dead Sea. This sea has a salt concentration well above that found in the Great Salt Lake in Utah; it lies farther below sea level than any other terrestrial spot on Earth. You can lie on your back and float without any effort. Amazingly, this extreme environment is home for a variety of halophilic bacteria. Author's photo (RIK).

BOX 2.2 Some Bizarre Bacteria

Some microbes exhibit an unusual lifestyle and remarkable characteristics that provide fascinating stories and illustrate the tremendous diversity of the microbial world.

Consider Deinococcus radiodurans, a bacterium further described in Box 2.3, that can survive a dose of radiation greater than 3,000 times the dose that can kill a human. The Dead Sea, characterized by its extreme salinity, is erroneously named; it is not dead at all but teems with salt-loving (halophilic) bacteria. You will be surprised to learn that microbes can grow in your car's battery acid or that some bacteria thrive on arsenic. How about magnetotactic bacteria? They manufacture minute, iron-containing magnetic particles used as compasses by which the organisms align themselves to the Earth's geomagnetic field. These curious microbes prefer life in the deeper parts of their aquatic environment where there is less oxygen. Their magnetic compass points the way.

And then there are the as yet unnamed bacteria living in symbiotic partnership with giant tube worms, as long as 2 meters, living in the hydrothermal vents of the ocean floor. As these worms mature, their entire digestive tract disappears, including their mouth and anal openings. Now that presents a problem, and it's bacteria to the rescue! The tissues of the worm are loaded with bacteria that obtain energy from the surrounding chemical environment sufficient for their own needs and for those of their worm hosts. In turn, the worms provide a safe harbor for the bacteria, ensure an adequate environment for energy production, and provide nitrogen-rich waste materials, allowing for synthesis of

microbial cellular components—a great mutualistic arrangement.

Here is a strange story about Serratia marcescens, a bacterium whose colonies form a deep red pigment when grown in moist environments. In 1263 in the Italian town of Bolsena a priest was celebrating Mass. When he broke the communion wafers he found what he thought was blood on them and assumed it to be the blood of Christ. Given the lack of scientific knowledge during the Dark Ages, it is understandable the event was regarded as a miracle. It was not a miracle at all. The red-pigment-producing *S. marcescens* had contaminated the wafers during their storage in the dampness of the ancient church (FIGURE B2.2). Nevertheless, Raphael's painting The Miracle of Bolsena, depicting this event, hangs on a wall in the Vatican. Here's another story of an unusual bacterium, Shewanella, reported in June 2011. These microbes use metal ions in place of oxygen in their metabolism and in so doing, can minimize some toxic metals from migrating into soil and FIGURE B2.2 A culture of Serratia marcescens. groundwaters—a nice example of bacteria used for clean-up.



Courtesy of Jeffrey Pommerville.

BOX 2.3 __Conan the Bacterium

Conan the Barbarian, a 1982 movie starring Arnold Schwarzenegger, was the first Conan movie. In this fantasy story, from the mythical age of sword and sorcery, Arnie portrays Conan as only Arnie can do!

Deinococcus radiodurans has been nicknamed "Conan the bacterium"; it is one of nature's "toughest cookies." It can survive the rigors of being completely dried out, have its chromosomes disrupted, and be exposed to 1.5 million rads of radiation, a dose 3,000 times greater than that which would kill a human. Further, it can transform toxic mercury into a less toxic form, a feature especially useful at nuclear waste sites. According to Owen White of the Institute for Genomic Research in Rockville, Maryland, "The Department of Energy is very jazzed about *D. radiodurans*, because the agency has a pretty big toxic cleanup problem at its waste development sites." Genes from bacteria that can digest toxic waste but cannot survive radiation have been genetically engineered into *D. radiodurans,* resulting in bacteria that can transform toxic mercury into a nontoxic form and unstable uranium into a stable form. These genetically engineered bacteria are powerful tools in cleaning up the three thousand waste sites containing millions of cubic yards of contaminated soil and contaminated groundwater estimated to be in the trillions of gallons. The ability of *D. radiodurans* to repair its own DNA is of interest to biologists because the process provides an insight into the mechanisms of aging and into the biology of cancer.

would open up a new form of life on earth. Other scientists were skeptical about the study, so the role of arsenic-loving bacteria remains open. Here is another strange story. *Deinococcus radiodurans* is a bacterium that can withstand 3,000 times more gamma radiation than that which would kill a human because of its unique ability to repair its damaged DNA. The scientists who study *D. radiodurans* have dubbed it "Conan the bacterium" (BOX 2.3).

You may not like to hear this, but the human mouth is considered one of the most diverse ecosystems and rivals the biological diversity of tropical rainforests. Within the past few years, scientists at Stanford University have discovered 37 new organisms in the mouth, pushing the total to more than 500. These new microbes were found in the scum (plaque) in the deep gum pockets between teeth. (Your dentist would love this tidbit!) Their presence remained unknown simply because traditional culture methods do not allow their growth. Enterprising microbiologists (sometimes known as plaque pickers) extracted DNA from plaque and mapped out DNA sequences, revealing bacteria that had not been previously known to inhabit the mouth. In fact, some new bacterial species were identified, supporting the statement that less than 2% of the microbial population has been identified.

A comprehensive global microbial survey to identify microbes that make up the biosphere is underway thanks to the cooperative effort of the National Science Foundation and the American Society for Microbiology. These two organizations are establishing a network of biodiversity research sites or "microbial observatories." Other international efforts are in the works to develop a worldwide microbial inventory of genetic sequences.

The origin of life on Earth continues to be a fascinating and mind-boggling question to which the explanation is purely speculative. The general consensus among scientists is that the "primordial soup" hypothesis is the most likely explanation. In this hypothesis, organic compounds formed from a specific combination

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of atmospheric gases collected in water and sparked by an energy source. How exactly this happened is still debated.

Another intriguing possibility is the hypothesis that Earth was seeded by life forms from Mars, the Red Planet. Photographs taken from the orbiting Mars Global Surveyor spacecraft indicated the possibility of water just below the surface of the planet. If, in fact, Mars has water, it is possible that the planet entertains, or entertained, life. According to the Laboratory for Atmospheric and Space Physics at the University of Colorado at Boulder, "Mars meets all the requirements for life." The possibility that life originated on Mars and was subsequently carried to Earth is plausible. Meteors and meteorites are constantly bombarding the Earth and some, originating from Mars' surface, could have transported ancestral procaryotic cells. Bacteria have been cultured out of Siberian and Antarctic permafrosts that have been in the deep freeze for millions of years. The National Aeronautic and Space Administration is now planning the Mars Sample Return Mission, which will bring Martian rocks back to Earth, and this will help to resolve the question of the beginnings of life. A famous and historic press conference was held at the National Aeronautic and Space Administration in Washington, D.C. on August 7, 1996, announcing that scientists had found evidence of ancient microbial life in a Mars meteorite known as ALH84001. Bear in mind, however, that the evidence was viewed by some authorities as weak and remains highly refuted.

Introducing the Microbes

Although there is no clear definition of microbes, it is time to introduce those biological agents that fall under the microbial umbrella (FIGURE 2.10 and TABLE 2.4). Algae are not discussed in detail in this text beyond this section, although they are highly significant in terms of food chains and other beneficial aspects. Further, some fungi are human pathogens, and many contribute to the death toll of patients with AIDS. With the exception of viruses and prions, all microbes have both DNA and RNA, as do all cells.

Microbes are measured in very small units of the metric system called **micrometers** (equal to one millionth of a meter), abbreviated as μ m, and **nanometers** (equal to 1 billionth of a meter), abbreviated as nm. A meter is equivalent to about 39 inches, so a micrometer is equal to one millionth of 39 inches. These numbers are probably not very meaningful to you, because they do not allow you to appreciate the size of microbes relative to more familiar

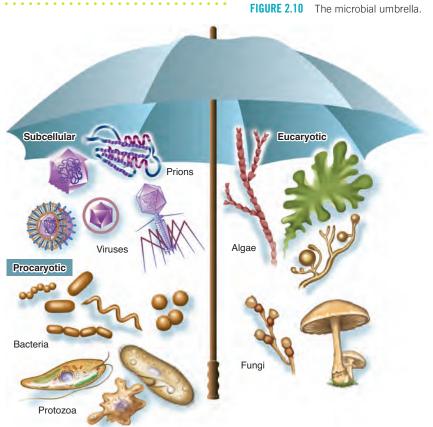


TABLE 2.4 Comparison of Microbial Groups^a

Characteristic	Archaea	Bacteria	Protozoans	Fungi	Unicellular Algae
Cell type	Procaryotic	Procaryotic	Eucaryotic	Eucaryotic	Eucaryotic
Size	Microscopic	Microscopic ^b	Microscopic	Macroscopic	Microscopic
Cell wall	Present	Present	Absent	Present	Present
Reproduction	Mostly asexual (binary fission)	Mostly asexual (binary fission)	Sexual and asexual	Sexual and asexual	Asexual
Energy process	Variable	Mostly heterotrophic	Heterotrophic	Heterotrophic	Autotrophic

^aViruses and prions are not cells and, therefore, are not included. ^bThere are a few exceptions.

> objects, but there are some points that may help you think in this scale. A spoonful of fertile soil contains trillions of microbes, and the number of microbes that can be accommodated on the period at the end of this sentence is in the millions. FIGURE 2.11 indicates the relative size of microbes. The monstrous bacteria described earlier are exceptions. Bacteria are many times smaller than eucaryotic cells but are about fifty times (or more) larger than viruses.

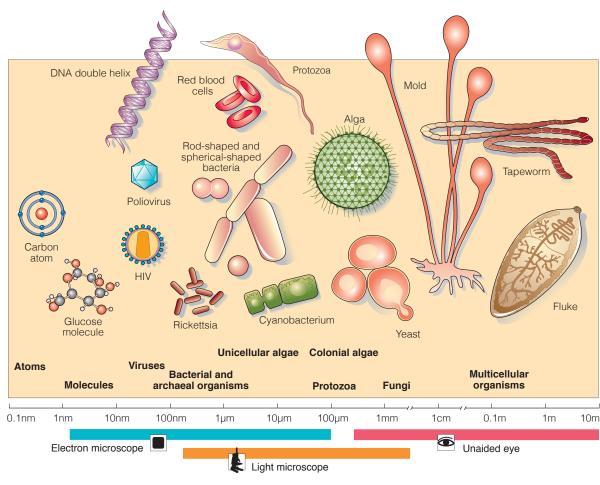


FIGURE 2.11 Comparison of sizes of different kinds of microorganisms (not drawn to scale).

Smallness has its advantages. Smallness provides a large surface area per unit volume, allowing for rapid uptake of nutrients from the environment. *E. coli,* for example, has a surface-to-volume ratio about twenty times greater than that of human cells. And now for the introduction—from least to the most complicated.

Prions

Prions are the most recent addition to the microbial list; some texts continue to place them with viruses for lack of a better place. But the awarding of the 1997 Nobel Prize to Stanley Prusiner, who discovered these agents, legitimized them as

separate entities. The word *prion* is an abbreviation for proteinaceous infectious particles. Prions are protein molecules and are devoid of both DNA and RNA; their lack of nucleic acid is their major (and most puzzling) biological property. Prions exist normally, primarily in the brain, as harmless proteins. Abnormal prions convert normal proteins into infectious, disease-producing proteins responsible for mad cow disease and dementia type diseases in humans and in other animals. Questions remain unanswered regarding their biology.

Viruses

As frequently noted, viruses are not organisms; Figure 2.4 indicates their subcellular position. Two major distinguishing characteristics of viruses are that, in contrast to cells, they contain either RNA or DNA (never both) and, further, they are submicroscopic particles and can be seen only with an electron microscope (FIGURE 2.12). Some have an additional coat or envelope encompassing them. Viruses are described as **obligate intracellular parasites**, meaning they must be (obligate) inside living cells (intracellular) to replicate; they are not capable of autonomous replication. They take over the metabolic machinery and reap the benefits of energy production, without any expenditure of energy, by the host cell. Perhaps this is the ultimate in parasitism.

Bacteria

Bacteria are the best known of the microbial. They are microscopic, unicellular, procaryotic, and have cell walls (with the exception of a single subgroup, the *Mycoplasma*). They reproduce asexually by binary fission. In terms of size, they can be seen with a regular (light) microscope (FIGURE 2.13). Many bacteria are heterotrophs and use organic compounds as a source of energy. Others are autotrophs and use the energy of the sun, whereas some derive energy from the use of inorganic substances. Although a number of

FIGURE 2.12 A transmission electron micrograph of smallpox (Variola) viruses. Courtesy of Dr. Fred Murphy/CDC.

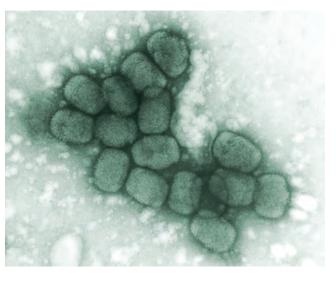
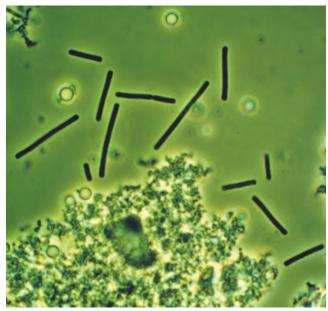


FIGURE 2.13 Lactobacillus bacteria. © John Walsh/Photo Researchers, Inc.



CHAPTER 2 The Microbial World

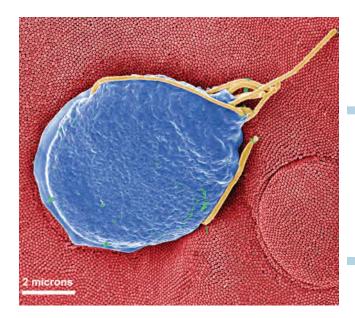


FIGURE 2.14 A colorized scanning electron micrograph of a flagellated *Giardia* protozoan adhering to an intestinal epithelial cell. Courtesy of Dr. Stan Erlandsen/CDC.

bacteria are pathogens and are the major subject of this text, the vast majority of bacteria are nonpathogenic and play essential roles in the environment without which life would not be possible.

Protozoans

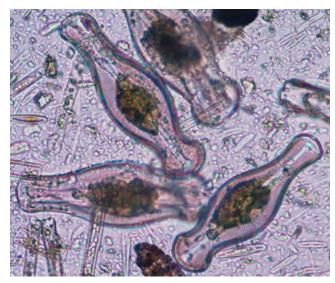
Protozoans are unicellular and eucaryotic and are classified according to their means of locomotion (FIGURE 2.14). Their energy generation requires the utilization of organic compounds. Many diseases, including malaria, sleeping sickness, and amebic dysentery, are caused by protozoans.

Algae

Algae are photosynthetic eucaryotes and in the photosynthetic process produce oxygen and carbohydrates used by forms requiring organic compounds. Hence,

they are highly significant in the balance of nature. **Dinoflagellates** and **diatoms** are examples of unicellular algae and fall under the umbrella of microbes (**FIGURE 2.15**). Dinoflagellates (plankton) are the primary source of food in the oceans of the world. Some algae are pathogenic for humans indirectly. For example, the toxin produced by the dinoflagellates that causes red tide, *Gymnodinium breve*, causes neurological disturbances and death in humans as a result of our consumption of fish and shellfish that had fed on the dinoflagellates. Another species of dinoflagellate, *Pfiesteria piscicida*, also referred to as the "cell from hell," threatened the fishing industry in the eastern United States in 1997. A bloom of these algae resulted in the release of large amounts of neurotoxin, causing neurological symptoms in fishermen and fear among consumers.

FIGURE 2.15 Freshwater diatoms. Courtesy of Robert W. Pillsbury.



Fungi

Fungi are eucaryotes. Morphologically, they can be divided into two groups, the yeasts and the molds. The yeasts are unicellular and are larger than bacteria; many reproduce by budding. Molds are the most typical fungi and are multicellular, consisting of long, branched, and intertwined filaments called **hyphae**. In early schemes of classification fungi were considered plants, primarily because they have cell walls. However, their cell wall composition is quite different from that of plants and from the cell walls of bacteria. Fungi are highly significant in terms of food chains and have certain beneficial aspects. Some are pathogenic and cause diseases that are difficult to treat; others play a highly significant role as **opportunistic pathogens** (organisms that are not usually considered to be pathogens), because, as in AIDS,

when the immune system is depressed they cause disease. Molds played a major role in the aftermath of Hurricanes Katrina and Rita, rendering houses uninhabitable. Patches of mold threaten the prehistoric paintings of animals in the Lascaux Cave in Dordogne region of southwest France and museum curators constantly need to guard against mold intrusions.

Mushrooms are a well-known group of fungi. Their diversity is unusual as demonstrated by a wide range of size, colors, and patterns on their cap (FIGURE 2.16). Many species are edible. The terms *bugs* and *germs* are part of our popular speech but have no scientific meaning. It should be clear from the above descriptions that each group of microbes is distinct from the others. When your physician diagnoses you as having a "bug," you might ask what kind.



FIGURE 2.16 Two mushrooms growing on a tree stump in a field. Author's photo (TS).

Overview

The microbial world is remarkable for its extreme diversity, as is evident in the distinct characteristics of the six microbial groups—prions, viruses, bacteria, protozoans, unicellular algae, and fungi. Further, within each group there is considerable diversity. Not all microbes are unicellular and microscopic; some are multicellular and macroscopic and others are subcellular and microscopic. In recent times "monster" bacteria have been found that are unique in being unicellular and macroscopic, a rare combination. Viruses and prions are subcellular and are not considered life forms. There is no clear definition of what makes a microbe a microbe, but it is clear that they are all at less than the tissue level of biological organization.

All bacteria are procaryotic, and all other microbes are eucaryotic. (Viruses and prions are not cells and are neither procaryotic nor eucaryotic.) Taxonomy evolved from a two-kingdom system (in which bacteria were considered plants) to a five-kingdom system, with various other schemes along the way; the trend has been toward recognizing the uniqueness of microbes. Woese proposes a classification system based on rRNA analysis and assigns bacteria to one of three domains and reflects their evolutionary history.

Since their origin on Earth microbes have adapted to extreme ecological diversity and can be isolated from all environments. Some live at the extremes—from hot springs to permafrost. All organisms must meet a basic requirement for energy, and microbial evolution has fostered a diversity of strategies. Some microbes obtain energy from organic compounds, whereas others use the energy of the sun or derive their energy from the metabolism of inorganic compounds.

The major characteristics of each of the six microbial groups show that each category is distinctive. The popular terms *bugs* and *germs* are used in a collective sense, but there is no basis for lumping these diverse microbial agents together. Further, these terms have a negative connotation, because they are usually used to describe microbial diseases, but it is important to remember that only a handful of microbes are disease producers.

Self-Evaluation

PART I: Choose the *single* best answer.

1. A major distinction between procaryotic and eucaryotic cells is based on the presence of

a. a cell wall b. DNA c. a nuclear membrane d. a cell membrane

- **2.** Most bacteria are considered to be
 - a. harmful b. anaerobes c. autotrophs d. heterotrophs
- 3. The smallest of these units of measurement is
 - a. millimeter b. nanometer c. micrometer d. centimeter
- 4. Which of the following are obligate intracellular parasites?a. bacteria b. viruses c. unicellular algae d. diatoms
- 5. Which one of the following does not have nucleic acid in its structure?a. viruses b. diatoms c. bread mold d. prions
- 6. The five-kingdom system of taxonomy is credited toa. Haeckel b. Woese c. Whittaker d. Darwin
- 7. According to Woese,
 - **a.** *Eucarya* arose from *Archaea*.
 - **b.** Archaea arose from Eucarya.
 - c. Bacteria, Archaea, and Eucarya all arose independently.
 - **d.** None of the above is correct.
- 8. This bacterium produces an enzyme that catalyzes DNA synthesis.
 - **a**. *E*. coli **b**. Thermus aquaticus **c**. Serratia marcescens **d**. Dienococcus radiodurans
- 9. Which of the following microbes are not disease producers?a. fungi b. bacteria c. archaea d. protozoa
- **10**. This bacterium produces a red pigment and contaminated communion wafers during a mass in 1263.
 - a. Staphylococcus aureus b. Serratia marcescens c. Yersinia pestis
 - d. Bacillus cereus

PART II: Fill in the blank.

- 1. Bacteria, viruses, fungi, and protozoans are microbes. Name another group that falls under the microbial umbrella.
- 2. The cell theory is credited to _____
- 3. Compounds of carbon are called _____ compounds.
- 4. Organisms that do not require organic compounds are called
- 5. The "energy compound" is called ______
- 6. Strict anaerobes are killed by _____
- 7. The term ______ is used to describe organisms too small to be seen without a microscope.

- 8. _____ are the ancestors of eucaryotes.
- 9. Most bacteria reproduce by _____
- 10. _____ are infectious protein molecules.

PART III: Answer the following.

- 1. Criticize the terms *bugs* and *germs* as used in a collective sense to describe microbes. List the categories of microbes, and write a one-sentence description of each.
- 2. What makes a microbe a microbe?
- **3.** What is the relevance to microbiology of Shakespeare's "What's in a name? That which we call a rose by any other name would smell as sweet."?
- 4. Heterotrophs are dependent on autotrophs. Why is this the case?
- 5. The archaea can survive extreme environments. Why?
- **6.** Fungi play a highly significant role as opportunistic pathogens. Define opportunistic pathogens and who is most susceptible to these types of infections.
- **7.** Explain why viruses and prions are not cells and are neither prokaryotic nor eukaryotic.
- 8. Explain why some microbes can survive without oxygen present.
- 9. Compare and contrast eukaryotic and prokaryotic cells.
- 10. Why do some bacteria form symbiotic partnerships with other life forms?