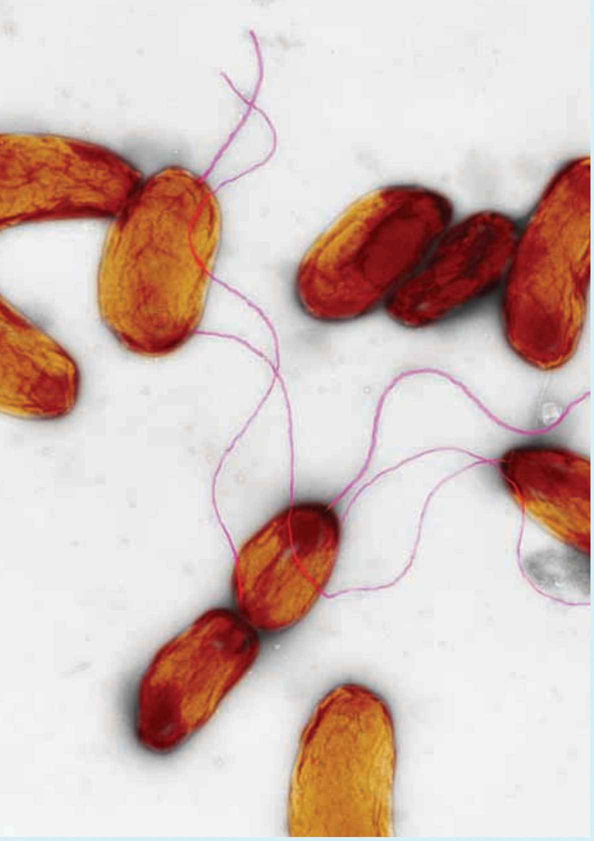
PART 1

Foundations of Microbiology



Cells of *Vibrio cholerae*, transmitted to humans in contaminated water and food, are the cause of cholera.

In 1676, a century before the Declaration of Independence, a Dutch merchant named Antony van Leeuwenhoek sent a noteworthy letter to the Royal Society of London. Writing in the vernacular of his home in the United Netherlands, Leeuwenhoek described how he used a simple microscope to observe vast populations of minute, living creatures. His reports opened a chapter of science that would evolve into the study of microscopic organisms and the discipline of microbiology. During the next three centuries scientists would discover how profoundly these organisms influence the quality of our lives and the environment around us.

We begin our study of the microorganisms by exploring the grassroot developments that led to the establishment of microbiology as a science. These developments are surveyed in Chapter 1, where we focus on some of the individuals who stood at the forefront of discovery. Today we are in the midst of a third Golden Age of microbiology and our understanding of microorganisms continues to grow even as you read this book.

Chapter 2 reviews basic chemistry, inasmuch as microbial growth, metabolism, and control are grounded in the molecules and macromolecules these organisms contain and in the biological processes they undergo. Chapter 3 sets down some basic microbiological concepts and describes one of the major tools for studying microorganisms. We will concentrate on the bacterial organisms in Chapter 4, where we survey their structural frameworks. In Chapter 5, we build on these frameworks by examining microbial growth patterns and nutritional requirements. Chapter 6 describes the metabolism of microbial cells, including those chemical reactions that produce energy and use energy. Part 1 concludes by considering the physical and chemical methods used to control microbial growth and metabolism (Chapter 7).

Much as the alphabet applies to word development, in each succeeding chapters we will formulate words into sentences and sentences into ideas as we construct an understanding of microorganisms and concentrate on their importance to public health and human welfare.

MICROBIOLOGY PATHWAYS

Being a Scientist

Science may not seem like the most glamorous profession. So, as you read many of the chapters in this text, you might wonder why many scientists have the good fortune to make key discoveries. At times, it might seem like it is the luck of the draw, but actually many scientists have a set of characteristics that put them on the trail to success.

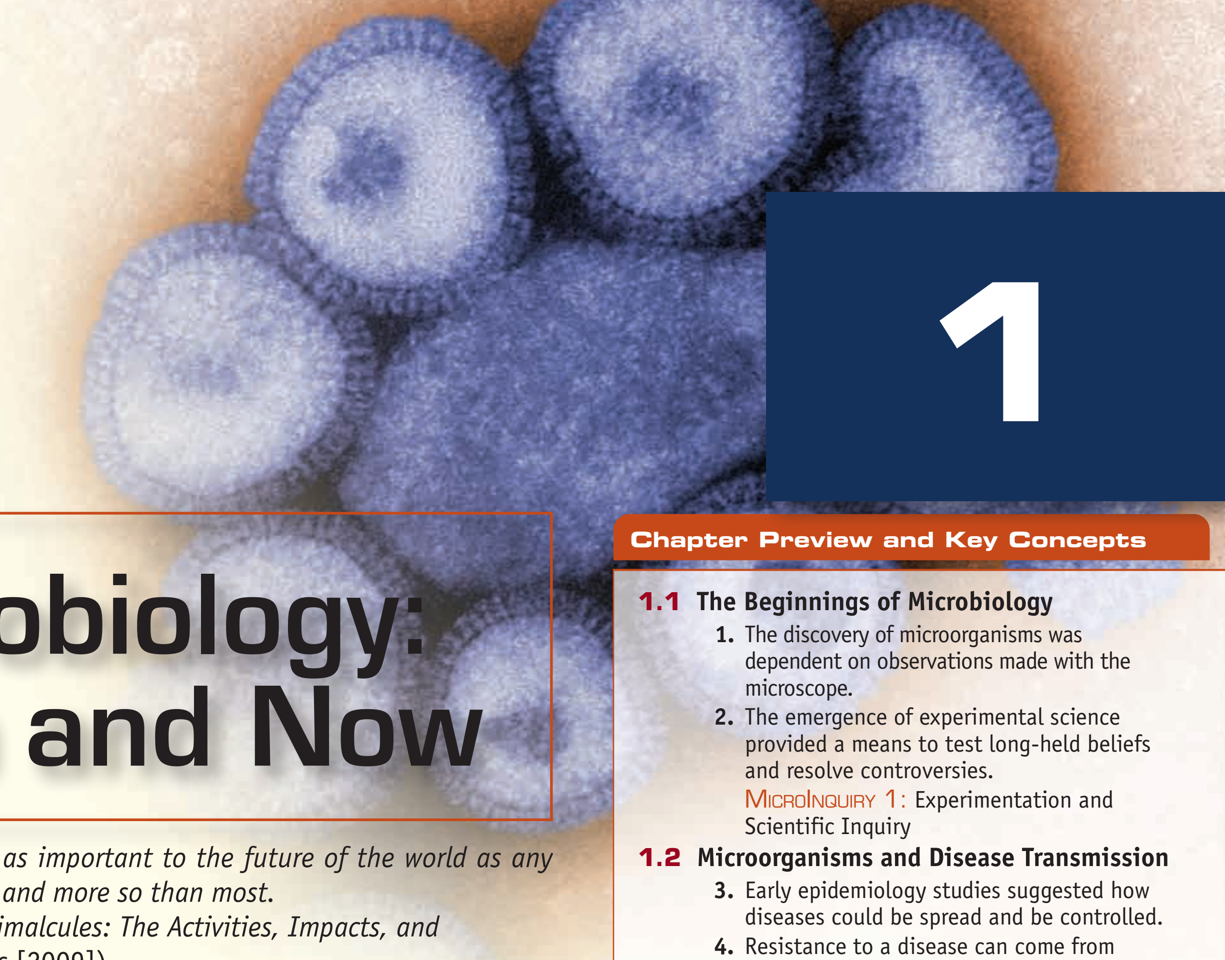
Robert S. Root-Bernstein, a physiology professor at Michigan State University, points out that many prominent scientists like to goof around, play games, and surround themselves with a type of chaos aimed at revealing the unexpected. Their labs may appear to be in disorder, but they know exactly where every tube or bottle belongs. Scientists also identify intimately with the organisms or creatures they study (it is said that Louis Pasteur actually dreamed about microorganisms), and this identification brings on an intuition—a "feeling for the organism." In addition, there is the ability to recognize patterns that might bring a breakthrough. (Pasteur had studied art as a teenager and, therefore, he had an appreciation of patterns.)

The geneticist and Nobel laureate Barbara McClintock once remarked, *"I was just so interested in what I was doing I could hardly wait to get up in the morning and get at it. One of my friends, a geneticist, said I was a child, because only children can't wait to get up in the morning to get at what they want to do."* Clearly, another characteristic of a scientist is having a child-like curiosity for the unknown.

Professor Alcamo once received a letter from a student, asking why he became a microbiologist. *"It was because I enjoyed my undergraduate microbiology course"* he said, *"and when I needed to select a graduate major, microbiology seemed like a good idea. I also think I had some of the characteristics described by Root-Bernstein: I loved to try out different projects; my corner of the world qualified as a disaster area; still I was a nut on organization, insisting that all the square pegs fit into the square holes."*

For this author, science has been an extraordinary opportunity to discover and understand something never before known. Science is fun, yet challenging—and at times arduous, tedious, and frustrating. As with most of us, we will not make the headlines for a breakthrough discovery or find a cure for a disease. However, as scientists we all hope our research will contribute to a better understanding of a biological (or microbiological) phenomenon and will push back the frontiers of knowledge.

Like any profession, being a scientist is not for everyone. Besides having a bachelor's degree in biology or microbiology, you should be well read in the sciences and capable of working as part of an interdisciplinary team. Of course, you should have good quantitative and communication skills, have an inquisitive mind, and be goal oriented. If all this sounds interesting, then maybe you fit the mold of a scientist. Why not consider pursuing a career in microbiology? Some possibilities are described in other Microbiology Pathways included in this book, but you should also visit with your instructor. Simply stop by the student union, buy two cups of coffee, and you are on your way.



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Microbiology: Then and Now

Microbiology is at least as important to the future of the world as any other branch of science, and more so than most.
—Bernard Dixon (in *Animalcules: The Activities, Impacts, and Investigators of Microbes* [2009])

Space. The final frontier! Really? *The* final frontier? There are an estimated 350 billion large galaxies and more than 10^{22} stars in the visible universe. However, the microbial universe consists of more than 10^{31} microorganisms scattered among an estimated 2 to 3 billion species. So, could understanding these organisms on Earth be as important as studying galaxies in space?

In 1990, microbiologist Stephen Giovannoni of Oregon State University identified in the Sargasso Sea off the southeast United States what is perhaps the most abundant and successful organism on the planet. Called SAR11 (SAR for Sargasso), this bacterial organism, which now goes by the scientific name *Pelagibacter ubique,* has been identified across the oceans of the world. What makes it significant is its population size. Estimated to be 2.4×10^{28} cells, SAR11 alone accounts for 20% of all oceanic bacterial species—and 50% of the bacterial species in the surface waters of temperate oceans in the summer!

SAR11's success story suggests the organism must have a significant impact on the planet. Although such roles remain to be identified and understood, Giovannoni believes SAR11 is responsible for up to 10% of all nutrient recycling on the planet, influencing the cycling of carbon and even affecting climate change.

Also sailing the Sargasso Sea was Craig Venter and his team from the J. Craig Venter Institute. Fresh from his success with the private sector effort to sequence the human genome, Venter's team in 2004 reported the discovery of over 1,800 new microbial species in Sargasso seawater and from them isolated 1.2 million new gene sequences.

Then between 2003 and 2007, Venter's team sailed the world's oceans—à la Charles Darwin—to sample seawater and evaluate the diversity of microorganisms in the Pacific and Atlantic oceans. For the

Chapter Preview and Key Concepts

1.1 The Beginnings of Microbiology
1. The discovery of microorganisms was dependent on observations made with the microscope.
2. The emergence of experimental science provided a means to test long-held beliefs and resolve controversies.

MicroInquiry 1: Experimentation and Scientific Inquiry

1.2 Microorganisms and Disease Transmission
3. Early epidemiology studies suggested how diseases could be spread and be controlled.
4. Resistance to a disease can come from exposure to and recovery from a mild form of (or a very similar) disease.

1.3 The Classical Golden Age of Microbiology (1854–1914)
5. The germ theory was based on the observations that different microorganisms have distinctive and specific roles in nature.
6. Antisepsis and identification of the cause of animal diseases reinforced the germ theory.
7. Koch's postulates provided a way to identify a specific microorganism as causing a specific infectious disease.
8. Laboratory science and teamwork stimulated the discovery of additional infectious disease agents.
9. Viruses also can cause disease.
10. Many beneficial bacterial species recycle nutrients in the environment.

1.4 Studying Microorganisms
11. The organisms and agents studied in microbiology represent diverse groups.

1.5 The Second Golden Age of Microbiology (1943–1970)
12. Microorganisms and viruses can be used as model systems to study phenomena common to all life.
13. All microorganisms have a characteristic cell structure.
14. Antimicrobial chemicals can be effective in treating infectious diseases.

1.6 The Third Golden Age of Microbiology—Now
15. Infectious disease (natural and intentional) preoccupies much of microbiology.
16. Microbial ecology and evolution are dominant themes in modern microbiology.

emerging field of marine molecular microbiology, Venter believes the gene sequencing of marine microorganisms will provide examples of novel metabolic pathways, identify species that use alternative energy sources, and perhaps help solve critical environmental problems, including climate change.

Giovannoni and Venter are just two of many microbiologists trying to understand the role of microorganisms in the ocean's ecosystems and their dominant role on this planet. But most of all, as "Being a Scientist" identified, Giovannoni's and Venter's primary goal is a voyage of discovery. Because only about 1% of the marine microorganisms have been identified, the microbial universe does represent an inner final frontier!

The science of **microbiology** embraces a biologically diverse group of usually microscopic life forms, encompassing primarily **microorganisms** (bacteria, fungi, and protists) and viruses.

Microorganisms (or **microbes** for short) are present in vast numbers in every environment and habitat on Earth where there is water. They survive in Antarctica, on top of the tallest mountains, near thermal vents in the deepest parts of the oceans, and even miles down within the crust of the earth. In all, microbes make up more than half of Earth's biomass.

The rich diversity of microorganisms is reflected in their profound influence on all aspects of life. Most are harmless or indeed beneficial. For example, they are essential to the recycling of nutrients that form the bodies of all organisms and sustaining all the metabolic cycles of life. They affect our climate and, as a group, produce about 50% of the oxygen gas we breathe and many other organisms use. They have influenced the evolution of life on Earth and actually have outpaced that of the more familiar plants and animals.

Microorganisms survive in, or are purposely put in, many of the foods we eat. Microorganisms and viruses also are in the air we breathe and, at times, in the water we drink. Even closer to home, some 100 billion microorganisms colonize our skin and grow in our mouth, ears, nose, throat, and digestive tract (FIGURE 1.1A). Fortunately, the majority of these microbes, called our natural **microbiota**, are actually beneficial in helping us resist disease, and regulating development and nutrition. To be human, we must be "infected."

When most of us hear the word "bacterium" or "virus" though, we think infection or disease. Although such **pathogens** (disease-causing agents) are rare, they periodically have carved out great swatches of humanity as epidemics passed over the land. Some diseases—such as plague, cholera, and smallpox—have become known historically as "slate-wipers," a reference to the barren towns they left in their wake (MicroFocus 1.1). Even today, with antibiotics and vaccines to cure and prevent many infectious diseases, pathogens still bring concern and sometimes panic. Just think about the scares that AIDS, and most recently avian and swine influenzas have caused worldwide (FIGURE 1.1B).

■ Genome: The complete set of genetic information in a cell, organism, or virus.

■ Biomass: The total weight of living organisms within a defined environment.

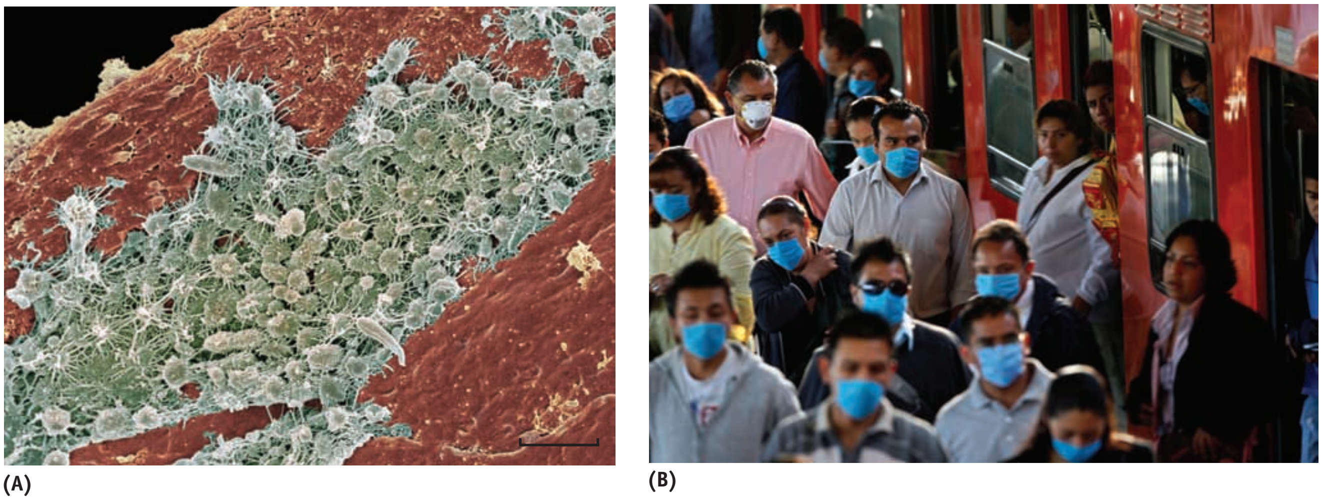

(A) (B)

FIGURE 1.1 **Microbes Are Key to Health and Illness.** (**A**) Large numbers of bacterial cells are found on and in parts of the human body. On the tongue, most are harmless or even beneficial, while a few in our mouth can cause throat infections or lead to tooth decay. (Bar = 5 µm.) (**B**) During the 2009–2010 swine flu pandemic, people in affected areas such as Mexico wore masks in an attempt to avoid being infected with the virus.

MICROFOCUS 1.1: History

The Tragedy of Eyam

On the last Sunday in August (Plague Sunday), English pilgrims gather in the English countryside outside the village of Eyam, to pay homage to the townsfolk who in 1665–1666 gave their lives so that others might live. The pilgrims pause, bow their heads, and remember. In 1665, bubonic plague was raging in London. In late August, a traveling tailor arrived in the village of Eyam, about 140 miles north of London. Unknown to him, cloth arriving from London was infected with plague-carrying fleas.

Within a few days, plague began to spread throughout Eyam and villagers debated whether they should flee north. The village rector realized that if the villagers left, they could spread the plague to other towns and villages. So, he made a passionate plea that they stay. After some deep soul-searching, most townsfolk resolved to remain, even though they knew that meant many would die.

Mompesson's well at Eyam in Derbyshire Peak in Great Britain. During the plague, Eyam residents left coins in the well in exchange for food and medicines.

The villagers marked off a circle of stones outside the village limits, and people from the adjacent towns brought food and supplies to the barrier, leaving them there for the self-quarantined villagers (see figure). Finally, in late 1666, the plague subsided. The rector recorded, *"Now, blessed be God, all our fears are over for none have died of the plague since the eleventh of October and the pest-houses have long been empty."* In the end, 260 of the town's 350 residents succumbed to the plague. Some have suggested this self-sacrificing incident is commemorated in a familiar children's nursery rhyme, one version of which is:

A ring-a-ring of rosies
A pocketful of posies
A tishoo! A tishoo!
We all fall down.

The ring of rosies refers to the rose-shaped splotches on the chest and armpits of plague victims. Posies were tiny flowers the people hoped would sweeten the air and ward off the foul smell associated with the disease. "A tishoo!" refers to the fits of sneezing that accompanied the disease. The last line, the saddest of all, suggests the deaths that befell so many.

Still, if microbes were solely agents of disease, none of us would be here today. Rather "infection" is a way of life—we, all life, and our planet are dependent on microbes!

A major focus of this introductory chapter is to give you an introspective "first look" at microbiology—then and now. We will see how microbes were first discovered and how those that cause infectious disease preoccupied the minds and efforts of so many. Along the way, we continue to see how curiosity and scientific inquiry stimulated the quest for understanding.

Although the study of microorganisms began in earnest with the work of Pasteur and Koch, they were not the first to report microorganisms. To begin our story, we reach back to the 1600s, where we encounter some equally inquisitive individuals.

1.1 The Beginnings of Microbiology

As the 17th century arrived, an observational revolution was about to begin: Dutch spectacle maker, Zacharias Janssen, was one of several individuals who discovered that if two convex lenses were put together, small objects would appear larger. Many individuals in Holland, England, and Italy further developed the two-lens system. In fact, it was in 1625 that the Italian Francesco Stelluti or Giovanni Faber used the term *microscopio* or "microscope" to refer to this new invention, which

■ **Convex:** Referring to a surface that curves outward.

Galileo had suggested be called, "the small glass for spying things up close." This combination of lenses, or "compound microscope," would be the forerunner of the modern microscope.

Microscopy—Discovery of the Very Small

KEY CONCEPT

1. The discovery of microorganisms was dependent on observations made with the microscope.

Robert Hooke, an English natural philosopher (the term "scientist" was not coined until 1833), was one of the most inventive and ingenious minds in the history of science. As the Curator of Experiments for the Royal Society of London, Hooke was the first to take advantage of the magnification abilities of the compound microscope. Although these microscopes only magnified about 25 times (25×), Hooke made detailed studies of many small living objects. In 1665, the Royal Society published his *Micrographia*, which contained descriptions of microscopes and stunning hand-drawn illustrations, including the anatomy of many insects and the structure of cork, where he used the word *cella* to describe the "great many little boxes" he observed and from which today we have the word "cell" (FIGURE 1.2A). Importantly, Hooke was the first person to describe and draw a microorganism, a mold he found growing on the sheepskin cover of a book (FIGURE 1.2B).

Micrographia represents one of the most important books in science history because it awakened the learned and general population of Europe to the world of the very small, revolutionized the art of scientific investigation, and showed that the microscope was an important tool for unlocking the secrets of nature.

Antony van Leeuwenhoek, a contemporary of Hooke, was a successful tradesman and dry goods dealer in Delft, Holland. As a cloth merchant, he used hand lenses to inspect the quality of cloth. After seeing Hooke's *Micrographia*, and without scientific training, Leeuwenhoek became skilled at grinding single pieces of glass into fine, biconvex lenses, which he placed between two silver or brass plates riveted together (FIGURE 1.2C, D). Using only a single lens, no larger than the head of a pin, his "simple microscope" could magnify objects more than 200×.

The process of "observation" is an important skill for all scientists, including microbiologists—and Leeuwenhoek believed only sound observation and experimentation could be trusted—a requirement that remains a cornerstone of all science inquiry today.

Leeuwenhoek chose to communicate his observations through letters to England's Royal Society. In 1674, one letter described a sample of cloudy surface water from a marshy lake. Placing the sample before his lens, he described hundreds of what he thought were tiny, living animals (probably protists), which he called **animalcules**. His curiosity aroused, Leeuwenhoek soon located even smaller animalcules in rainwater, which, reported in his 18th letter in 1676, likely represent the first description of bacterial cells.

In 1683, he sent his 39th letter to the Royal Society in which he described and illustrated for the first time what almost certainly were swimming bacterial cells taken from dental plaque (FIGURE 1.2E). Leeuwenhoek wrote:

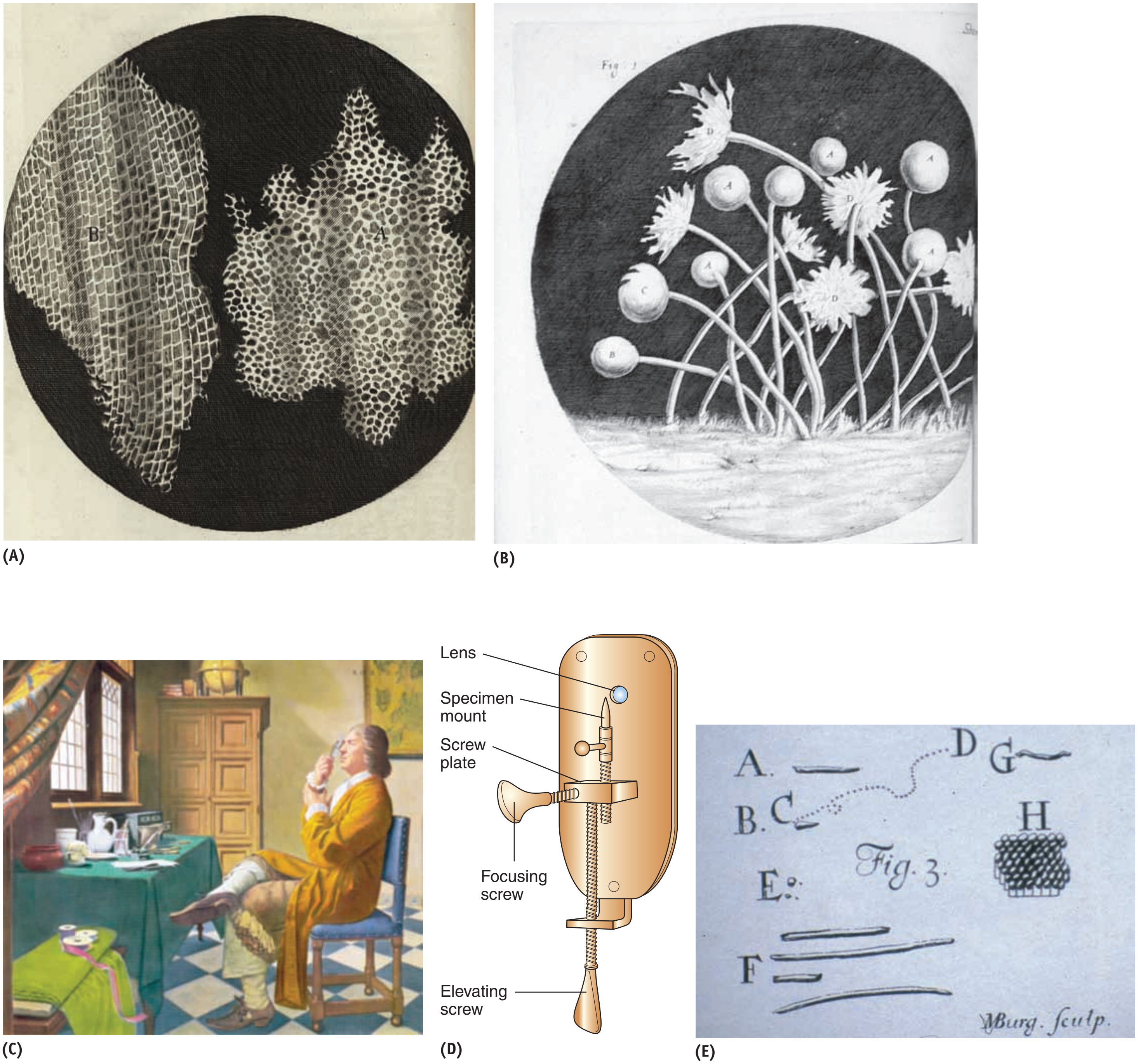
> *"I then most always saw, with great wonder, that in the said matter there were many very little living animalcules, very prettily a-moving. The biggest sort . . . had a very strong and swift motion, and shot through the water (or spittle) like a pike does through the water. The second sort . . . oft-times spun round like a top . . . and these were far more in number."*

Leeuwenhoek's sketches were elegant in detail and clarity. Among the 165 letters sent to the Royal Society, he outlined structural details of yeast cells, and described thread-like fungi and microscopic algae.

Unfortunately, Leeuwenhoek invited no one to work with him, nor did he show anyone how he ground his lenses. Without these lenses, naturalists were hard pressed to repeat his observations or verify his results, which are key components of scientific inquiry. In fact, it took Hooke more than a year to develop a microscope that could resolve Leeuwenhoek's animalcules. Still, Leeuwenhoek's observations on the presence and diversity of his "marvelous beasties" and Hooke's *Micrographia* opened the door to a completely new world: the world of the microbe.

CONCEPT AND REASONING CHECKS

1.1 If you were alive in Leeuwenhoek's time, how would you explain the origin for the animalcules he saw with his simple microscope?



(A) (B) (C) (D) (E)

FIGURE 1.2 **The First Observations and Drawings of Microorganisms.** In his *Micrographia*, Robert Hooke included a drawing of thin shavings of cork that he saw with his microscope (**A**). He also described and drew the structure of a fungal mold (**B**). (**C**) Leeuwenhoek looking through one of his simple microscopes. (**D**) For viewing, he placed an object on the tip of the specimen mount, which was attached to a screw plate. An elevating screw moved the specimen up and down while the focusing screw pushed against the metal plate, moving the specimen toward or away from the lens. (**E**) Leeuwenhoek's drawings of animalcules (bacterial cells) were included in a letter sent to the Royal Society in 1683. He found many of these organisms between his teeth and those of others.

Experimentation—Can Life Generate Itself Spontaneously?

KEY CONCEPT

2. The emergence of experimental science provided a means to test long-held beliefs and resolve controversies.

In the early 1600s, most naturalists were "vitalists," individuals who thought life depended on a mysterious "vital force" that pervaded all organisms. This force provided the basis for the doctrine of **spontaneous generation**, which suggested that organisms could arise from nonliving matter; that is, where there was putrefaction and decay. Common people embraced the idea, for they too witnessed what appeared to be slime that produced toads and decomposing wheat grains that generated wormlike maggots.

Regarding the latter, Leeuwenhoek suggested that maggots did not arise from wheat grains, but rather from tiny eggs laid in the grain that he could see with his microscope. Resolving such divergent observations concerning spontaneous generation required a new form of investigation—"experimentation"—and a new generation of experimental naturalists arose.

Noting Leeuwenhoek's descriptions, the Italian naturalist Francesco Redi performed one of history's first controlled biological experiments to see if maggots could arise from rotting meat. In 1668, he covered some jars of rotting meat with gauze, thereby preventing the entry of flies, while leaving other jars uncovered. If flies were prevented from entering and landing on pieces of exposed meat, Redi predicted they could not lay their invisible eggs and no maggots would hatch (FIGURE 1.3). Indeed, that is exactly what Redi observed and the idea that spontaneous generation could produce larger living creatures soon subsided. However, what about the mysterious and minute animalcules that appeared to straddle the boundary between the nonliving and living world? Could they arise spontaneously?

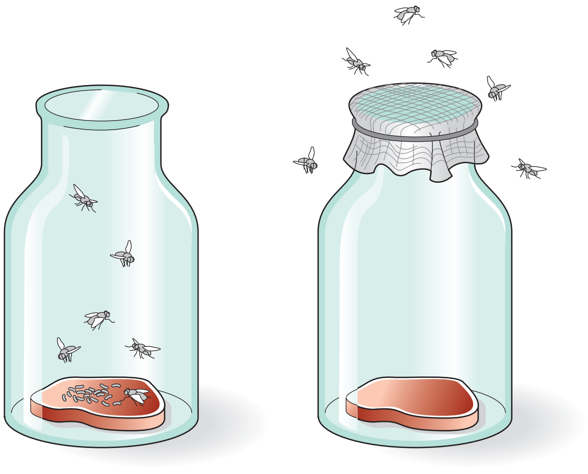


FIGURE 1.3 Redi's Experiments Refute Spontaneous Generation. Francesco Redi carried out one of the first biological experiments by placing a piece of meat in an open jar and another in a jar covered with gauze. Maggots arose only in the open jar because flies had access to the meat where they laid their eggs.

In 1748, a British clergyman and naturalist, John Needham, suggested that the spontaneous generation of animalcules resulted from a vital force that reorganized the decaying matter from more complex organisms. To prove this, Needham boiled several flasks of mutton broth and sealed the flasks with corks. After several days, Needham proclaimed that the *"gravy swarm'd with life, with microscopical animals of most dimensions."* He was convinced that putrefaction could generate the vital force needed for spontaneous generation.

Because experiments almost always are subject to varying interpretations, the Italian cleric and naturalist Lazzaro Spallanzani challenged Needham's conclusions and suggested that the duration of heating might not have been long enough. In 1765, he repeated Needham's experiments by boiling similar flasks for longer periods. As controls, he left some tubes open to the air, stoppered others loosely with corks, and sealed others. After two days, the open tubes were swarming with animalcules, but the stoppered ones had many fewer—and the sealed ones contained none. Spallanzani proclaimed, *"the number of animalcula developed is proportional to the communication with the external air."*

Needham and others countered that Spallanzani's experiments had destroyed the vital force of life because excessive heating drove off the air necessary for life. The controversy over spontaneous generation of animalcules continued into the mid-1800s. To solve the problem, a new experimental strategy would be needed.

To get at a resolution, in 1860 the French Academy of Sciences offered a prize for the best experiment to prove or disprove spontaneous generation. Louis Pasteur took up the challenge and, through an elegant series of experiments that were a variation of the methods of Needham and Spallanzani, discredited the idea in 1861. MicroInquiry 1 outlines the process of scientific inquiry and Pasteur's winning experiments.

Although Pasteur's experiments generated considerable debate for several years, his exacting and carefully designed experiments marked the end of the long and tenacious clashes over spontaneous generation that had begun two centuries earlier.

However, today there is another form of "spontaneous generation"—this time occurring in the laboratory (MicroFocus 1.2).

CONCEPT AND REASONING CHECKS

1.2 Evaluate the role of experimentation as an important skill to the eventual rejection of spontaneous generation.

1.2 Microorganisms and Disease Transmission

In the 13th century, people knew diseases could be transmitted between individuals, so quarantines were used to combat disease spread. In 1546, the Italian poet and naturalist Girolamo Fracostoro suggested that transmission could occur by direct human contact, from lifeless objects like clothing and eating utensils, or through the air.

Quarantine: An enforced isolation of people or animals with a highly communicable disease.

By the mid-1700s, the prevalent belief among naturalists and common people was that disease resulted from an altered chemical quality of the atmosphere or from tiny poisonous particles of decomposed matter in the air, an entity called **miasma** (the word malaria comes from *mala aria,* meaning "bad air"). To protect oneself from the black plague in Europe, for example, plague doctors often wore an elaborate costume they thought would protect them from the plague miasma (FIGURE 1.4).

However, as the 19th century unfolded, more scientists relied on keen observations and experimentation as a way of knowing and explaining divergent observations, for contagion and disease.

Epidemiology—Understanding Disease Transmission

KEY CONCEPT

3. Early epidemiology studies suggested how diseases could be spread and be controlled.

Epidemiology, as applied to infectious diseases, is the scientific study from which the source, cause, and mode of transmission of disease can be identified. The first scientific epidemiological studies, carried out by Ignaz Semmelweis and John Snow, were instrumental in suggesting how diseases were transmitted—and how simple measures could interrupt transmission.

Ignaz Semmelweis was a Hungarian obstetrician who was shocked by the numbers of pregnant women in his hospital who were dying of puerperal fever (a type of blood poisoning also called childbed fever) during labor. He determined the disease was more prevalent and deadly in the ward handled by medical students (29% deaths) than in the ward run by midwifery students (3% deaths). This comparative study suggested to Semmelweis that disease transmission must involve his medical students and that the source of contagion must be from cadavers on which the medical students previously had been performing autopsies. Midwifery students did not work on cadavers. So, in 1847, Semmelweis directed his staff to wash their hands in chlorine water before entering the maternity ward (FIGURE 1.5A). Deaths from childbed fever

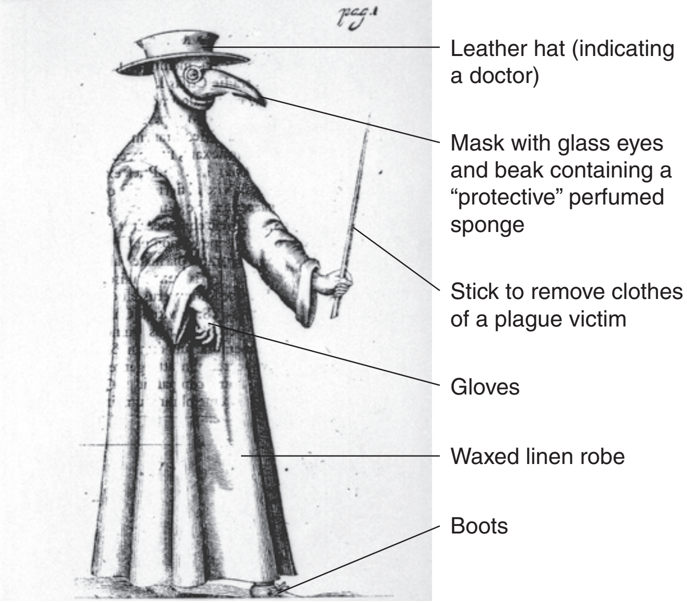


FIGURE 1.4 **Dressed for Protection.** This dress was thought to protect a plague doctor from the air (miasma) that caused the plague.

MICROINQUIRY 1

Experimentation and Scientific Inquiry

Science certainly is a body of knowledge as you can see from the thickness of this textbook! However, science also is a process—a way of learning. Often we accept and integrate into our understanding new information because it appears consistent with what we believe is true. But, are we confident our beliefs are always in line with what is actually true? To test or challenge current beliefs, scientists must present logical arguments supported by well-designed and carefully executed **experiments**.

The Components of Scientific Inquiry

There are many ways of finding out the answer to a problem. In science, **scientific inquiry**—or what has been called the "scientific method"—is the way problems are investigated. Let's understand how scientific inquiry works by following the logic of the experiments Louis Pasteur published in 1861 to refute the idea of spontaneous generation.

When studying a problem, the inquiry process usually begins with **observations**. For spontaneous generation, Pasteur's earlier observations suggested that organisms do not appear from nonliving matter (see text discussion of the early observations supporting spontaneous generation).

Next comes the **question**, which can be asked in many ways but usually as a "what," "why," or "how" question. For example, "What accounts for the generation of microorganisms in the beef broth?"

From the question, various hypotheses are proposed that might answer the question. A **hypothesis** is a provisional but testable explanation for an observed phenomenon. In almost any scientific question, several hypotheses can be proposed to account for the same observation. However, previous work or observations usually bias which hypothesis looks most promising, and scientists then put their "pet hypothesis" to the test first.

Pasteur's previous work suggested that the purported examples of life arising spontaneously in meat or vegetable broths were simply cases of airborne microorganisms in dust landing on a suitable substance and then multiplying in such profusion that they could be seen as a cloudy liquid.

Pasteur's Experiments

Pasteur set up a series of experiments to test the hypothesis that "Life only arises from other life" (see facing page).

Experiment 1A and **1B**: Pasteur sterilized a meat broth in glass flasks by heating. He then either left the neck open to the air (A) or sealed the glass neck (B). Organisms only appeared (turned the broth cloudy) in the open flask.

Experiment 2A and **2B**: Pasteur sterilized a meat broth in swan-necked flasks (A), so named because their S-shaped necks resembled a swan's neck. No organisms appeared, even after many days. However, if the neck was snapped off or the broth tipped to come in contact with the neck (B), organisms (cloudy broth) soon appeared.

Analysis of Pasteur's Experiments

Let's analyze the experiments. Pasteur had a preconceived notion of the truth and designed experiments to test his hypothesis. In his experiments, only one **variable** (an adjustable condition) changed. In experiment 1, the flask was open or sealed; in experiment 2, the neck was left intact or exposed to the unsterile air. Pasteur kept all other factors the same; that is, the broth was the same in each experiment; it was heated the same length of time; and similar flasks were used. Thus, the experiments had rigorous **controls** (the comparative condition). For example, in experiment 1, the control was the flask left open. Such controls are pivotal when explaining an experimental result. Pasteur's finding that no life appeared in the sealed flask (experiment 2A) is interesting, but tells us very little by itself. We only learn something by comparing this to the broken neck (or tipped flask) where life quickly appeared.

Also note that the idea of spontaneous generation could not be dismissed by just one experiment (see "His critics" on facing page). Pasteur's experiments required the accumulation of many experiments, all of which pointed to the same conclusion.

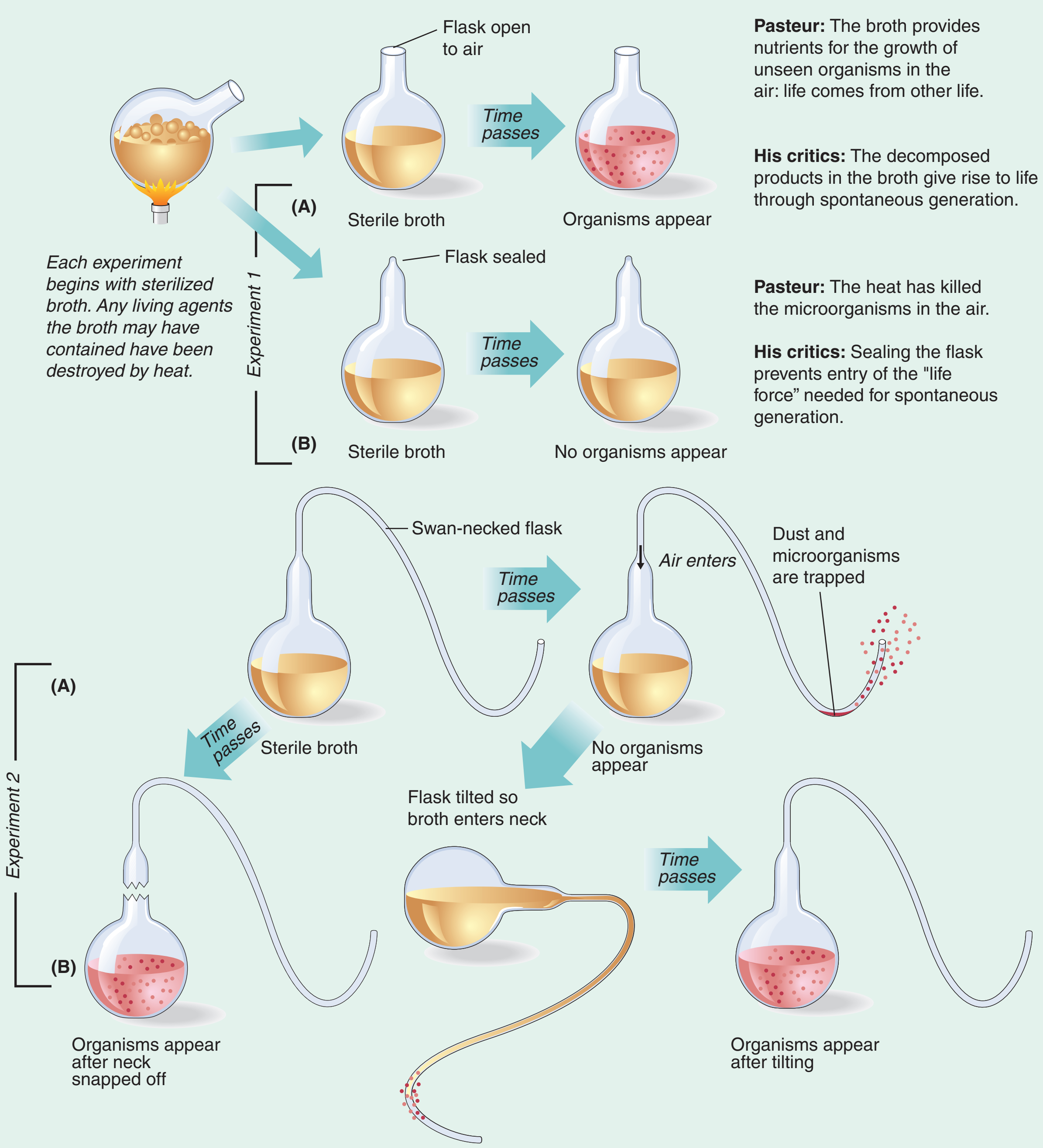
Hypothesis and Theory

When does a hypothesis become a theory? The answer is that there is no set time or amount of evidence that specifies the change from hypothesis to theory. A **theory** is defined as a hypothesis that has been tested and shown to be correct every time by many separate investigators. So, at some point, sufficient evidence exists to say a hypothesis is now a theory. However, theories are not written in stone. They are open to further experimentation and so can be refuted.

As a side note, today a theory often is used incorrectly in everyday speech and in the news media. In these cases, a theory is equated incorrectly with a hunch or belief—whether or not there is evidence to support it. In science, a theory is a general set of principles supported by large amounts of experimental evidence.

Discussion Point

Based on Pasteur's experiments, could one still argue that spontaneous generation could occur? Explain. Also see end of chapter question 37.



Pasteur: The broth provides nutrients for the growth of unseen organisms in the air: life comes from other life.

His critics: The decomposed products in the broth give rise to life through spontaneous generation.

Pasteur: The heat has killed the microorganisms in the air.

His critics: Sealing the flask prevents entry of the "life force" needed for spontaneous generation.

Pasteur and the Spontaneous Generation Controversy. **(1A)** When a flask of sterilized broth is left open to the air, organisms appear. **(1B)** When a flask of broth is boiled and sealed, no organisms appear. **(2A)** Broth sterilized in a swan-necked flask is left open to the air. The curvature of the neck traps dust particles and microorganisms, preventing them from reaching the broth. **(2B)** If the neck is snapped off to allow in air or the flask is tipped so broth enters the neck, organisms come in contact with the broth and grow.

Pasteur: No life will appear in the flask because microorganisms will not be able to reach the broth.

His critics: If the "life force" has free access to the flask, life will appear, given enough time.

Many days later the intact flask is still free of any life. Pasteur has refuted the doctrine of spontaneous generation.

MICROFOCUS 1.2: Biotechnology

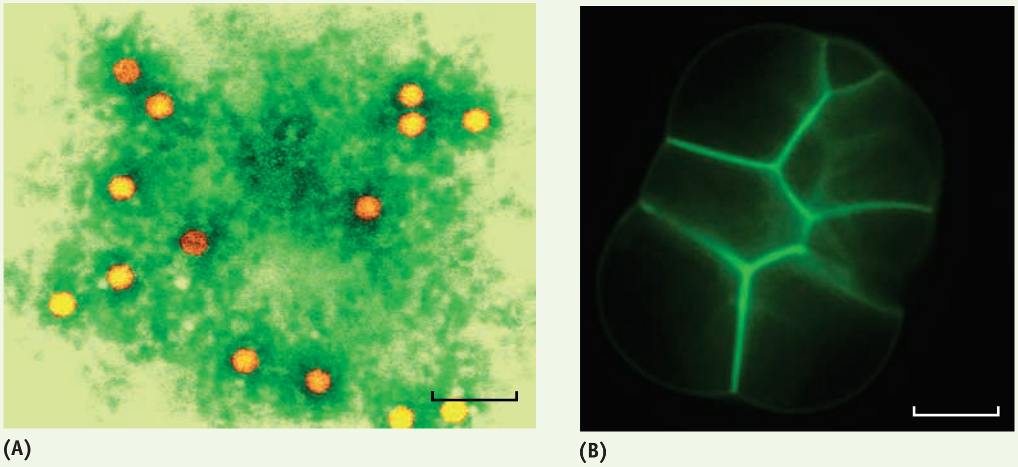
Generating Life—Today (Part I)

Those who believed in spontaneous generation proposed that animalcules arose from the rearrangement of molecules released from decayed organisms. Today, a different kind of rearrangement of molecules is occurring. The field, called **synthetic biology**, aims to rebuild or create new "life forms" (such as viruses or bacterial cells) from scratch by recombining molecules taken from different species. It is like fashioning a new car by taking various parts from a Ford and Chevy, and assembling them on a Toyota chassis.

In 2002, scientists at the State University of New York, Stony Brook, reconstructed a poliovirus by assembling separate poliovirus genes and proteins (see figure A). A year later, Craig Venter and his group assembled a bacteriophage—a virus that infects bacterial cells—from "off-the-shelf" biomolecules. Although many might not consider viruses to be "living" microbes, these constructions showed the feasibility of the idea. Then in 2004, researchers at Rockefeller University created small "vesicle bioreactors" that resembled crude biological cells (see figure B). The vesicle walls were made of egg white and the cell contents, stripped of any genetic material, were derived from a bacterial cell. The researchers then added genetic material and viral enzymes, which resulted in the cell making proteins, just as in a live cell.

Importantly, these steps toward synthetic life have more uses than simply trying to build something like a bacterial cell from scratch. Design and construction of novel organisms or viruses would have functions very different from naturally occurring organisms. As such, they represent the opportunity to expand evolution's repertoire by designing cells or organisms that are better at doing certain jobs. Can we, for example, design bacterial cells that are better at degrading toxic wastes, providing alternative energy sources, or making cheaper pharmaceuticals? These and many other positive benefits are envisioned as outcomes of synthetic biology.

Part II of Generating Life appears in Chapter 2 (page 56).


(A) (B)

(**A**) This image shows naturally occurring polioviruses, similar to those assembled from the individual parts. (Bar = 100 nm.) (**B**) A "vesicle bioreactor" that simulates a crude cell was assembled from various parts of several organisms. The green glow is from a protein produced by the genetic material added to the vesicle. (Bar = 10 µm.)

dropped, showing that disease spread could be interrupted. Unfortunately, few physicians initially heeded Semmelweis' recommendations.

In 1854, a cholera epidemic hit London's Soho district. With residents dying, English surgeon John Snow set out to discover the reason for cholera's spread. He carried out one of the first thorough epidemiological studies by interviewing sick and healthy Londoners and plotting the location of each cholera case on a district map (FIGURE 1.5B). The results indicated most cholera cases were linked to a sewage-contaminated street pump from which many local residents obtained their drinking water.

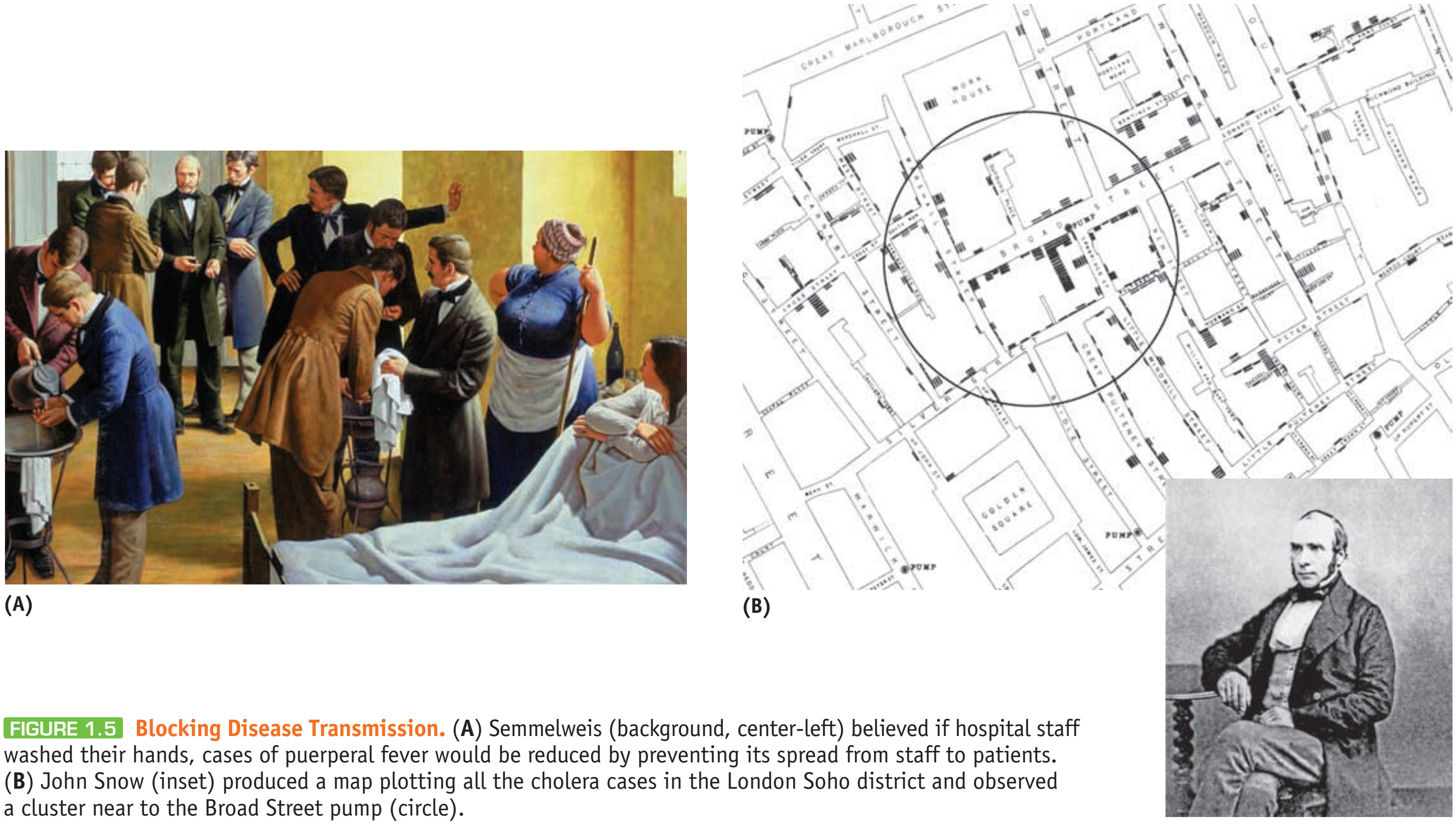


FIGURE 1.5 **Blocking Disease Transmission.** (**A**) Semmelweis (background, center-left) believed if hospital staff washed their hands, cases of puerperal fever would be reduced by preventing its spread from staff to patients. (**B**) John Snow (inset) produced a map plotting all the cholera cases in the London Soho district and observed a cluster near to the Broad Street pump (circle).

Snow then instituted the first known example of a public health measure to interrupt disease transmission—he requested the parish Board of Guardians to remove the street pump handle! Again, disease spread was broken by a simple procedure.

Snow went on to propose that cholera was not inhaled as a miasma but rather was waterborne. In fact, he asserted that "organized particles" caused cholera—an educated guess that proved to be correct even though the causative agent would not be identified for another 29 years.

It is important to realize that although the miasma premise was incorrect, the fact that disease was associated with bad air and filth led to new hygiene measures, such as cleaning streets, laying new sewer lines in cities, and improving working conditions. These changes helped usher in the Sanitary Movement and create the infrastructure for the public health systems we have today (MicroFocus 1.3).

CONCEPT AND REASONING CHECKS

1.3 Contrast the importance of the observations and studies by Semmelweis and Snow toward providing a better understanding of disease transmission.

Variolation and Vaccination—Prevention of Infectious Disease

KEY CONCEPT

4. Resistance to a disease can come from exposure to and recovery from a mild form of (or a very similar) disease.

Besides the controversies over the mechanism of disease transmission, ways to prevent disease from occurring were being considered. In the 1700s, smallpox was prevalent throughout Europe. In England, for example, smallpox epidemics were so severe that one third of the children died before reaching the age of three. Many victims who recovered were blinded from corneal infections and most were left pockmarked.

Significantly, survivors were protected from suffering the disease a second time. These observations suggested that if one contracted a weakened or mild form of the disease, perhaps such individuals would have lifelong resistance.

In the 14th century, the Chinese knew that smallpox survivors would not get reinfected. Spreading from China to India and Africa, the practice of **variolation** developed, which involved blowing a ground smallpox powder into the

MICROFOCUS 1.3: Public Health

Epidemiology Today

Today, we have a good grasp of disease transmission mechanisms, as we will discuss in Chapter 13. However, even with the advances in sanitation and public health, cholera remains a public health threat in parts of the developing world. In addition, almost 160 years after Semmelweis' suggestions, a lack of hand washing by hospital staff, even in developed nations, remains a major mechanism for disease transmission (see figure). The simple process of washing one's hands still could reduce substantially disease transmission among the public and in hospitals.

Two of the most important epidemiological organizations today are the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, and, on a global level, the World Health Organization (WHO) in Geneva, Switzerland. Both employ numerous epidemiologists, popularly called "disease detectives," who, like Snow (but with more expertise), systematically gather information about disease outbreaks in an effort to discover how the disease agent is introduced, how it is spread in a community or population, and how the spread can be stopped. For example, in 2008 more than 1,400 people in 43 states developed similar gastrointestinal symptoms, which CDC investigators traced to bacterial contamination in imported jalapeño peppers. Warnings to not purchase or eat these peppers halted the outbreak and prevented further transmission. And to think, it all started with the seminal work of Semmelweis and Snow.

individual's nose. By the 18th century, Europeans were inoculating dried smallpox scabs under the skin of the arm. Although some individuals did get smallpox, most contracted a mild form of the disease and, upon recovery, were resistant to future smallpox infections.

As an English country surgeon, Edward Jenner learned that milkmaids who occasionally contracted cowpox, a disease of the udders of cows, would subsequently be protected from deadly smallpox. Jenner wondered if intentionally giving cowpox to people would protect them against smallpox and be an effective alternative to variolation. In 1796, he put the matter to the test.

A dairy maid named Sarah Nelmes came to his office, the lesions of cowpox evident on her hand. Jenner took material from the lesions and scratched it into the skin of a boy named James Phipps (FIGURE 1.6). The boy soon developed a slight fever, but recovered. Six weeks later Jenner inoculated young Phipps with material from a smallpox lesion. Within days, the boy developed a reaction at the site but failed to show any sign of smallpox.

Jenner repeated his experiments with other children, including his own son. His therapeutic technique of **vaccination** (*vacca* = "cow") worked in all cases and eliminated the risks associated with variolation. In 1798, he published a pamphlet on his work that generated considerable interest. Prominent physicians confirmed his findings, and within a few years, Jenner's method of vaccination

FIGURE 1.6 The First Vaccination Against Smallpox. Edward Jenner performed the first vaccination against smallpox. On May 14, 1796, material from a cowpox lesion was scratched into the arm of eight-year-old James Phipps. The vaccination protected him from smallpox.

spread through Europe and abroad. By 1801, some 100,000 people in England had been vaccinated. President Thomas Jefferson wrote to Jenner, *"You have erased from the calendar of human afflictions one of its greatest. Yours is the comfortable reflection that mankind can never forget that you have lived."*

A hundred years would pass before scientists discovered the milder cowpox virus was triggering a defensive mechanism by the body's immune system against the deadlier smallpox virus. It is remarkable that without any knowledge of viruses or disease causation, Jenner accomplished what he did. Again, hallmarks of a scientist—keen observational skills and insight—led to a therapeutic intervention against disease.

CONCEPT AND REASONING CHECKS

1.4 Evaluate the effectiveness of variolation and vaccination as ways to produce disease resistance.

The Stage Is Set

During the early years of the 1800s, several events occurred that helped set the stage for the coming "germ revolution." In the 1830s, advances were made in microscope optics that allowed better resolution of objects. This resulted in improved and more widespread observations of tiny living organisms, many of which resembled short sticks. In fact, in 1838 the German biologist Christian Ehrenberg suggested these "rod-like" looking organisms be called **bacteria** (*bakterion* = "little rod").

The Swiss physician Jacob Henle reported in 1840 that living organisms could cause disease. This was strengthened in 1854 by Filippo Pacini's discovery of rod-shaped cholera bacteria in stool samples from cholera patients. Still, scientists debated whether bacterial organisms could cause disease because such living organisms sometimes were found in healthy people. Therefore, how could these bacterial cells possibly cause disease?

To understand clearly the nature of infectious disease, a new conception of disease had to emerge. In doing so, it would be necessary to demonstrate that a specific bacterial species was associated with a specific infectious disease. This would require some very insightful work, guided by Louis Pasteur in France and Robert Koch in Germany.

1.3 The Classical Golden Age of Microbiology (1854–1914)

Beginning around 1854, microbiology blossomed and continued until the advent of World War I. During these 60 years, many branches of microbiology were established, and the foundations were laid for the maturing process that has led to modern microbiology. We refer to this period as the first, or classical, Golden Age of microbiology.

Louis Pasteur Proposes That Germs Cause Infectious Disease

KEY CONCEPT

5. The germ theory was based on the observations that different microorganisms have distinctive and specific roles in nature.

Born in 1822 in Dôle, France, Louis Pasteur studied chemistry at the École Normale Supérieure in Paris and, in 1854, was appointed Professor of Chemistry at the University of Lille in northern France (FIGURE 1.7A). Pasteur was among the first scientists who believed that problems in science could be solved in the laboratory with the results having practical applications.

Always one to tackle big problems, Pasteur soon set out to understand the process of fermentation and the other processes that can accompany it. The prevailing theory held that fermentation was strictly a chemical reaction. However, Pasteur's microscope observations consistently revealed large numbers of tiny yeast cells in the juice that were overlooked by other scientists. When he mixed yeast in a sugar-water solution, the yeast grew and the quantity of yeast increased. Yeast must be living organisms and one of the living "ferments" responsible for the fermentation process.

Fermentation: A splitting of sugar molecules into simpler products, including alcohol, acid, and gas (CO_2).

Pasteur also demonstrated that wines, beers, and vinegar each contained different and specific types of microorganisms. For example, in studying a local problem of wine souring, he observed that only soured wines contained populations of bacterial cells. These cells must have contaminated a batch of yeast and produced the acids that caused the souring. In addition, Pasteur discovered the process occurred in the absence of oxygen gas (FIGURE 1.7B).

Pasteur recommended a practical solution for the "wine disease" problem: heat the wine

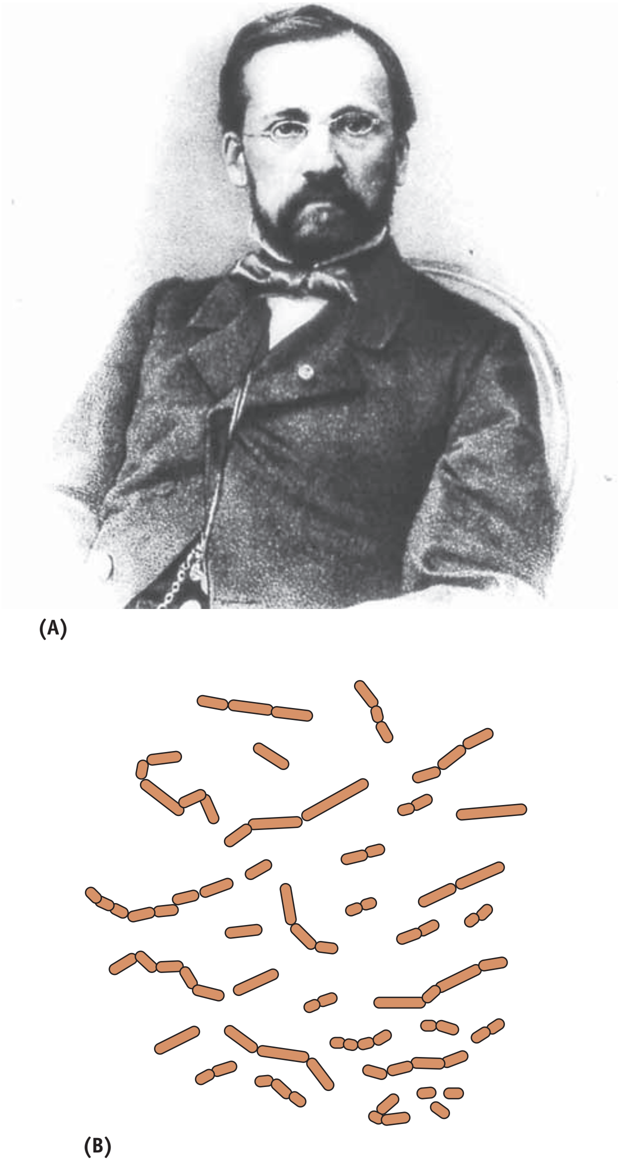


FIGURE 1.7 **Louis Pasteur and Fermentation Bacteria.** (**A**) Louis Pasteur as a 46-year-old professor of chemistry at the University of Paris. (**B**) The following is part of a description of the living bacterial cells he observed. *"A most beautiful object: vibrios all in motion, advancing or undulating. They have grown considerably in bulk and length since the 11th; many of them are joined together in long sinuous chains . . ."* Pasteur concluded these bacterial cells can live without air or free oxygen; in fact, *"the presence of gaseous oxygen operates prejudicially against the movements and activity of those vibrios."*

to 55°C after fermentation but before aging. His controlled heating technique, known as **pasteurization**, soon was applied to other products, especially milk.

Pasteur's experiments demonstrated that yeast and bacterial cells are tiny, living factories in which important chemical changes take place. Therefore, if microorganisms represented agents of change, perhaps human infections could be caused by those microorganisms that cause disease—**germs**.

In 1857, Pasteur published a short paper on wine souring by bacterial cells in which he implied that germs (bacterial cells) also could be related to human illness. Five years later, after he disproved spontaneous generation, he formulated the **germ theory** of disease, which holds that some microorganisms are responsible for infectious disease.

CONCEPT AND REASONING CHECKS

1.5 Describe how wine fermentation and souring led Pasteur to propose the germ theory.

Pasteur's Work Stimulates Disease Control and Reinforces Disease Causation

KEY CONCEPT

6. Antisepsis and identification of the cause of animal diseases reinforced the germ theory.

Pasteur had reasoned that if microorganisms were acquired from the environment, their spread could be controlled and the chain of disease transmission broken.

Joseph Lister was Professor of Surgery at Glasgow Royal Infirmary in Scotland, where more than half his amputation patients died—not from the surgery—but rather from postoperative infections. Hearing of Pasteur's germ theory, Lister argued that these surgical infections resulted from living organisms in the air. Knowing that carbolic acid had been effective on sewage control, in 1865 he used a carbolic acid spray in surgery and on surgical wounds (FIGURE 1.8). The result was spectacular—the wounds healed without infection. His technique would soon not only revolutionize medicine and the practice of surgery, but also lead to the practice of **antisepsis**, the use of chemical methods for disinfection of external living surfaces, such as the skin (Chapter 7). Once again, practical applications from the laboratory triumphed.

In an effort to familiarize himself with biological problems, Pasteur turned his attention to pébrine, a disease of silkworms. He identified a protist as the infectious agent in silkworms and on the mulberry leaves fed to the worms. By separating the healthy silkworms from the diseased silkworms and their food, he managed to quell the spread of disease. The identification of the pathogen was crucial to supporting the germ theory and Pasteur would never again doubt the ability of microorganisms to cause infectious disease. Now infectious disease would be his only interest.

In 1865, cholera engulfed Paris, killing 200 people a day. Pasteur tried to capture the responsible pathogen by filtering the hospital air and trapping the bacterial cells in cotton. Unfortunately, Pasteur could not grow or separate one bacterial species apart from the others because his broth cultures allowed the organisms to mix freely. Although Pasteur demonstrated that bacterial inoculations made animals ill, he could not pinpoint an exact cause.

To completely validate the germ theory, what was missing was the ability to isolate a specific bacterial species from a diseased individual and demonstrate the isolated organism caused the same disease.

CONCEPT AND REASONING CHECKS

1.6 Assess Lister's antisepsis procedures and Pasteur's work on pébrine toward supporting the germ theory.

Robert Koch Formalizes Standards to Identify Germs with Infectious Disease

KEY CONCEPT

7. Koch's postulates provided a way to identify a specific microorganism as causing a specific infectious disease.

Robert Koch (FIGURE 1.9A) was a German country doctor who was well aware of anthrax, a deadly disease that periodically ravaged cattle and sheep, and could cause disease in humans.

In 1875, Koch injected mice with the blood from such diseased sheep and cattle. He then performed meticulous autopsies and noted the same symptoms in the mice that had appeared in the sheep and cattle. Next, he isolated from the blood a few rod-shaped bacterial cells and grew them in the sterile aqueous humor of an ox's eye. With his microscope, Koch watched for hours as the bacterial cells multiplied, formed tangled threads, and finally reverted to spores. He then took several spores on a sliver of wood and injected them into healthy mice. The symptoms of anthrax appeared within hours. Koch autopsied the animals and found their blood swarming with the same bacterial cells. He reisolated the cells in fresh aqueous humor. The cycle was now complete. These bacterial cells definitely were the causative agent of anthrax.

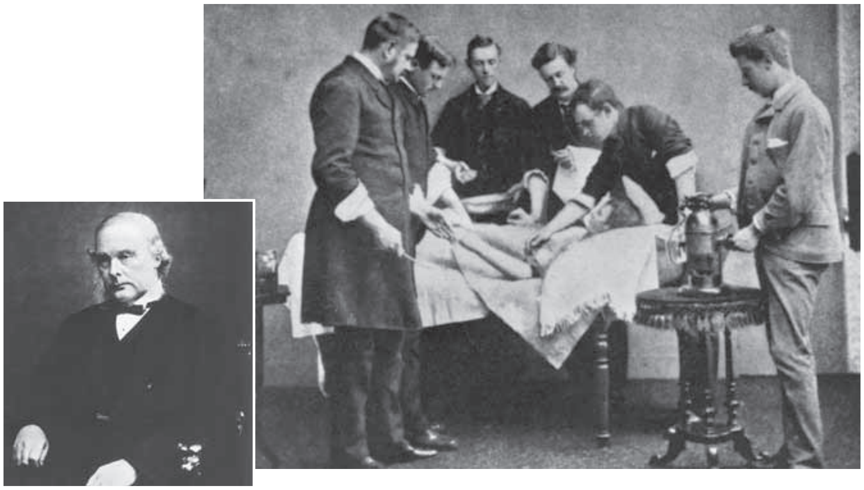


FIGURE 1.8 **Lister and Antisepsis.** By 1870, Joseph Lister (inset) and his students were using a carbolic acid spray in surgery and on surgical wounds to prevent postoperative infections.

■ **Broth:** A liquid containing nutrients for microbial growth.

When Koch presented his work, scientists were astonished. Here was the verification of the germ theory that had eluded Pasteur. Koch's procedures became known as **Koch's postulates** and were quickly adopted as the formalized standards for implicating a specific organism to a specific disease (FIGURE 1.9B).

Koch Develops Pure Culture Techniques

Growing bacterial cells in an ox's eye was not very convenient. Then, in 1880, Koch observed a slice of potato on which small masses of bacterial cells, which he termed **colonies**, were growing and multiplying. So, Koch tried adding gelatin to his broth to prepare a similar solid culture surface. He then inoculated bacterial cells on the surface and set the dish aside to incubate. Within 24 hours, visible colonies would be growing on the surface, each colony representing a **pure culture** containing only one bacterial type. By 1884, agar replaced gelatin as the preferred solidifying agent (MicroFocus 1.4).

■ **Agar:** A complex polysaccharide derived from marine algae.

CONCEPT AND REASONING CHECKS

1.7 Why was pure culture crucial to Koch's postulates and the germ theory?

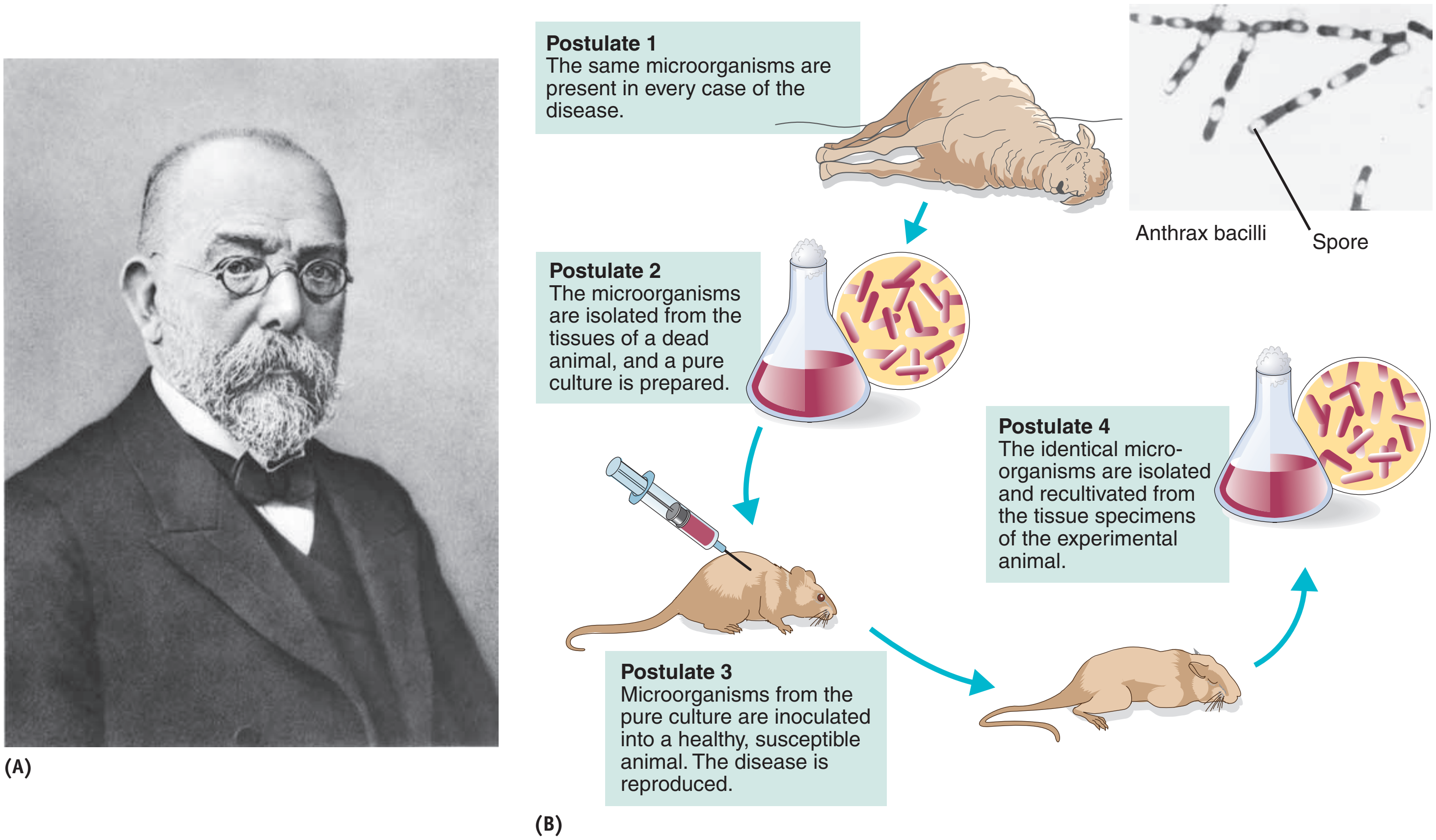


FIGURE 1.9 **A Demonstration of Koch's Postulates.** Robert Koch (**A**) developed what became known as Koch's postulates (**B**) that were used to relate a single microorganism to a single disease. The insert (in the upper right) is a photo of the rod-shaped anthrax bacterial cells. Many rods are swollen with spores (white ovals).

Competition Fuels the Study of Infectious Disease

KEY CONCEPT

8. Laboratory science and teamwork stimulated the discovery of additional infectious disease agents.

The period of the 1860s took a toll on Pasteur. His father and three of his five children died, and a stroke in 1868 left him partially paralyzed in his left arm and leg. However, he soon wrote that the work on silkworms was *"a good preparation for the investigations that we are about to undertake."*

■ Attenuate:
To reduce or weaken.

Research studies conducted in a laboratory were becoming the normal method of work. Pasteur's lab and coworkers now were primarily interested in the mechanism of infection and immunity, and the practical applications that could be derived, while Koch's lab focused on procedural methods such as isolation, cultivation, and identification of specific pathogens. A competition arose that would last into the next century.

The Pasteur Lab. Pasteur continued work with anthrax and found that the cells could be trapped on a filter and just a small drop of these cells was sufficient to kill many animals. These and other experiments further validated the germ theory.

One of Pasteur's more remarkable discoveries was made in 1881. For months, he and his coworker Charles Chamberland had been working on ways to attenuate the bacterial cells of chicken cholera using heat, different growth conditions, successive inoculations in animals, and virtually anything that might damage the cells. Finally, they developed a weak strain by suspending the bacterial cells in a mildly acidic medium and allowing the culture to remain undisturbed for a long period. When the bacterial cells were inoculated into chickens and later followed by a dose of lethal pathogen, the animals did not develop cholera. This attenuation principle is the basis for many vaccines today. Pasteur also applied the principle to anthrax in 1881 and, in a public demonstration, found he

MICROFOCUS 1.4: History

Jams, Jellies, and Microorganisms

Fanny Hesse.

One of the major developments in microbiology was Robert Koch's use of a solid culture surface on which bacterial colonies would grow. He accomplished this by solidifying beef broth with gelatin. When inoculated onto the surface of the nutritious medium, bacterial cells grew vigorously at room temperature and produced discrete, visible colonies.

On occasion, however, Koch was dismayed to find that the gelatin turned to liquid. It appeared that certain bacterial species were producing a chemical substance to digest the gelatin. Moreover, gelatin liquefied at the warm incubator temperatures commonly used to cultivate certain bacterial species.

Walther Hesse, an associate of Koch's, mentioned the problem to his wife and laboratory assistant, Fanny Eilshemius Hesse. She had a possible solution. For years, she had been using a seaweed-derived powder called agar (pronounced ah'gar) to solidify her jams and jellies. Agar was valuable because it mixed easily with most liquids and once gelled, it did not liquefy, even at the warm incubator temperatures.

In 1880, Hesse was sufficiently impressed to recommend agar to Koch. Soon Koch was using it routinely to grow bacterial species, and in 1884 he first mentioned agar in his paper on the isolation of the bacterial organism responsible for tuberculosis. It is noteworthy that Fanny Hesse may have been among the first Americans (she was originally from New Jersey) to make a significant contribution to microbiology.

Another point of interest: The common petri dish (plate) also was invented about this time (1887) by Julius Petri, another of Koch's assistants.

could protect sheep against this disease as well (FIGURE 1.10).

Pasteur reached the zenith of his career in 1885 when he successfully immunized a young boy against the dreaded disease rabies. Although he never could culture the causative agent of rabies on agar, Pasteur could cultivate it in spinal cord tissue of experimental animals. After his coworker Émile Roux tested the vaccine with success in dogs—all immunized animals survived a rabies exposure—the ultimate test arrived. A 9-year-old boy, Joseph Meister, had been bitten and mauled by a rabid dog. Pasteur gave the boy the untested (in humans) rabies vaccine (MicroFocus 1.5). The treatment lasted 10 days and the boy recovered and remained healthy. The rabies vaccine was a triumph because it fulfilled his dream of applying the principles of science to practical problems. Such successes helped establish the Pasteur Institute in Paris, one of the world's foremost scientific establishments. Pasteur presided over the Institute until his death in 1895.

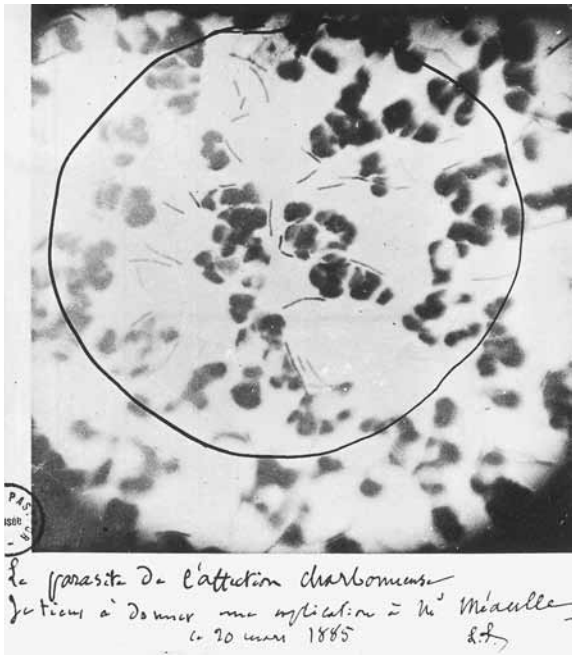


FIGURE 1.10 **The Anthrax Bacterial Cells.** A photomicrograph of the anthrax bacterial cells taken by Louis Pasteur in 1885. Pasteur circled the bacilli (the tiny rods) in tissue and annotated the photograph, "the parasite of Charbonneuse." ("Charbonneuse" is the French equivalent of anthrax.)

MICROFOCUS 1.5: History

The Private Pasteur

The notebooks of Louis Pasteur had been an enduring mystery of science ever since the scientist himself requested his family not to show them to anyone. But in 1964, Pasteur's last surviving grandson donated the notebooks to the National Library in Paris, and after soul-searching for a decade, the directors made them available to a select group of scholars. Among the group was Gerald Geison of Princeton University. What Geison found stripped away part of the veneration conferred on Pasteur and showed another side to his work.

In 1881, Pasteur conducted a trial of his new anthrax vaccine by inoculating half a flock of animals with the vaccine, then exposing the entire flock to the disease. When the vaccinated half survived, Pasteur was showered with accolades. However, Pasteur's notebooks, according to Geison, reveal that he had prepared the vaccine not by his own method, but by a competitor's.

Pasteur also apparently sidestepped established protocols when he inoculated two boys with a rabies vaccine before it was tested on animals. Fortunately, the two boys survived, possibly because they were not actually infected or because the vaccine was, indeed, safe and effective. Nevertheless, the untested treatment should not have been used, says Geison. His book, *The Private Science of Louis Pasteur* (Princeton University Press, 1995) places the scientist in a more realistic light and shows that today's pressures to succeed in research are little different than they were more than a century ago.

The Koch Lab. Koch also reached the height of his influence in the 1880s. He developed methods for staining bacterial cells and preparing permanent visual records. In 1882, he identified and grew the bacterium responsible for tuberculosis (TB) in pure culture. In 1883, he interrupted his work on TB to lead a group of German scientists studying cholera in Egypt and India. In both countries, Koch isolated a comma-shaped bacillus and confirmed John Snow's suspicion that water is the key to transmission. In 1891, as director of Berlin's Institute for Infectious Diseases, Koch returned to his work on TB. Unfortunately, his supposed vaccine was a total failure—several people actually died from the vaccine. Still, his TB studies were significant and ultimately gained him the 1905 Nobel Prize in Physiology or Medicine. He died of a stroke in 1910.

The germ theory set a new course for studying and treating infectious disease. The studies carried out by Pasteur and Koch made the discipline of **bacteriology**, the study of bacterial organisms, a well-respected field of study. In fact, a new generation of international scientists, including several from the Pasteur and Koch labs, stepped in to expand the work on infectious disease (TABLE 1.1).

CONCEPT AND REASONING CHECKS

1.8 Assess the importance of the science laboratory and teamwork to the increasing identification of pathogenic bacteria.

Other Global Pioneers Contribute to New Disciplines in Microbiology

KEY CONCEPTS

9. Viruses also can cause disease.
10. Many beneficial bacterial species recycle nutrients in the environment.

Although the list of identified microbes was growing, the agents responsible for diseases such as measles, mumps, smallpox, and yellow fever continued to elude identification. In 1892, a Russian scientist, Dimitri Ivanowsky, used a filter developed by Pasteur's group to trap what he thought were bacterial cells responsible for tobacco mosaic disease, which produces mottled and stunted tobacco leaves. Surprisingly, Ivanowsky discovered that when he applied the liquid that passed through the filter to healthy tobacco plants, the leaves became mottled and stunted. Ivanowsky assumed bacterial cells somehow had slipped through the filter.

Unaware of Ivanowsky's work, Martinus Beijerinck, a Dutch investigator, did similar experiments in 1899 and suggested tobacco mosaic disease was a "contagious, living liquid" that acted like a poison or virus (*virus* = "poison"). In 1898, the first virus responsible for an animal disease—hoof-and-mouth disease—was discovered, and in 1901 American Walter Reed concluded that the agent responsible for yellow fever in humans also

TABLE 1.1 Other International Scientists and Their Accomplishments During the Classical Golden Age of Microbiology

Investigator (Year)	Country	Accomplishment
Otto Obermeier (1868)	Germany	Observed bacterial cells in relapsing fever patients
Ferdinand Cohn (1872)	Germany	Established bacteriology as a science; produced the first bacterial taxonomy scheme
Gerhard Hansen (1873)	Norway	Observed bacterial cells in leprosy patients
Albert Neisser (1879)	Germany	Discovered the bacterium that causes gonorrhea
*Charles Laveran (1880)	France	Discovered that malaria is caused by a protozoan
Hans Christian Gram (1884)	Denmark	Introduced staining system to identify bacterial cells
Pasteur Lab		
Elie Metchnikoff (1884)	Ukraine	Described phagocytosis
Émile Roux and Alexandre Yersin (1888)	France	Identified the diphtheria toxin
Koch Lab		
Friedrich Löeffler (1883)	Germany	Isolated the diphtheria bacillus
Georg Gaffky (1884)	Germany	Cultivated the typhoid bacillus
*Paul Ehrlich (1885)	Germany	Suggested some dyes might control bacterial infections
Shibasaburo Kitasato (1889)	Japan	Isolated the tetanus bacillus
Emil von Behring (1890)	Germany	Developed the diphtheria antitoxin
Theodore Escherich (1885)	Germany	Described the bacterium responsible for infant diarrhea
Daniel E. Salmon (1886)	United States	Developed the first heat-killed vaccine
Richard Pfeiffer (1892)	Germany	Identified a bacterial cause of meningitis
William Welch and George Nuttall (1892)	United States	Isolated the gas gangrene bacillus
Theobald Smith and F. Kilbourne (1893)	United States	Proved that ticks transmit Texas cattle fever
S. Kitasato and A. Yersin (1894)	Japan France	Independently discovered the bacterium causing plague
Emile van Ermengem (1896)	Belgium	Identified the bacterium causing botulism
*Ronald Ross (1898)	Great Britain	Showed mosquitoes transmit malaria to birds
Kiyoshi Shiga (1898)	Japan	Isolated a cause of bacterial dysentery
Walter Reed (1901)	United States	Studied mosquito transmission of yellow fever
David Bruce (1903)	Great Britain	Proved that tsetse flies transmit sleeping sickness
Fritz Schaudinn and Erich Hoffman (1903)	Germany	Discovered the bacterium responsible for syphilis
*Jules Bordet and Octave Gengou (1906)	France	Cultivated the pertussis bacillus
Albert Calmette and Camille Guérin (1906)	France	Developed immunization process for tuberculosis
Howard Ricketts (1906)	United States	Proved that ticks transmit Rocky Mountain spotted fever
Charles Nicolle (1909)	France	Proved that lice transmit typhus fever
George McCoy and Charles Chapin (1911)	United States	Discovered the bacterial cause of tularemia

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*Nobel Prize winners in Physiology or Medicine.

was a virus. With these discoveries, the discipline of **virology**, the study of viruses, was launched.

While many scientists were advancing medical microbiology, others devoted their research to the environmental importance of microorganisms. The Russian scientist Sergei Winogradsky discovered bacterial cells that metabolized sulfur and developed the concept of **nitrogen fixation**, where bacterial cells convert inert nitrogen gas (N_2) into useable ammonia (NH_3). Beijerinck was the first to obtain pure cultures of microorganisms from soil and water by enriching the growth conditions. Together with Winogradsky, he developed many of the laboratory methods essential to the study of microbial ecology, while revealing the physiological significance of soil microbes and discovering the essential roles microorganisms play in the recycling of matter.

Today, along with Giovannoni and Venter, many microbiologists continue to search for and understand the roles of microorganisms. In fact, with less than 2% of all microorganisms on Earth having been identified and many fewer cultured, there is still a lot to be discovered in the microbial world!

CONCEPT AND REASONING CHECKS

1.9 Describe how viruses were discovered as disease-causing agents.

1.10 Judge the significance of the work pioneered by Winogradsky and Beijerinck.

1.4 Studying Microorganisms

Besides bacteriology and virology, other disciplines also were developing at the beginning of the 20th century. This included **mycology**, the study of fungi; **protozoology**, the study of the animal-like protists; and **phycology**, the study of algae (FIGURE 1.11).

The applications of microbiological knowledge also were important to the development of epidemiology, infection control, and **immunology**, which is the study of bodily defenses against microorganisms and other agents.

The Spectrum of Microorganisms and Viruses Is Diverse

KEY CONCEPT

11. The organisms and agents studied in microbiology represent diverse groups.

By the end of the classical Golden Age of microbiology, the diversity of microbes included more than just bacterial species. Let's briefly survey what we know about these groups today.

Bacteria. It is estimated that there may be more than 10 million bacterial species. Most are very small, single-celled (unicellular) organisms (although some form filaments, and many associate in a bacterial mass called a "biofilm"). The cells may be spherical, spiral, or rod-shaped (FIGURE 1.12A), and they lack the cell nucleus and most of the typical cellular compartments typical of other microbes and multicellular organisms. Some bacterial organisms, like the **cyanobacteria**, carry out photosynthesis (FIGURE 1.12B).

Besides the disease-causing members, some are responsible for food spoilage while others are useful in the food industry. Many bacterial

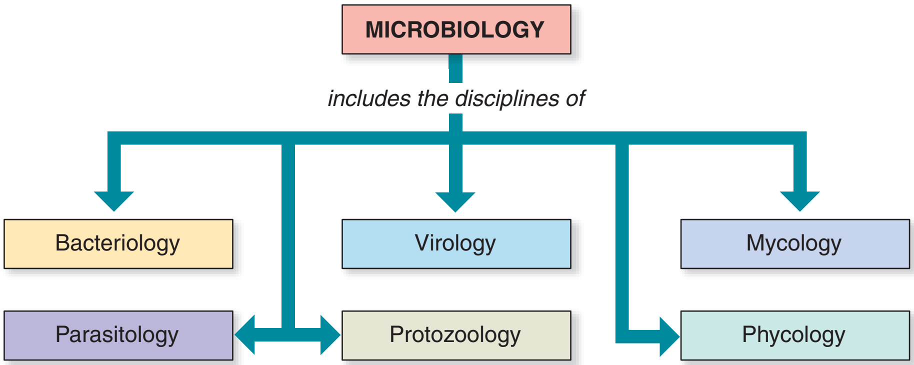


FIGURE 1.11 **Microbiology Disciplines by Organism or Agent Studied.** This simple concept map shows the relationship between microbiology and the organisms or agents that make up the various disciplines. Parasitology is the study of animal parasites. Some of these parasites cause disease in humans, which is why parasitology is included with the other disciplines of microbiology.

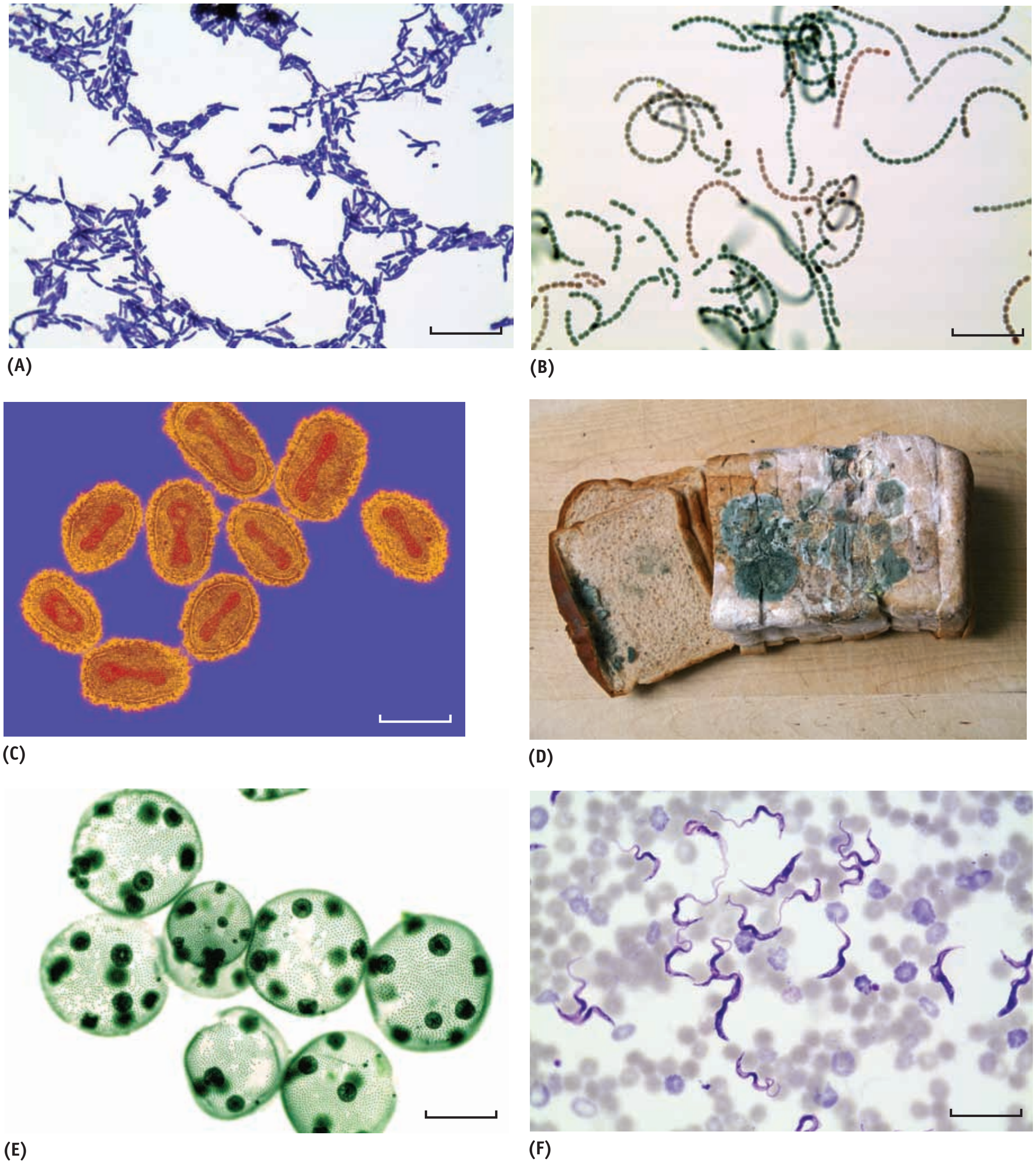


FIGURE 1.12 **Groups of Microorganisms.** (**A**) A bacterial smear showing the rod shaped cells of *Bacillus cereus* (stained purple), a normal inhabitant of the soil. (Bar = 10 µm.) (**B**) Filamentous strands of *Anabaena*, a cyanobacterium that carries out photosynthesis. (Bar = 100 µm.) (**C**) Smallpox viruses. (Bar = 100 nm.) (**D**) A typical blue-gray *Penicillium* mold growing on a loaf of bread. (**E**) The colonial green alga, *Volvox*. (Bar = 300 µm.) (**F**) The ribbon-like cells of the protist *Trypanosoma*, the causative agent of African sleeping sickness. (Bar = 10 µm.)

species, along with several fungi, are **decomposers**, organisms that recycle nutrients from dead organisms.

Archaea. Based on recent biochemical and molecular studies, many bacterial species have been reassigned into another evolutionary group, called the Archaea. Many archaeal species can be found in environments that are extremely hot (such as the Yellowstone hot springs), extremely salty (such as the Dead Sea), or extremely low pH (such as acid mine drainage). Adaptations to these environments are partly why they have been collected into their own unique group. Most bacterial and archaeal species have a rigid cell wall through which nutrients are absorbed from the environment.

Viruses. Although not correctly labeled as microorganisms, currently there are more than 3,600 known types of viruses. Viruses are not cellular and cannot be grown in pure culture. They have a core of nucleic acid (DNA or RNA) surrounded by a protein coat. Among the features used to identify viruses are morphology (size, shape), genetic material (RNA, DNA), and biological properties (organism or tissue infected).

Viruses infect organisms for one reason only—to replicate. Viruses in the air or water, for example, cannot replicate because they need the metabolic machinery inside a cell. Of the known viruses, only a small percentage causes disease in humans. Polio, the flu, measles, AIDS, and smallpox are examples (FIGURE 1.12C).

The other group of microbes has a cell nucleus and a variety of internal cellular compartments. Many of the organisms are familiar to us.

Fungi. The fungi include the unicellular yeasts and the multicellular mushrooms and molds (FIGURE 1.12D). About 100,000 species of fungi have been described; however, there may be as many as 1.5 million species in nature.

Most fungi grow best in warm, moist places and secrete digestive enzymes that break down nutrients into smaller bits that can be absorbed easily across a rigid cell wall. Fungi thus live in their own food supply. If that food supply is a human, disease may result.

Some fungi provide useful products including antibiotics, such as penicillin. Others are used in the food industry to impart distinctive flavors in foods such as Roquefort cheeses. Together with many bacterial species, numerous molds play a major role as decomposers.

Protists. The protists consist mostly of single-celled organisms. Some are free living while others live in association with plants or animals. Movement, if present, is achieved by flagella or cilia, or by a crawling movement.

Protists obtain nutrients in different ways. Some absorb nutrients from the surrounding environment or ingest algae and bacterial cells. The unicellular, colonial, or filamentous algae carry out photosynthesis (FIGURE 1.12E). The aquatic protists also provide energy and organic compounds for the lower trophic levels of the food web. Some protists are capable of causing diseases in animals, including humans; these include malaria, several types of diarrhea, and sleeping sickness (FIGURE 1.12F).

CONCEPT AND REASONING CHECKS

1.11 Why have microorganisms been separated into a variety of different groups?

1.5 The Second Golden Age of Microbiology (1943–1970)

The 1940s brought the birth of molecular genetics to biology. Many biologists focused on understanding the genetics of organisms, including the nature of the genetic material and its regulation.

Molecular Biology Relies on Microorganisms

KEY CONCEPT

12. Microorganisms and viruses can be used as model systems to study phenomena common to all life.

In 1943, the Italian-born microbiologist Salvador Luria and the German physicist Max Delbrück carried out a series of experiments with bacterial cells and viruses that marked the second Golden Age of microbiology. They used a common gut-inhabiting bacterium, *Escherichia coli,* to address a basic question regarding evolutionary biology: Do mutations occur spontaneously or does the environment induce them? Luria and Dulbrück showed that bacterial cells could develop spontaneous mutations that generate resistance to viral infection. Besides the significance of their findings to microbial genetics, the use of microbial model systems showed to other researchers that microorganisms could be used to study general principles of biology.

Mutations: Permanent alterations in DNA base sequences.

Biologists were quick to jump on the "microbial bandwagon." Experiments carried out by Americans George Beadle and Edward Tatum in the 1940s ushered in the field of molecular biology by using the fungus *Neurospora* to show that "one gene codes for one enzyme." Oswald Avery, Colin MacLeod, and Maclyn McCarty, working with the bacterial species *Streptococcus pneumoniae,* suggested in 1944 that deoxyribonucleic acid (DNA) is the genetic material in cells. In 1953, American biochemist Alfred Hershey and geneticist Martha Chase, using a virus that infects bacterial cells, provided irrefutable evidence that DNA is the substance of the genetic material. These experiments and discoveries, which will be discussed in more detail in Chapter 8, placed microbiology in the middle of the molecular biology revolution.

CONCEPT AND REASONING CHECKS

1.12 What roles did microorganisms and viruses play in understanding general principles of biology?

Two Types of Cellular Organization Are Realized

KEY CONCEPT

13. All microorganisms have a characteristic cell structure.

The small size of bacterial cells hindered scientists' abilities to confirm whether these cells were similar to other cellular organisms in organization. In the 1940s and 1950s, a new type of microscope—the electron microscope—was being developed that could magnify objects and cells thousands of times better than typical light microscopes. With the electron microscope, for the first time bacterial cells were seen as being cellular like all other microbes, plants, and animals. However, studies showed that they were organized in a fundamentally different way from other organisms.

It was known that animal and plant cells contained a cell nucleus that houses the genetic instructions in the form of chromosomes and was separated physically from other cell structures by a membrane envelope (FIGURE 1.13A). This type of cellular organization is called **eukaryotic** (*eu* = "true"; *karyon* = "nucleus"). Microscope observations of the protists and fungi had revealed that these organisms also have a eukaryotic organization. Thus, not only are all plants and animals eukaryotes, so are the microorganisms that comprise the fungi and protists.

Studies with the electron microscope revealed that bacterial (and archaeal) cells had few of the membranous compartments typical of eukaryotic cells. They lacked a cell nucleus, indicating the bacterial chromosome (DNA) was not surrounded by a membrane envelope (FIGURE 1.13B). Therefore, members of the Bacteria and Archaea have a **prokaryotic** (*pro* = "before") type of cellular organization and represent prokaryotes. (By the way, because viruses lack a cellular organization, they are neither prokaryotes nor eukaryotes.) As we will see in Chapter 4, there are many differences between bacterial and archaeal cells, blurring the use of the term "prokaryote."

CONCEPT AND REASONING CHECKS

1.13 Distinguish between prokaryotic and eukaryotic cells.

Antibiotics Are Used to Cure Infectious Disease

KEY CONCEPT

14. Antimicrobial chemicals can be effective in treating infectious diseases.

In 1910, another coworker of Koch's, Paul Ehrlich, synthesized the first "magic bullet"—a chemical that could kill pathogens without damaging the

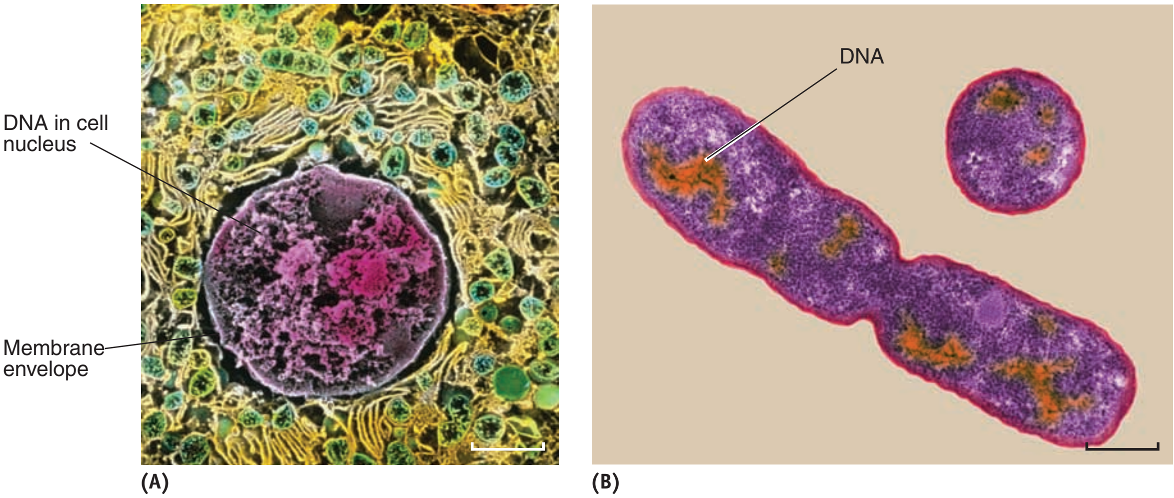


FIGURE 1.13 **False Color Images of Eukaryotic and Prokaryotic Cells.** (**A**) A scanning electron microscope image of a eukaryotic cell. All eukaryotes, including the protists and fungi, have their DNA (pink) enclosed in a cell nucleus with a membrane envelope. (Bar = 3 µm.) (**B**) A transmission electron microscope image of a dividing *Escherichia coli* cell. The DNA (orange) is not surrounded by a membrane. (Bar = 0.5 µm.)

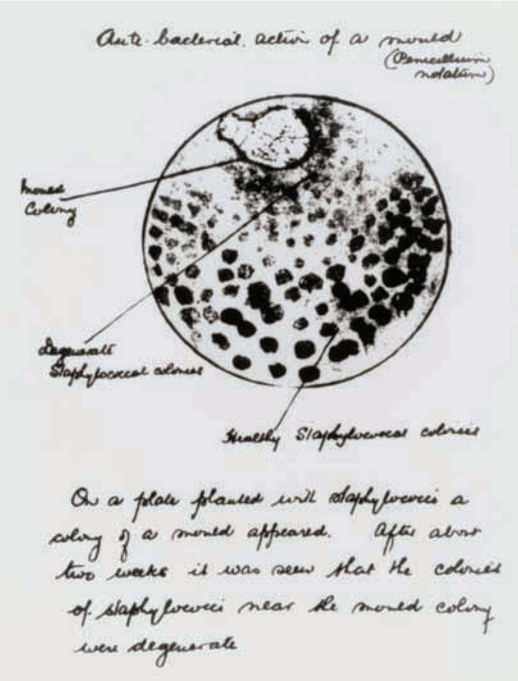
surrounding tissue. Called salvarsan, Ehrlich showed that this arsenic-containing compound cured syphilis, a sexually transmitted disease. Antibacterial **chemotherapy**, the use of antimicrobial chemicals to kill microbes, was born.

In 1928, Alexander Fleming, a Scottish scientist, discovered a mold growing in one of his bacterial cultures (FIGURE 1.14A, B). His curiosity aroused, Fleming observed that the mold, a species of *Penicillium,* killed the bacterial cells and colonies that were near the mold. He named the antimicrobial substance penicillin and developed an assay for its production. In 1940, biochemists Howard Florey and Ernst Chain purified penicillin and carried out clinical trials that showed the antimicrobial potential of the natural drug (MicroFocus 1.6).

Additional magic bullets also were being discovered. The German chemist Gerhard Domagk discovered a synthetic chemical dye, called prontosil, which was effective in treating *Streptococcus* infections. Examination of soil bacteria led Selman Waksman to the discovery of actinomycin and streptomycin, the latter being the first effective agent against tuberculosis. He coined the term **antibiotic** to refer to those antimicrobial substances naturally produced by mold and bacterial species that inhibit growth or kill other microorganisms.

The push to market effective antibiotics was stimulated by a need to treat potentially deadly infections in casualties of World War II (FIGURE 1.14C). By the 1950s, penicillin and several additional antibiotics were established treatments

(A)



Anti-bacterial action of a mould (Penicillium notatum)

On a plate planted with staphylococci a colony of a mould appeared. After about two weeks it was seen that the colonies of staphylococci near the mould colony were degenerate

(B)

(C)

FIGURE 1.14 **Fleming and Penicillin.** (**A**) A painting by Dean Fausett of Fleming in his laboratory. (**B**) Fleming's notes on the inhibition of bacterial growth by the fungus *Penicillium*. (**C**) A World War II poster touting the benefits of penicillin and illustrating the great enthusiasm in the United States for treating infectious diseases in war casualties.

MICROFOCUS 1.6: History

Hiding a Treasure

Their timing could not have been worse. Howard Florey, Ernest Chain, Norman Heatley, and others of the team had rediscovered penicillin, purified it, and proved it useful in infected patients. But it was 1939, and German bombs were falling on London. This was a dangerous time to be doing research into new drugs and medicines. What would they do if there was a German invasion of England? If the enemy were to learn the secret of penicillin, the team would have to destroy all their work. So, how could they preserve the vital fungus yet keep it from falling into enemy hands?

Heatley made a suggestion. Each team member would rub the mold on the inside lining of his coat. The *Penicillium* mold spores would cling to the rough coat surface where the spores could survive for years (if necessary) in a dormant form. If an invasion did occur, hopefully at least one team member would make it to safety along with his "moldy coat." Then, in a safe country the spores would be used to start new cultures and the research could continue. Of course, a German invasion of England did not occur, but the plan was an ingenious way to hide the treasured organism.

The whole penicillin story is well told in *The Mold in Dr. Flory's Coat* by Eric Lax (Henry Holt Publishers, 2004).

in medical practice. In fact, the growing arsenal of antibiotics convinced many that the age of infectious disease was waning. By the mid-1960s, many believed all major infections would soon disappear due to antibiotic chemotherapy.

Partly due to the perceived benefits of antibiotics, interest in microbes was waning by the end of the 1960s as the knowledge gained from bacterial studies was being applied to eukaryotic organisms, especially animals. What was ignored was the mounting evidence that bacterial species were becoming resistant to antibiotics.

CONCEPT AND REASONING CHECKS

1.14 Contrast Ehrlich's salvarsan and Domagk's prontosil from those drugs developed by Fleming, Florey and Chain, and Waksman.

1.6 The Third Golden Age of Microbiology—Now

Microbiology finds itself on the world stage again, in part from the biotechnology advances made in the latter part of the 20th century. **Biotechnology** frequently uses the natural and genetically engineered abilities of microbial agents to carry out biological processes for industrial/commercial/medical applications. It has revolutionized the way microorganisms are genetically manipulated to act as tiny factories producing human proteins, such as insulin, or new synthetic vaccines, such as the hepatitis B vaccine. In the latest Golden Age, microbiology again is making important contributions to the life sciences and humanity.

Microbiology Continues to Face Many Challenges

KEY CONCEPT

15. Infectious disease (natural and intentional) preoccupies much of microbiology.

The third Golden Age of microbiology faces several challenges, many of which still concern the infectious diseases that are responsible for 26% of all deaths globally (FIGURE 1.15).

A New Infectious Disease Paradigm. Infectious disease remains a major concern worldwide. Even in the United States, more than 100,000 people die each year from bacterial infections, making them the fourth leading cause of death. In fact, on a global scale, infectious diseases are spreading geographically faster than at any time in history. It is estimated that more than 2.5 billion people traveled by air in 2010, making an outbreak or epidemic in any one part of the world only a few airline hours away from becoming a potentially dangerous threat in another part of the world. It is a sobering thought to realize that since 2002, the World Health Organization (WHO) has verified more than 1,100 epidemic

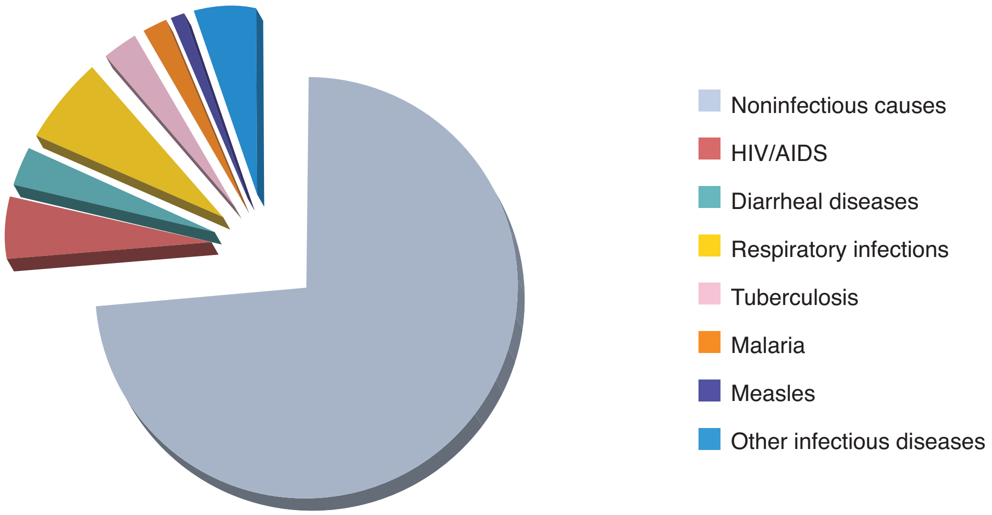


FIGURE 1.15 **Global Mortality—All Ages.** On a global scale, infectious diseases account for about 26% of all deaths. Noninfectious causes include chronic diseases, injuries, nutritional deficiencies, and maternal and perinatal conditions. *Source:* World Health Statistics 2008: World Health Organization.

events worldwide. So, unlike past generations, today's highly mobile, interdependent, and interconnected world provides potential opportunities for the rapid spread of infectious diseases.

Today, our view of infectious diseases also has changed. In Pasteur and Koch's time, it was mainly a problem of finding the germ that caused a specific disease. Today, new pathogens are being discovered that were never known to be associated with infectious disease and some of these agents actually cause more than one disease. In addition, there are **polymicrobial diseases**; that is, diseases caused by more than one infectious agent. Even some noninfectious diseases, such as heart disease, may have a microbial component that heightens the illness.

Emerging and Reemerging Infectious Diseases. Infectious diseases are not only spreading faster, they appear to be emerging more quickly than ever before. Since the 1970s, new diseases have been identified at the unprecedented rate of one or more per year. There are now nearly 40 diseases that were unknown a generation ago. For example, the food chain has undergone considerable and rapid changes over the last 50 years, becoming highly sophisticated and international. Although the safety of food has dramatically improved overall, progress is uneven and foodborne outbreaks from microbial contamination are common in many countries. The trading of contaminated food between countries increases the potential that outbreaks will spread.

■ **Superbug:**
A microbe resistant to many antimicrobial drugs.

Emerging infectious diseases are those that have recently surfaced in a population. Among the more newsworthy have been AIDS, hantavirus pulmonary syndrome, Lyme disease, mad cow disease, and most recently swine flu. There is no cure for any of these. **Reemerging infectious diseases** are ones that have existed in the past but are now showing a resurgence in incidence or a spread in geographic range. Among the more prominent re-emerging diseases are cholera, tuberculosis, dengue fever, and, for the first time in the Western Hemisphere, West Nile virus disease (FIGURE 1.16A). The cause for the resurgence may be antibiotic resistance or a population of susceptible individuals. Climate change also may become implicated in the upsurge and spread of disease as more moderate temperatures advance to more northern and southern latitudes.

Increased Antibiotic Resistance. Another challenge concerns our increasing inability to fight infectious disease because most pathogens are now resistant to one or more antibiotics and antibiotic resistance is developing faster than new antibiotics are being discovered. Ever since it was recognized that pathogens could mutate into "superbugs," a crusade has been waged to restrain the inappropriate use of these drugs by doctors and to educate patients not to demand them in uncalled-for situations.

The challenge facing microbiologists and drug companies is to find new and effective antibiotics to which pathogens will not quickly develop resistance before the current arsenal is completely useless. Unfortunately, the growing threat of antibiotic resistance has been accompanied

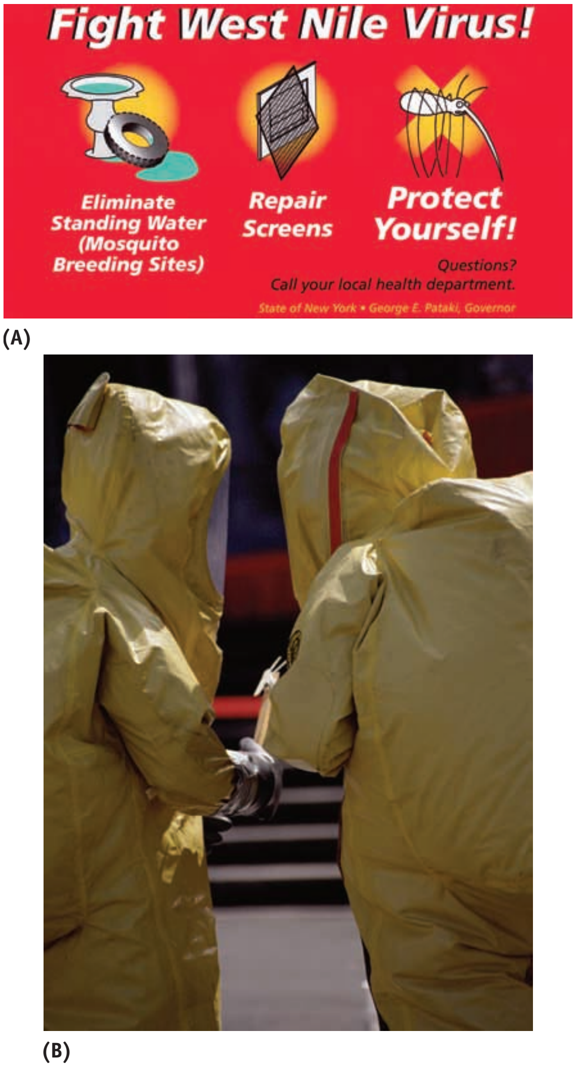


FIGURE 1.16 Emerging Disease Threats: Natural and Intentional. (**A**) There have been and will continue to be natural disease outbreaks. West Nile virus (WNV) is just one of several agents responsible for emerging or reemerging diseases. Methods have been designed that individuals can use to protect themselves from mosquitoes that spread the WNV. (**B**) Combating the threat of bioterrorism often requires special equipment and protection because many agents seen as possible bioweapons could be spread through the air.

by a decline in new drug discovery and an increase in the time to develop a drug from discovery to market. Thus, antibiotic resistance has become a major health threat and a significant challenge for microbiology today. If actions are not taken to contain and reverse resistance, the world could be faced with previously treatable diseases that have again become untreatable, as in the days before antibiotics were developed.

Bioterrorism. Perhaps it is the potential misuse of microbiology that has brought microbiology to the attention of the life science community and the public. **Bioterrorism** involves the intentional or threatened use of biological agents to cause fear in or actually inflict death or disease upon a large population. Most of the recognized biological agents are microorganisms, viruses, or microbial toxins that are bringing diseases like anthrax, smallpox, and plague back into the human psyche (FIGURE 1.16B). To minimize the use of these agents to inflict mass casualties, the challenge to the scientific community and microbiologists is to improve the ways that bioterror agents are detected, discover effective measures to protect the public, and develop new and effective treatments for individuals or whole populations. If there is anything good to come out of such challenges, it is that we will be better prepared for potential natural emerging infectious disease outbreaks, which initially might be difficult to tell apart from a bioterrorist attack.

CONCEPT AND REASONING CHECKS

1.15 Describe the natural and intentional disease threats challenging microbiology.

Microbial Ecology and Evolution Are Helping to Drive the New Golden Age

KEY CONCEPT

16. Microbial ecology and evolution are dominant themes in modern microbiology.

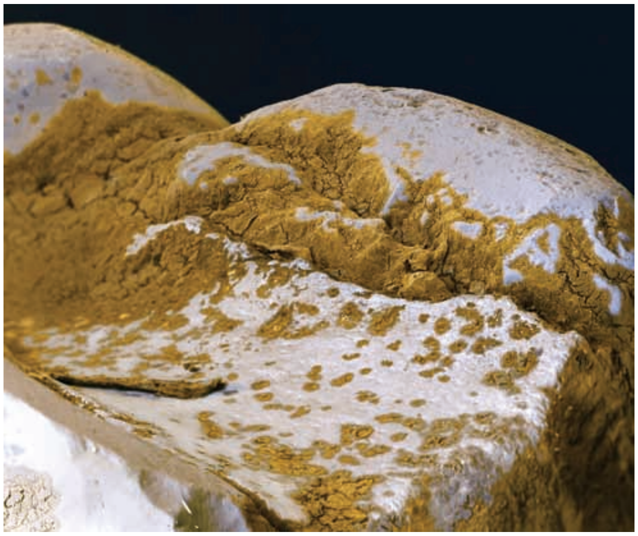
Since the time of Pasteur, microbiologists have wanted to know how a microbe interacts, survives, and thrives in the environment. Today, microbiology is less concerned with a specific microbe and more concerned with the relationships among microorganisms and with their environment.

Microbial Ecology. Traditional methods of microbial ecology require organisms from an environment be cultivated in the laboratory so that they can be characterized and identified. However, up to 99% of microorganisms do not grow well in the lab (if at all) and therefore could not be studied. Today, many microbiologists, armed with genetic, molecular, and biotechnological tools, can study and characterize these uncultured microbes. Such investigations are producing a new understanding of microbial communities and their influence on the ecology of all organisms. SAR11 and the plans of Craig Venter, mentioned in the chapter's opening piece, are but two examples.

Today we are learning that most microbes do not act as individual entities; rather, in nature they survive in complex, often polymicrobial communities called a **biofilm** (FIGURE 1.17A). Microbes in biofilms act very differently than individual cells and can be difficult to treat when biofilms cause infectious disease. If you or someone you know has had a middle ear infection, the cause was a bacterial biofilm.

Bioremediation: The use of microorganisms to remove or decontaminate toxic materials in the environment.

The discovered versatility of many bacterial and archaeal species is being applied to problems that have the potential to benefit humankind. Bioremediation is one example where the understanding of microbial ecology has produced a useful outcome (FIGURE 1.17B). Other microbes also are playing increasingly important roles in the health of the planet as described in Part 6 of this text.



(A)

(B)

FIGURE 1.17 **Microbial Ecology—Biofilms and Bioremediation.** (**A**) The plaque (the brown-colored crust) typically found on tooth surfaces is an example of a biofilm. (**B**) Microbes can be used to clean up toxic spills. A shoreline coated with oil from an oil spill can be sprayed with microorganisms that, along with other measures, help degrade oil.

Microbial Evolution. It was Charles Darwin—another of the scientists in this chapter who combined observation with a "prepared mind"—who first described the principles of evolution, which represents the foundation for all biology and medicine.

Like all life, microorganisms evolve. Because most have relatively short generation times, they represent experimental (model) systems in which evolutionary processes can be observed directly; microbial evolution is an experimental science. That makes it possible today to "replay history" by following the accumulation of unpredictable, chance events that lead to evolutionary novelty. For example, when considering the challenges facing microbiology today, current research is putting together a better understanding of the superfast evolution and spread of antibiotic resistance. It is also helping us better understand the mechanisms and evolution of emerging infectious diseases.

Researchers once thought that they would not be able to work out the evolutionary history of microbes. Today, thanks to the availability of sequenced genomes for groups of related and unrelated microbes, and new analytical approaches, researchers are constructing a family tree that more clearly illustrates evolutionary relationships (Chapter 3). Such developments are giving us a better appreciation for the roles microbes have played and are continuing to play in Earth's evolution. Indeed, microbial evolution represents the organization for the biological and microbiological knowledge contained within this text.

CONCEPT AND REASONING CHECKS

1.16 Give some examples of how microbial ecology and evolution are helping drive the new Golden Age of microbiology.

In conclusion, microbiology (from then until now) has gone from observing the first bacterial cells (Leeuwenhoek) to identifying and studying individual microorganisms (Pasteur and Koch) to sequencing all species in a sample of seawater (Venter and others). Yet, over these 300+ years, microbiologists have only discovered perhaps 1% of all microbial species. Microbiology from then until now has come a long way, but has a much longer way yet to go.

SUMMARY OF KEY CONCEPTS

1.1 The Beginnings of Microbiology

1. The observations with the microscope made by Hooke and especially Leeuwenhoek, who reported the existence of **animalcules** (microorganisms), sparked interest in an unknown world of microscopic life.
2. The controversy over **spontaneous generation** initiated the need for accurate scientific experimentation, which then provided the means to refute the concept.

1.2 Microorganisms and Disease Transmission

3. Semmelweis and Snow believed that infectious disease could be caused by something transmitted from the environment and that the transmission could be interrupted.
4. Edward Jenner determined that disease (smallpox) could be prevented through **vaccination** with a similar but milder disease-causing agent.

1.3 The Classical Golden Age of Microbiology (1854–1914)

5. Pasteur's fermentation experiments indicated that microorganisms could induce chemical changes. He proposed the **germ theory** of disease, which stated that human disease could be due to chemical changes brought about by microorganisms in the body.
6. Lister's use of **antisepsis** techniques and Pasteur's studies of pébrine supported the germ theory and showed how diseases can be controlled.
7. Koch's work with anthrax allowed him to formalize the methods (**Koch's postulates**) for relating a specific microorganism to a specific disease. These postulates were only valid after he discovered how to make **pure cultures** of bacterial species.
8. Laboratory science arose as Pasteur and Koch hunted down the microorganisms of infectious disease. Pasteur's lab studied the mechanisms for infection and developed vaccines for chicken cholera, animal anthrax, and human rabies. Koch's lab focused on isolation, cultivation, and identification of pathogens such as those responsible for cholera and tuberculosis.
9. Ivanowsky and Beijerinck provided the first evidence for viruses as infectious agents.
10. Winogradsky and Beijerinck were the first to recognize the beneficial roles played by microorganisms found in the environment.

1.4 Studying Microorganisms

11. Microbes include the "bacteria" (Bacteria and Archaea), viruses, fungi, and protists.

1.5 The Second Golden Age of Microbiology (1943–1970)

12. Many of the advances toward understanding molecular biology and general principles in biology were based on experiments using microbial model systems.
13. With the advent of the electron microscope, microbiologists realized that there were two basic types of cellular organization: **eukaryotic** and **prokaryotic**.
14. Following from the initial work by Ehrlich, **antibiotics** were developed as "magic bullets" to cure many infectious diseases.

1.6 The Third Golden Age of Microbiology—Now

15. In the 21st century, fighting infectious disease, identifying **emerging** and **reemerging infectious diseases**, combating increasing antibiotic resistance, and countering the **bioterrorism** threat are challenges facing microbiology, health care systems, and society.
16. **Microbial ecology** is providing new clues to the roles of microorganisms in the environment. The understanding of **microbial evolution** through the use of genomic technologies has expanded our understanding of microorganism relationships.

LEARNING OBJECTIVES

After understanding the textbook reading, you should be capable of writing a paragraph that includes the appropriate terms and pertinent information to answer the objective.

1. Identify the significant contributions made by Hooke and Leeuwenhoek that foreshadowed the beginnings of microbiology.
2. Discuss **spontaneous generation** and compare the experiments that led to its downfall.
3. Assess the importance of the work carried out by Semmelweis and by Snow that went against the **miasma** idea and established the field of **epidemiology**.
4. Explain how Jenner's work differs from earlier practices for preventing infectious disease.
5. Discuss Pasteur's early studies suggesting that germs could cause disease.
6. Describe how Lister's surgical work and Pasteur's studies of pébrine further strengthened the **germ theory** of disease.
7. Judge the importance of (a) the germ theory of disease and (b) **Koch's postulates** to the identification of microbes as agents of infectious disease.
8. Identify several discoveries made in the laboratories of Pasteur and Koch.
9. Describe how viruses were discovered.
10. Describe the contributions Winogradsky and Beijerinck made to environmental microbiology.
11. Briefly describe the organisms or agents found in each of the microbiology disciplines.
12. Illustrate how microorganisms and viruses make good model systems.
13. Explain why Bacteria and Archaea are **prokaryotic** cells and all other organisms are **eukaryotic** cells.
14. Define **chemotherapy** and explain why **antibiotics** were referred to as "magic bullets."
15. Outline the major challenges facing microbiology today.
16. Assess the importance of **microbial ecology** and **microbial evolution** to the current Golden Age of microbiology.

For **STEPS A–D**, answers to even-numbered questions and problems can be found in **Appendix C** on the Student Companion Website at **http://microbiology.jbpub.com/bodysystems2e/**. In addition, the site features eLearning, an online review area that provides quizzes and other tools to help you study for your class. You can also follow useful links for in-depth information, read more MicroFocus stories, or just find out the latest microbiology news.

STEP A: SELF-TEST

Each of the following questions is designed to assess your ability to remember or recall factual or conceptual knowledge related to this chapter. Read each question carefully, then select the **one** answer that best fits the question or statement.

1. Who was the first person to see bacterial cells with the microscope?
  A. Pasteur
  B. Koch
  C. Leeuwenhoek
  D. Hooke

2. What process was studied by Redi and Spallanzani?
  A. Spontaneous generation
  B. Fermentation
  C. Variolation
  D. Antisepsis

3. What is the name for the field of study established by Semmelweis and Snow in the mid 1800s?
  A. Immunology
  B. Bacteriology
  C. Virology
  D. Epidemiology

4. The process of _____ involved the inoculation of dried smallpox scabs under the skin.
  A. vaccination
  B. antisepsis
  C. variolation
  D. immunization

5. The process of controlled heating, called _____, was used to keep wine from spoiling.
  A. curdling
  B. fermentation
  C. pasteurization
  D. variolation

6. What surgical practice was established by Lister?
  A. Antisepsis
  B. Chemotherapy
  C. Variolation
  D. Sterilization

7. Which one of the following statements is NOT part of Koch's postulates?
  A. The microorganism must be isolated from a dead animal and pure cultured.
  B. The microorganism and disease can be identified from a mixed culture.
  C. The pure cultured organism is inoculated into a healthy, susceptible animal.
  D. The same microorganism must be present in every case of the disease.

8. Match the lab with the correct set of identified diseases.
  A. Pasteur: tetanus and tuberculosis
  B. Koch: anthrax and rabies
  C. Koch: cholera and tuberculosis
  D. Pasteur: diphtheria and typhoid

9. What group of microbial agents would eventually be identified from the work of Ivanowsky and Beijerinck?
  A. Viruses
  B. Fungi
  C. Protists
  D. Bacteria

10. What microbiological field was established by Winogradsky and Beijerinck?
  A. Virology
  B. Microbial ecology
  C. Bacteriology
  D. Mycology

11. What group of microorganisms has a variety of internal cell compartments and acts as decomposers?
  A. Bacteria
  B. Viruses
  C. Archaea
  D. Fungi

12. Which one of the following organisms was NOT a model organism related to the birth of molecular genetics?
  A. *Streptococcus*
  B. *Penicillium*
  C. *Escherichia*
  D. *Neurospora*

13. Which group of microbial agents is eukaryotic?
  A. Bacteria
  B. Viruses
  C. Archaea
  D. Algae

14. The term antibiotic was coined by _____ to refer to antimicrobial substances naturally derived from _____.
  A. Waksman; bacteria and fungi
  B. Domagk; other living organisms
  C. Fleming; fungi and bacteria
  D. Ehrlich; bacteria

15. Which one of the following is NOT considered an emerging infectious disease?
  A. Polio
  B. Hantavirus pulmonary disease
  C. Lyme disease
  D. AIDS

16. A _____ is a mixture of _____ that form as a complex community.
  A. genome; genes
  B. biofilm; microbes
  C. biofilm; chemicals
  D. miasma; microbes

STEP B: REVIEW

17. Construct a concept map for **Microbial Agents** using the following terms.

algae
Archaea
cyanobacteria
decomposers
Bacteria
fungi
microorganisms
nucleated cells
protists
viruses

On completing your study of these pages, test your understanding of their contents by deciding whether the following statements are true (T) or false (F). If the statement is false, substitute a word or phrase for the underlined word or phrase to make the statement true.

18. _____ Leeuwenhoek believed that animalcules arose spontaneously from decaying matter.

19. _____ Pasteur proposed that "wine disease" was a souring of wine caused by yeast cells.

20. _____ Antisepsis is the use of chemical methods for disinfecting living surfaces.

21. _____ Separate bacterial colonies can be observed in a broth culture.

22. _____ Semmelweis proposed that cholera was a waterborne disease.

23. _____ Some bacterial species can convert nitrogen gas (N_2) into ammonia (NH_3).

24. _____ Fungi are eukaryotic microorganisms.

25. _____ Koch proposed the germ theory.

26. _____ Variolation involved inoculating individuals with smallpox scabs.

27. _____ Mycology is the scientific study of viruses.

STEP C: APPLICATIONS

28. As a microbiologist in the 1940s, you are interested in discovering new antibiotics that will kill bacterial pathogens. You have been given a liquid sample of a chemical substance to test in order to determine if it kills bacterial cells. Drawing on the culture techniques of Robert Koch, design an experiment that would allow you to determine the killing properties of the sample substance.

29. As an environmental microbiologist, you discover a new species of microbe. How could you determine if it has a prokaryotic or eukaryotic cell structure? Suppose it has a eukaryotic structure. What information would be needed to determine if it is a member of the protista or fungi?

30. Louis Pasteur once stated: "In the field of observation, chance favors only the prepared mind." How does this quote apply to the work done by (a) Semmelweis, (b) Snow, and (c) Fleming?

STEP D: QUESTIONS FOR THOUGHT AND DISCUSSION

31. Many people are fond of pinpointing events that alter the course of history. In your mind, which single event described in this chapter had the greatest influence on the development of microbiology? What event would be in second place?

32. One of the foundations of scientific inquiry is proper experimental design involving the use of controls. What is the role of a control in an experiment? For each of the experiments described in the section on spontaneous generation, identify the control(s) and explain how the interpretation of the experimental results would change without such controls.

33. One reason for the rapid advance in knowledge concerning molecular biology during the second Golden Age of microbiology was because many researchers used microorganisms as model systems. Why would bacterial cells be more advantageous to use for research than, say, rats or guinea pigs?

34. When you tell a friend that you are taking microbiology this semester, she asks, "*Exactly what is microbiology?*" How do you answer her?

35. As microbiologists continue to explore the microbial universe, it is becoming more apparent that microbes are "invisible emperors" that rule the world. Now that you have completed Chapter 1, provide examples to support the statement: Microbes Rule!

36. Who would you select as the "first microbiologist?" (a) Leeuwenhoek, (b) Hooke, or (c) Pasteur and Koch. Support your decision.

37. Felix Pouchet was a French biologist and science writer who believed in spontaneous generation. As such, he was often in debate with Pasteur because he was not convinced that Pasteur's experiments refuted the idea of spontaneous generation. As proof, Pouchet set up a series of swan-necked flasks identical to those used by Pasteur to refute spontaneous generation. He then filled the flasks with a broth made from hay, boiled the flasks for one hour, and allowed them to cool. Everything was identical to Pasteur's experiments except Pasteur used a sugar and yeast extract broth and only boiled the flasks for a few minutes.

In all cases, Pouchet saw growth of microorganisms in all his flasks, even with boiling for one hour. Propose a solution for the contradictory results of Pasteur and Pouchet, knowing that (a) what both scientists saw was valid and correct, and (b) spontaneous generation does not occur.

If you remain stumped, check out the short paper entitled: John Tyndall and the Spontaneous Generation Debate (**Microbiology Today**, November 2005-http:www.sgm.ac.uk/pubs/micro_today/pdf/110501.pdf