7 The Control of Microbial Growth

The scientific control of microbial growth began only about 100 years ago. Recall from Chapter 1 that Pasteur's work on microorganisms led scientists to believe that microbes were a possible cause of disease. In the mid-1800s, the Hungarian physician Ignaz Semmelweis and English physician Joseph Lister used this thinking to develop some of the first microbial control practices for medical procedures. These practices included hand washing with microbe-killing chloride of lime and use of the techniques of **aseptic surgery** to prevent microbial contamination of surgical wounds. Until that time, hospital-acquired infections, or *nosocomial infections,* were the cause of death in at least 10% of surgical cases, and deaths of delivering mothers were as high as 25%. Ignorance of microbes was such that, during the American Civil War, a surgeon might have cleaned his scalpel on his bootsole between incisions.

Over the last century, scientists have continued to develop a variety of physical methods and chemical agents to control microbial growth. In Chapter 20 we will discuss methods for the control of microbes once infection has occurred, mainly antibiotic chemotherapy.

UNDER THE MICROSCOPE

Bacteria trapped in a membrane filter. Filtration can be used to remove microorganisms from water and solutions.

THE TERMINOLOGY OF MICROBIAL CONTROL

LEARNING OBJECTIVE

- Define the following key terms related to microbial control: *sterilization, disinfection, antisepsis, degerming, sanitization, biocide, germicide, bacteriostasis,* and *asepsis.*

A word frequently used, and misused, in discussing the control of microbial growth is *sterilization*. **Sterilization** is the removal or destruction of *all forms* of microbial life. Heating is the most common method used for killing microbes, including the most resistant forms such as endospores. A sterilizing agent is called a **sterilant.** Sterilization by removal of microbes from liquids or gases can be done by filtration.

One would think that canned food in the supermarket is completely sterile. In reality, the heat treatment required to ensure absolute sterility would unnecessarily degrade the quality of the food. Instead, food is subjected only to enough heat to destroy the endospores of *Clostridium botulinum*, which can produce a deadly toxin. This limited heat treatment is termed **commercial sterilization.** The endospores of a number of thermophilic bacteria, capable of causing food spoilage but not human disease, are considerably more resistant to heat than *C. botulinum*. If present, they will survive, but their survival is usually of no practical consequence; they will not grow at normal food storage temperatures. If canned foods in a supermarket were incubated at temperatures in the growth range of these thermophiles (above about 45°C), significant food spoilage would occur.

Complete sterilization is often not required in other settings. For example, the body's normal defenses can cope with a few microbes entering a surgical wound. A drinking glass or a fork in a restaurant requires only enough microbial control to prevent the transmission of possibly pathogenic microbes from one person to another.

Control directed at destroying harmful microorganisms is called **disinfection.** It usually refers to the destruction of vegetative (non–endospore-forming) pathogens, which is not the same thing as complete sterility. Disinfection might make use of chemicals, ultraviolet radiation, boiling water, or steam. In practice, the term is most commonly applied to the use of a chemical (a *disinfectant*) to treat an inert surface or substance. When this treatment is directed at living tissue, it is called **antisepsis,** and the chemical is then called an *antiseptic*. Therefore, in practice the same chemical might be called a disinfectant for one use and an antiseptic for another. Of course, many chemicals suitable for swabbing a tabletop would be too harsh to use on living tissue.

There are modifications of disinfection and antisepsis. For example, when someone is about to receive an injection, the skin is swabbed with alcohol—the process of **degerming** (or *degermation*), which mostly results in the mechanical removal, rather than the killing, of most of the microbes in a limited area. Restaurant glassware, china, and tableware are subjected to **sanitization,** which is intended to lower microbial counts to safe public health levels and minimize the chances of disease transmission from one user to another. This is usually accomplished by high-temperature washing or, in the case of glassware in a bar, washing in a sink followed by a dip in a chemical disinfectant.

Table 7.1 summarizes the terminology relating to the control of microbial growth.

TABLE 7.1 Terminology Relating to the Control of Microbial Growth

	Definition	Comments
Sterilization	Destruction or removal of all forms of microbial life, including endospores.	Usually done by steam under pressure or a sterilizing gas such as ethylene oxide.
Commercial Sterilization	Sufficient heat treatment to kill endospores of *Clostridium botulinum* in canned food.	More-resistant endospores of thermophilic bacteria may survive, but they will not germinate and grow under normal storage conditions.
Disinfection	Destruction of vegetative pathogens.	May make use of physical or chemical methods.
Antisepsis	Destruction of vegetative pathogens on living tissue.	Treatment is almost always by chemical antimicrobials.
Degerming	Removal of microbes from a limited area, such as the skin around an injection site.	Mostly a mechanical removal by an alcohol-soaked swab.
Sanitization	Treatment intended to lower microbial counts on eating and drinking utensils to safe public health levels.	May be done with high-temperature washing or by dipping into a chemical disinfectant.

Names of treatments that cause the outright death of microbes have the suffix *-cide*, meaning kill. A **biocide,** or **germicide,** kills microorganisms (usually with certain exceptions, such as endospores); a *fungicide* kills fungi; a *virucide* inactivates viruses; and so on. Other treatments only inhibit the growth and multiplication of bacteria; their names have the suffix *-stat* or *-stasis*, meaning to stop or to steady, as in **bacteriostasis.** Once a bacteriostatic agent is removed, growth might resume.

Sepsis, from the Greek for decay or putrid, indicates bacterial contamination, as in septic tanks for sewage treatment. (The term is also used to describe a disease condition; see Chapter 23, page 672.) *Aseptic* means that an object or area is free of pathogens. Recall from Chapter 1 that **asepsis** is the absence of significant contamination. Aseptic techniques are important in surgery to minimize contamination from the instruments, operating personnel, and the patient.

THE RATE OF MICROBIAL DEATH

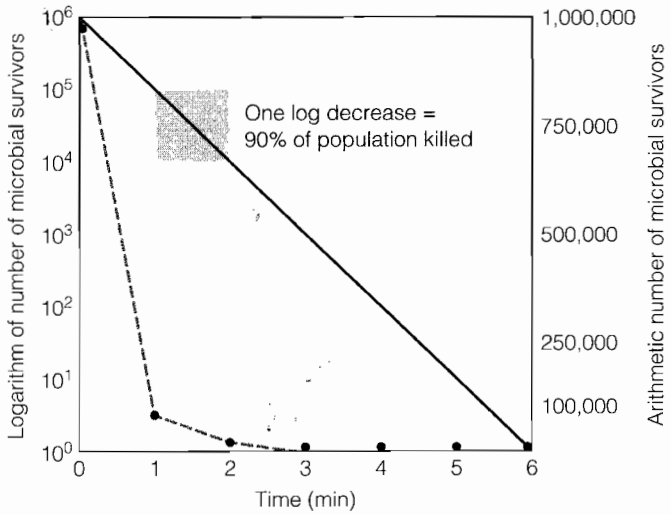
LEARNING OBJECTIVE

- Describe the patterns of microbial death caused by treatments with microbial control agents.

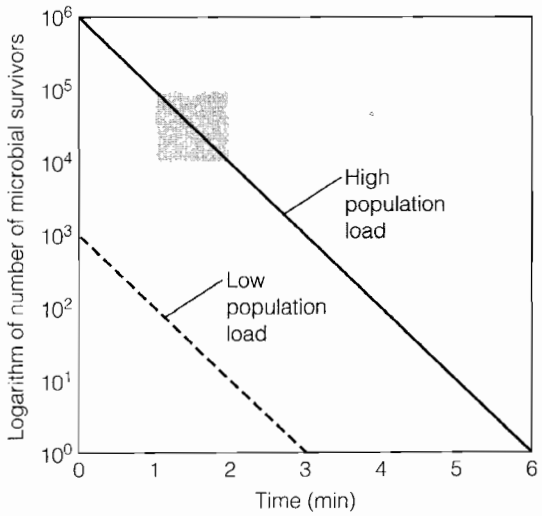
When bacterial populations are heated or treated with antimicrobial chemicals, they usually die at a constant rate. For example, suppose a population of 1 million microbes has been treated for 1 minute, and 90% of the population has died. We are now left with 100,000 microbes. If the population is treated for another minute, 90% of those microbes die, and we are left with 10,000 survivors. In other words, for each minute the treatment is applied, 90% of the remaining population is killed (Table 7.2). If the death curve is plotted logarithmically, the death rate is constant, as shown by the straight line in Figure 7.1a.

TABLE 7.2 Microbial Death Rate: An Example

Time (min)	Deaths per Minute	Number of Survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9000	1000
4	900	100
5	90	10
6	9	1



(a) The curve is plotted logarithmically (solid line) and arithmetically (broken line). In this case, the cells are dying at a rate of 90% each minute.



(b) The effect of high or low initial load of microbes. If the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one. This is true for both heat and chemical treatments.

FIGURE 7.1 A microbial death curve.

Q If the graph in part (a) reflects the experience with a vegetative bacterium, what would the logarithmic curve look like for the endospores of the same bacterium? Less steep? Steeper?

Several factors influence the effectiveness of antimicrobial treatments:

- *The number of microbes*. The more microbes there are to begin with, the longer it takes to eliminate the entire population (Figure 7.1b).

- *Environmental influences*. The presence of organic matter often inhibits the action of chemical antimicrobials. In hospitals, the presence of organic matter in blood, vomit, or feces influences the selection of disinfectants. Microbes in surface biofilms, shown in Figure 27.11, are difficult for biocides to reach effectively. Because their activity is due to temperature-dependent chemical reactions, disinfectants work somewhat better under warm conditions. Directions on disinfectant containers frequently specify the use of a warm solution.

  The nature of the suspending medium is also a factor in heat treatment. Fats and proteins are especially protective, and a medium rich in these substances protects microbes, which will then have a higher survival rate. Heat is also measurably more effective under acidic conditions.
- *Time of exposure*. Chemical antimicrobials often require extended exposure for more-resistant microbes or endospores to be affected. In heat treatments, a longer exposure can compensate for a lower temperature, a phenomenon of particular importance to pasteurization of dairy products.
- *Microbial characteristics*. The concluding section of this chapter discusses how microbial characteristics affect chemical and physical control methods.

ACTIONS OF MICROBIAL CONTROL AGENTS

LEARNING OBJECTIVE

- Describe the effects of microbial control agents on cellular structures.

In this section, we examine the ways various agents actually kill or inhibit microbes.

ALTERATION OF MEMBRANE PERMEABILITY

A microorganism's plasma membrane (see Figure 4.14, page 90), located just inside the cell wall, is the target of many microbial control agents. This membrane actively regulates the passage of nutrients into the cell and the elimination of wastes from the cell. Damage to the lipids or proteins of the plasma membrane by antimicrobial agents causes cellular contents to leak into the surrounding medium and interferes with the growth of the cell.

DAMAGE TO PROTEINS AND NUCLEIC ACIDS

Bacteria are sometimes thought of as "little bags of enzymes." Enzymes, which are primarily protein, are vital to all cellular activities. Recall that the functional properties of proteins are the result of their three-dimensional shape (see Figure 2.15, page 46). This shape is maintained by chemical bonds that link adjoining portions of the amino acid chain as it folds back and forth upon itself. Some of those bonds are hydrogen bonds, which are susceptible to breakage by heat or certain chemicals; breakage results in denaturation of the protein. Covalent bonds, which are stronger, are also subject to attack. For example, disulfide bridges, which play an important role in protein structure by joining amino acids with exposed sulfhydryl (—SH) groups, can be broken by certain chemicals or sufficient heat.

The nucleic acids DNA and RNA are the carriers of the cell's genetic information. Damage to these nucleic acids by heat, radiation, or chemicals is frequently lethal to the cell; the cell can no longer replicate, nor can it carry out normal metabolic functions such as the synthesis of enzymes.

PHYSICAL METHODS OF MICROBIAL CONTROL

LEARNING OBJECTIVES

- Compare the effectiveness of moist heat (boiling, autoclaving, pasteurization) and dry heat.
- Describe how filtration, low temperatures, high pressure, desiccation, and osmotic pressure suppress microbial growth.
- Explain how radiation kills cells.

As early as the Stone Age, it is likely that humans were already using some physical methods of microbial control to preserve foods. Drying (desiccation) and salting (osmotic pressure) were probably among the earliest techniques.

When selecting methods of microbial control, consideration must be given to effects on things besides the microbes. For example, certain vitamins or antibiotics in a solution might be inactivated by heat. Many laboratory or hospital materials, such as rubber and latex tubing, are damaged by repeated heating. There are also economic considerations; for example, it may be less expensive to use presterilized, disposable plasticware than to repeatedly wash and resterilize glassware.

HEAT

A visit to any supermarket will demonstrate that heat-preserved canned goods represent one of the most common methods of food preservation. Laboratory media and glassware, and hospital instruments, are also usually sterilized

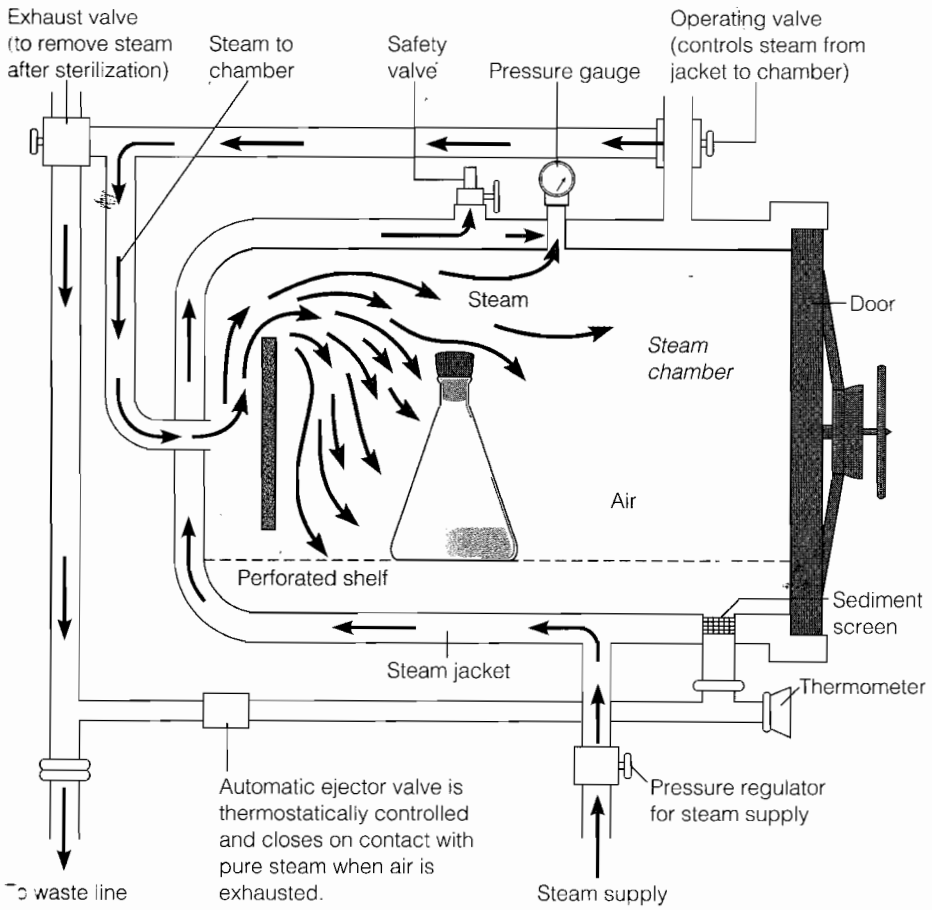


FIGURE 7.2 An autoclave. The entering steam forces the air out of the bottom (blue arrows). The automatic ejector valve remains open as long as an air-steam mixture is passing out of the waste line. When all the air has been ejected, the higher temperature of the pure steam closes the valve, and the pressure in the chamber increases.

Q **How would an empty, uncapped flask be positioned for sterilization in an autoclave?**

by heat. Heat appears to kill microorganisms by denaturing their enzymes; the resultant changes to the three-dimensional shapes of these proteins inactivate them (see Figure 5.6, page 121).

Heat resistance varies among different microbes; these differences can be expressed through the concept of thermal death point. **Thermal death point (TDP)** is the lowest temperature at which all the microorganisms in a particular liquid suspension will be killed in 10 minutes.

Another factor to be considered in sterilization is the length of time required. This is expressed as **thermal death time (TDT),** the minimal length of time for all bacteria in a particular liquid culture to be killed at a given temperature. Both TDP and TDT are useful guidelines that indicate the severity of treatment required to kill a given population of bacteria.

Decimal reduction time (DRT, or *D value*) is a third concept related to bacterial heat resistance. DRT is the time, in minutes, in which 90% of a population of bacteria at a given temperature will be killed (in Table 7.2 and Figure 7.1a, DRT is 1 minute). In Chapter 28 you can find an important application of DRT in the canning industry; see the discussion of the 12D treatment of canned goods in Chapter 28.

MOIST HEAT

Moist heat kills microorganisms primarily by the coagulation of proteins (denaturation), which is caused by breakage of the hydrogen bonds that hold the proteins in their three-dimensional structure. This coagulation process is familiar to anyone who has watched an egg white frying.

One type of moist heat sterilization is boiling, which kills vegetative forms of bacterial pathogens, almost all viruses, and fungi and their spores within about 10 minutes, usually much faster. Free-flowing (unpressurized) steam is essentially the same temperature as boiling water. Endospores and some viruses, however, are not destroyed this quickly. Some hepatitis viruses, for example, can survive up to 30 minutes of boiling, and some bacterial endospores can resist boiling for more than 20 hours. Boiling is therefore not always a reliable sterilization procedure. However, brief boiling, even at high altitudes, will kill most pathogens. The use of boiling to sanitize baby bottles is a familiar example.

Reliable sterilization with moist heat requires temperatures above that of boiling water. These high temperatures are most commonly achieved by steam under pressure in an **autoclave** (Figure 7.2). Autoclaving is the preferred method of sterilization, unless the material to be sterilized can be damaged by heat or moisture.

TABLE 7.3 The Relationship Between the Pressure and Temperature of Steam at Sea Level*

Pressure (psi in excess of atmospheric pressure)	Temperature (°C)
0	100
5	110
10	116
15	121
20	126
30	135

*At higher altitudes the atmospheric pressure is less, which must be taken into account in operation of an autoclave. For example, in order to reach sterilizing temperatures (121°C) in Denver, Colorado, whose altitude is 5280 feet (1600 meters), the pressure shown on the autoclave gauge would need to be higher than the 15 psi shown in the table.

TABLE 7.4 The Effect of Container Size on Autoclave Sterilization Times for Liquid Solutions*

Container Size	Liquid Volume	Sterilization Time (min)
Test tube: 18 × 150 mm	10 ml	15
Erlenmeyer flask: 125 ml	95 ml	15
Erlenmeyer flask: 2000 ml	1500 ml	30
Fermentation bottle: 9000 ml	6750 ml	70

*Sterilization times in the autoclave include the time for the contents of the containers to reach sterilization temperatures. For smaller containers, this is only 5 min or less, but for a 9000-ml bottle, it might be as much as 70 min. A container is usually not filled past 75% of its capacity.

The higher the pressure in the autoclave, the higher the temperature. For example, when free-flowing steam at a temperature of 100°C is placed under a pressure of 1 atmosphere above sea level pressure—that is, about 15 pounds of pressure per square inch (psi)—the temperature rises to 121°C. Increasing the pressure to 20 psi raises the temperature to 126°C. The relationship between temperature and pressure is shown in Table 7.3.

Sterilization in an autoclave is most effective when the organisms are either contacted by the steam directly or are contained in a small volume of aqueous (primarily water) liquid. Under these conditions, steam at a pressure of about 15 psi (121°C) will kill *all* organisms (but not prions, see page 000) and their endospores in about 15 minutes.

Autoclaving is used to sterilize culture media, instruments, dressings, intravenous equipment, applicators, solutions, syringes, transfusion equipment, and numerous other items that can withstand high temperatures and pressures. Large industrial autoclaves are called *retorts* (see Figure 28.2), but the same principle applies for the common household pressure cooker used in the home canning of foods.

Heat requires extra time to reach the center of solid materials, such as canned meats, because such materials do not develop the efficient heat-distributing convection currents that occur in liquids. Heating large containers also requires extra time. Table 7.4 shows the different time requirements for sterilizing liquids in various container sizes. Unlike sterilizing aqueous solutions, sterilizing the surface of a solid requires that steam actually contact it. To sterilize dry glassware, bandages, and the like, care must be taken to ensure that steam contacts all surfaces.

For example, aluminum foil is impervious to steam and should not be used to wrap dry materials that are to be sterilized; paper should be used instead. Care should also be taken to avoid trapping air in the bottom of a dry container because trapped air will not be replaced by steam, which is lighter than air. The trapped air is the equivalent of a small hot-air oven, which, as we will see shortly, requires a higher temperature and longer time to sterilize materials. Containers that can trap air should be placed in a tipped position so that the steam will force out the air. Products that do not permit penetration by moisture, such as mineral oil or petroleum jelly, are not sterilized by the same methods that would sterilize aqueous solutions.

Several commercially available methods can indicate whether sterilization has been achieved by heat treatment. Some of these are chemical reactions in which an indicator changes color when the proper times and temperatures have been reached (Figure 7.3). In some designs, the word "sterile"or "autoclaved" appears on wrappings or tapes. In another method, a pellet contained within a glass vial melts. A widely used test consists of preparations of specified species of bacterial endospores impregnated into paper strips. After autoclaving, these can then be aseptically inoculated into culture media. Growth in the culture media indicates survival of the endospores and therefore inadequate processing. Other designs use endospore suspensions that can be released, after heating, into a surrounding culture medium within the same vial.

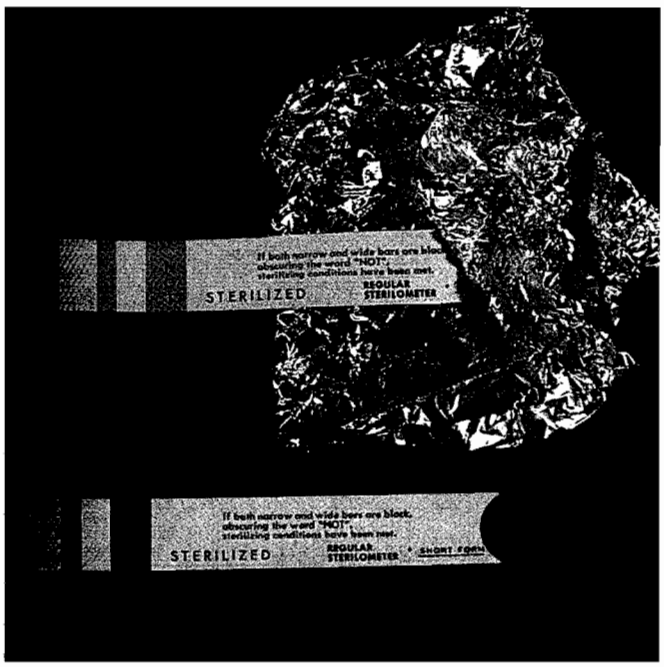


FIGURE 7.3 Examples of sterilization indicators. The strips indicate if the item has been properly sterilized, the word "NOT" appears if heating has been inadequate. In the illustration, the indicator that was wrapped with aluminum foil was not sterilized because steam couldn't penetrate the foil.

What should have been used instead of aluminum foil to wrap the items?

Steam under pressure fails to sterilize when the air is not completely exhausted. This can happen with the premature closing of the autoclave's automatic ejector valve (see Figure 7.2). The principles of heat sterilization have a direct bearing on home canning. As anyone familiar with home canning knows, the steam must flow vigorously out of the valve in the lid for several minutes to carry with it all the air before the pressure cooker is sealed. If the air is not completely exhausted, the container will not reach the temperature expected for a given pressure. Because of the possibility of botulism, a kind of food poisoning resulting from improper canning methods (see Chapter 22, page 649), people involved in home canning should obtain reliable directions and follow them exactly.

PASTEURIZATION

Recall from Chapter 1 that in the early days of microbiology, Louis Pasteur found a practical method of preventing the spoilage of beer and wine. Pasteur used mild heating, which was sufficient to kill the organisms that caused the particular spoilage problem without seriously damaging the taste of the product. The same principle was later applied to milk to produce what we now call pasteurized milk. The intent of **pasteurization** of milk was to eliminate pathogenic microbes. It also lowers microbial numbers, which prolongs milk's good quality under refrigeration. Many relatively heat-resistant (**thermoduric**) bacteria survive pasteurization, but these are unlikely to cause disease or cause refrigerated milk to spoil.

Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures, which often differ considerably. There are several reasons for these variations. For example, heating is less efficient in foods that are more viscous, and fats in food can have a protective effect on microorganisms. The dairy industry routinely uses a test to determine whether products have been pasteurized: the *phosphatase test* (phosphatase is an enzyme naturally present in milk). If the product has been pasteurized, phosphatase will have been inactivated.

In the classic pasteurization treatment of milk, the milk was exposed to a temperature of about 63°C for 30 minutes. Most milk pasteurization today uses higher temperatures, at least 72°C, but for only 15 seconds. This treatment, known as **high-temperature short-time (HTST) pasteurization,** is applied as the milk flows continuously past a heat exchanger. In addition to killing pathogens, HTST pasteurization lowers total bacterial counts, so the milk keeps well under refrigeration.

Milk can also be sterilized—something quite different from pasteurization—by **ultra-high-temperature (UHT) treatments** so that it can be stored without refrigeration. This is more useful in parts of the world where refrigeration facilities are not always available. In the United States, sterilization is sometimes used on the small containers of coffee creamers found in restaurants. To avoid giving the milk a cooked taste, a UHT system is used in which the liquid milk never touches a surface hotter than the milk itself while being heated by steam. The milk falls in a thin film through a chamber of superheated steam and reaches 140°C in less than a second. It is held for 3 seconds in a holding tube and then cooled in a vacuum chamber, where the steam flashes off. With this process, in less than 5 seconds the milk temperature rises from 74°C to 140°C and drops back to 74°C.

The heat treatments we have just discussed illustrate the concept of **equivalent treatments:** as the temperature is increased, much less time is needed to kill the same number of microbes. For example, the destruction of highly resistant endospores might take 70 minutes at 115°C, whereas only 7 minutes might be needed at 125°C. Both treatments yield the same result. The concept of equivalent treatments also explains why classic pasteurization at 63°C for 30 minutes, HTST treatment at 72°C for 15 seconds, and UHT treatment at 140°C for less than a second can have similar effects.

DRY HEAT STERILIZATION

Dry heat kills by oxidation effects. A simple analogy is the slow charring of paper in a heated oven, even when the temperature remains below the ignition point of paper. One of the simplest methods of dry heat sterilization is direct **flaming.** You will use this procedure many times in the microbiology laboratory when you sterilize inoculating loops. To effectively sterilize the inoculating loop, you heat the wire to a red glow. A similar principle is used in *incineration*, an effective way to sterilize and dispose of contaminated paper cups, bags, and dressings.

Another form of dry heat sterilization is **hot-air sterilization.** Items to be sterilized by this procedure are placed in an oven. Generally, a temperature of about 170°C maintained for nearly 2 hours ensures sterilization. The longer period and higher temperature (relative to moist heat) are required because the heat in water is more readily transferred to a cool body than is the heat in air. For example, imagine the different effects of immersing your hand in boiling water at 100°C (212°F) and of holding it in a hot-air oven at the same temperature for the same amount of time.

FILTRATION

Recall from Chapter 6 that *filtration* is the passage of a liquid or gas through a screenlike material with pores small enough to retain microorganisms (often the same apparatus used for counting; see Figure 6.17, page 181). A vacuum is created in the receiving flask; air pressure then forces the liquid through the filter. Filtration is used to sterilize heat-sensitive materials, such as some culture media, enzymes, vaccines, and antibiotic solutions.

Some operating theaters and rooms occupied by burn patients receive filtered air to lower the numbers of airborne microbes. **High-efficiency particulate air (HEPA) filters** remove almost all microorganisms larger than about 0.3 μm in diameter.

In the early days of microbiology, hollow candle-shaped filters of unglazed porcelain were used to filter liquids. The long and indirect passageways through the walls of the filter adsorbed the bacteria. Unseen pathogens that passed through the filters (causing such diseases as rabies) were called *filterable viruses*.

In recent years, **membrane filters,** composed of such substances as cellulose esters or plastic polymers, have become popular for industrial and laboratory use (Figure 7.4). These filters are only 0.1 mm thick. The pores of membrane filters include, for example, 0.22-μm and 0.45-μm sizes, which are intended for bacteria. Some very flexible bacteria, such as spirochetes, or the wall-less mycoplasma, will sometimes pass through such filters, however. Filters

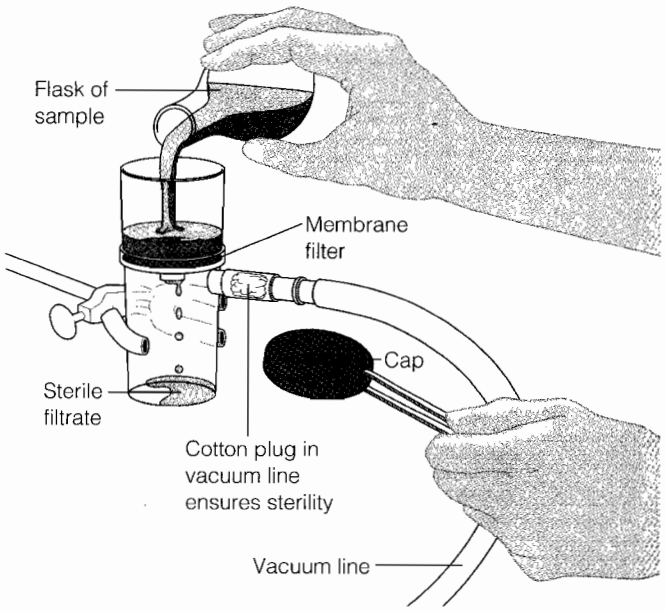


FIGURE 7.4 Filter sterilization with a disposable, presterilized plastic unit. The sample is placed into the upper chamber and forced through the membrane filter by a vacuum in the lower chamber. Pores in the membrane filter are smaller than the bacteria, so bacteria are retained on the filter. The sterilized sample can then be decanted from the lower chamber. Similar equipment with removable filter disks is used to count bacteria in samples (see Figure 6.17).

Q How is a plastic filtration apparatus presterilized? (Assume the plastic cannot be heat sterilized.)

are available with pores as small as 0.01 μm, a size that will retain viruses and even some large protein molecules.

LOW TEMPERATURES

The effect of low temperatures on microorganisms depends on the particular microbe and the intensity of the application. For example, at temperatures of ordinary refrigerators (0 to 7°C), the metabolic rate of most microbes is so reduced that they cannot reproduce or synthesize toxins. In other words, ordinary refrigeration has a bacteriostatic effect. Yet psychrotrophs do grow slowly at refrigerator temperatures and will alter the appearance and taste of foods after a time. For example, a single microbe reproducing only three times a day would reach a population of more than 2 million within a week. Pathogenic bacteria generally will not grow at refrigerator temperatures, but for at least one important exception, see the discussion of listeriosis in Chapter 22 (page 647).

Surprisingly, some bacteria can grow at temperatures several degrees below freezing. Most foods remain unfrozen until −2°C or lower. Rapidly attained subfreezing

temperatures tend to render microbes dormant but do not necessarily kill them. Slow freezing is more harmful to bacteria; the ice crystals that form and grow disrupt the cellular and molecular structure of the bacteria. Thawing, being inherently slower, is actually the more damaging part of a freeze-thaw cycle. Once frozen, one-third of the population of some vegetative bacteria might survive a year, whereas other species might have very few survivors after this time. Many eukaryotic parasites, such as the roundworms that cause human trichinosis, are killed by several days of freezing temperatures. Some important temperatures associated with microorganisms and food spoilage are shown in Figure 6.2 (page 161).

HIGH PRESSURE

High pressure applied to liquid suspensions is transferred instantly and evenly throughout the sample. If the pressure is high enough, the molecular structures of proteins and carbohydrates are altered, resulting in the rapid inactivation of vegetative bacterial cells. Endospores are relatively resistant to high pressure. They can, however, be killed by other techniques, such as combining high pressure with elevated temperatures or by alternating pressure cycles that cause spore germination, followed by pressure-caused death of the resulting vegetative cells. Fruit juices preserved by high-pressure treatments have been marketed in Japan and the United States. An advantage is that these treatments preserve the flavors, colors, and nutrient values of the products.

DESICCATION

In the absence of water, a condition that is known as **desiccation,** microorganisms cannot grow or reproduce but can remain viable for years. Then, when water is made available to them, they can resume their growth and division. This ability is used in the laboratory when microbes are preserved by lyophilization, or freeze-drying, a process described in Chapter 6 (page 174). Certain foods are also freeze-dried (for example, coffee and some fruit additives for dry cereals).

The resistance of vegetative cells to desiccation varies with the species and the organism's environment. For example, the gonorrhea bacterium can withstand dryness for only about an hour, but the tuberculosis bacterium can remain viable for months. Viruses are generally resistant to desiccation, but they are not as resistant as bacterial endospores, some of which have survived for centuries. This ability of certain dried microbes and endospores to remain viable is important in a hospital setting. Dust, clothing, bedding, and dressings might contain infectious microbes in dried mucus, urine, pus, and feces.

OSMOTIC PRESSURE

The use of high concentrations of salts and sugars to preserve food is based on the effects of *osmotic pressure*. High concentrations of these substances create a hypertonic environment that causes water to leave the microbial cell (see Figure 6.4, (page 163). This process resembles preservation by desiccation, in that both methods deny the cell the moisture it needs for growth. The principle of osmotic pressure is used in the preservation of foods. For example, concentrated salt solutions are used to cure meats, and thick sugar solutions are used to preserve fruits.

As a general rule, molds and yeasts are much more capable than bacteria of growing in materials with low moisture or high osmotic pressures. This property of molds, sometimes combined with their ability to grow under acidic conditions, is the reason fruits and grains are spoiled by molds rather than by bacteria. It is also part of the reason molds are able to form mildew on a damp wall or a shower curtain.

RADIATION

Radiation has various effects on cells, depending on its wavelength, intensity, and duration. Radiation that kills microorganisms (sterilizing radiation) is of two types: ionizing and nonionizing.

Ionizing radiation—gamma rays, X rays, or high-energy electron beams—has a wavelength shorter than that of nonionizing radiation, less than about 1 nm. Therefore, it carries much more energy (Figure 7.5). *Gamma rays* are emitted by certain radioactive elements such as cobalt, and electron beams are produced by accelerating electrons to high energies in special machines. *X rays*, which are produced by machines in a manner similar to the production of electron beams, are similar in nature to gamma rays. Gamma rays penetrate deeply but may require hours to sterilize large masses; *high-energy electron beams* have much lower penetrating power but usually require only a few seconds of exposure. The principal effect of ionizing radiation is the ionization of water, which forms highly reactive hydroxyl radicals (see the discussion of toxic forms of oxygen in Chapter 6, pages 166–167). These radicals react with organic cellular components, especially DNA.

The so-called target theory of damage by radiation supposes that ionizing particles, or packets of energy, pass through or close to vital portions of the cell; these constitute "hits." One, or a few, hits may only cause nonlethal mutations, some of them conceivably useful. More hits are likely to cause sufficient mutations to kill the microbe.

The food industry has recently renewed its interest in the use of radiation for food preservation (discussed more fully in Chapter 28). Low-level ionizing radiation, used for years in many countries, has been approved in the United

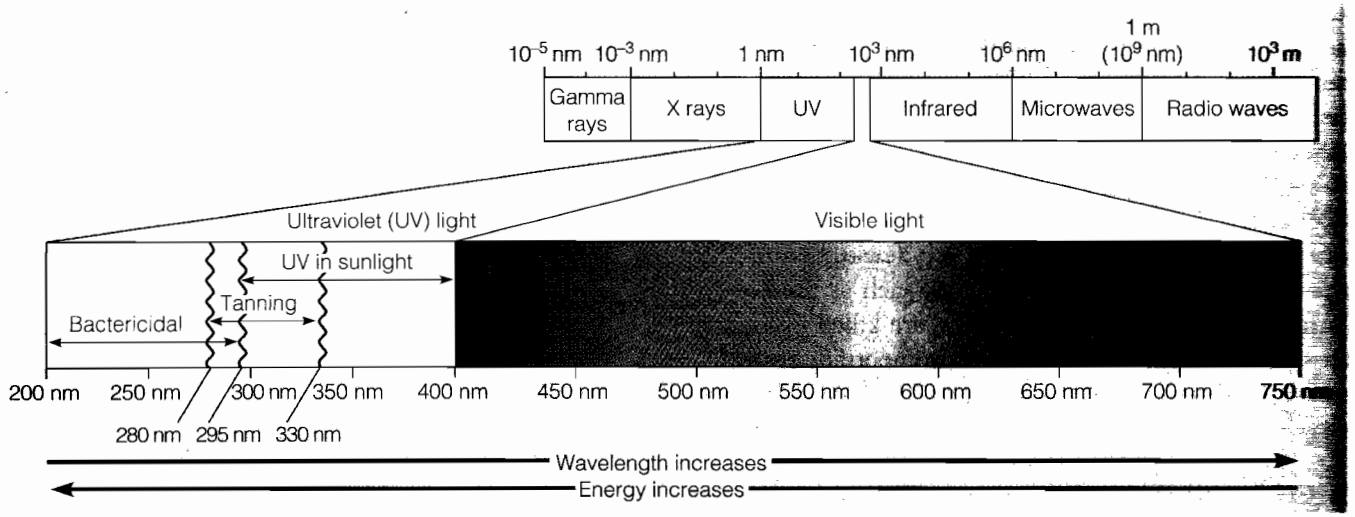


FIGURE 7.5 The radiant energy spectrum. Visible light and other forms of radiant energy radiate through space as waves of various lengths. Ionizing radiation, such as gamma rays and X rays, has a wavelength shorter than 1 nm. Nonionizing radiation, such as ultraviolet (UV) light, has a wavelength between 1 nm and about 380 nm, where the visible spectrum begins.

What effect might increased UV radiation (due to decrease in the ozone layer) have on the Earth's ecosystems?

States for processing spices and certain meats and vegetables. Ionizing radiation, especially high-energy electron beams, is used for the sterilization of pharmaceuticals and disposable dental and medical supplies, such as plastic syringes, surgical gloves, suturing materials, and catheters. As a protection against bioterrorism, the postal service often uses electron beam radiation to sterilize certain classes of mail.

Nonionizing radiation has a wavelength longer than that of ionizing radiation, usually greater than about 1 nm. The best example of nonionizing radiation is ultraviolet (UV) light. UV light damages the DNA of exposed cells by causing bonds to form between adjacent pyrimidine bases (page 235), usually thymines, in DNA chains (see Figure 8.20). These *thymine dimers* inhibit correct replication of the DNA during reproduction of the cell. The UV wavelengths most effective for killing microorganisms are about 260 nm; these wavelengths are specifically absorbed by cellular DNA. UV radiation is also used to control microbes in the air. A UV, or "germicidal," lamp is commonly found in hospital rooms, nurseries, operating rooms, and cafeterias. UV light is also used to disinfect vaccines and other medical products. A major disadvantage of UV light as a disinfectant is that the radiation is not very penetrating, so the organisms to be killed must be directly exposed to the rays. Organisms protected by solids and such coverings as paper, glass, and textiles are not affected. Another potential problem is that UV light can damage human eyes, and prolonged exposure can cause burns and skin cancer in humans.

Sunlight contains some UV radiation, but the shorter wavelengths—those most effective against bacteria—are screened out by the ozone layer of the atmosphere. The antimicrobial effect of sunlight is due almost entirely to the formation of singlet oxygen in the cytoplasm (see Chapter 6, page 166). Many pigments produced by bacteria provide protection from sunlight.

Microwaves do not have much direct effect on microorganisms, and bacteria can readily be isolated from the interior of recently operated microwave ovens. Moisture-containing foods are heated by microwave action, and the heat will kill most vegetative pathogens. Solid foods heat unevenly because of the uneven distribution of moisture. For this reason, pork cooked in a microwave oven has been responsible for outbreaks of trichinellosis.

* * *

Table 7.5 summarizes the physical methods of microbial control.

CHEMICAL METHODS OF MICROBIAL CONTROL

Chemical agents are used to control the growth of microbes on both living tissue and inanimate objects. Unfortunately, few chemical agents achieve sterility; most

TABLE 7.5 Physical Methods Used to Control Microbial Growth

Methods	Mechanism of Action	Comment	Preferred Use
Heat			
1. Moist heat			
a. Boiling or flowing steam	Protein denaturation	Kills vegetative bacterial and fungal pathogens and almost all viruses within 10 min; less effective on endospores.	Dishes, basins, pitchers, various equipment
b. Autoclaving	Protein denaturation	Very effective method of sterilization; at about 15 psi of pressure (121°C), all vegetative cells and their endospores are killed in about 15 min.	Microbiological media, solutions, linens, utensils, dressings, equipment, and other items that can withstand temperature and pressure
2. Pasteurization	Protein denaturation	Heat treatment for milk (72°C for about 15 sec) that kills all pathogens and most nonpathogens.	Milk, cream, and certain alcoholic beverages (beer and wine)
3. Dry heat			
a. Direct flaming	Burning contaminants to ashes	Very effective method of sterilization.	Inoculating loops
b. Incineration	Burning to ashes	Very effective method of sterilization.	Paper cups, contaminated dressings, animal carcasses, bags, and wipes
c. Hot-air sterilization	Oxidation	Very effective method of sterilization but requires temperature of 170°C for about 2 hr.	Empty glassware, instruments, needles, and glass syringes
Filtration	Separation of bacteria from suspending liquid	Removes microbes by passage of a liquid or gas through a screen-like material. Most filters in use consist of cellulose acetate or nitrocellulose.	Useful for sterilizing liquids (enzymes, vaccines) that are destroyed by heat
Cold			
1. Refrigeration	Decreased chemical reactions and possible changes in proteins	Has a bacteriostatic effect.	Food, drug, and culture preservation
2. Deep-freezing (see Chapter 6, page 174)	Decreased chemical reactions and possible changes in proteins	An effective method for preserving microbial cultures, in which cultures are quick-frozen between −50° and −95°C.	Food, drug, and culture preservation
3. Lyophilization (see Chapter 6, page 174)	Decreased chemical reactions and possible changes in proteins	Most effective method for longterm preservation of microbial cultures; water removed by high vacuum at low temperature.	Food, drug, and culture preservation
High Pressure	Alteration of molecular structure of proteins and carbohydrates	Preservation of colors, flavors, nutrient values.	Fruit juices
Desiccation	Disruption of metabolism	Involves removing water from microbes; primarily bacteriostatic.	Food preservation
Osmotic Pressure	Plasmolysis	Results in loss of water from microbial cells.	Food preservation
Radiation			
1. Ionizing	Destruction of DNA	Not widespread in routine sterilization.	Used for sterilizing pharmaceuticals and medical and dental supplies
2. Nonionizing	Damage to DNA	Radiation not very penetrating.	Control of closed environment with UV (germicidal) lamp

of them merely reduce microbial populations to safe levels or remove vegetative forms of pathogens from objects. A common problem in disinfection is the selection of an agent. No single disinfectant is appropriate for all circumstances.

PRINCIPLES OF EFFECTIVE DISINFECTION

LEARNING OBJECTIVE

- List the factors related to effective disinfection.

By reading the label, we can learn a great deal about a disinfectant's properties. The label will usually indicate what groups of organisms the disinfectant will be effective against. Remember that the concentration of a disinfectant affects its action, so it should always be diluted exactly as specified by the manufacturer.

Also consider the nature of the material being disinfected. For example, are organic materials present that might interfere with the action of the disinfectant? Similarly, the pH of the medium often has a great effect on a disinfectant's activity.

Another very important consideration is whether the disinfectant will easily make contact with the microbes. An area might need to be scrubbed and rinsed before the disinfectant is applied. In general, disinfection is a gradual process. Thus, to be effective, a disinfectant might need to be left on a surface for several hours.

EVALUATING A DISINFECTANT

LEARNING OBJECTIVE

- Interpret the results of use-dilution tests and the disk-diffusion method.

USE-DILUTION TESTS

There is a need to evaluate the effectiveness of disinfectants and antiseptics. For many years the standard test was the *phenol coefficient test*, which compared the activity of a given disinfectant with that of phenol. However, the current standard is the American Official Analytical Chemist's **use-dilution test.** For most purposes, the three bacteria used in this test are *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Metal carrier rings are dipped into standardized cultures of the test bacteria grown in liquid media, removed, and dried at 37°C for a short time. The dried cultures are then placed into a solution of the disinfectant at the concentration recommended by the manufacturer and left there for 10 minutes at 20°C. Following this exposure, the carrier rings are transferred to a medium that will permit the growth of any surviving bacteria. The effectiveness of the disinfectant can then be determined by the number of cultures that grow.

Variations of this method are used for testing the effectiveness of antimicrobial agents against endospores, mycobacteria that cause tuberculosis, and fungi, because they are difficult to control with chemicals. Also, tests of antimicrobials intended for special purposes, such as dairy utensil disinfection, may substitute other test bacteria. Virucidal chemicals are usually tested against cultures of Newcastle disease virus (a disease of birds and domestic fowl). After exposure to the chemical, the cultures are injected into embryonated chicken eggs; if any viruses survive, they will kill the embryos.

THE DISK-DIFFUSION METHOD

The **disk-diffusion method** is used in teaching laboratories to evaluate the efficacy of a chemical agent. A disk of filter paper is soaked with a chemical and placed on an agar plate that has been previously inoculated and incubated with the test organism. After incubation, if the chemical is effective, a clear zone representing inhibition of growth can be seen around the disk (Figure 7.6).

Disks containing antibiotics are commercially available and used to determine microbial susceptibility to antibiotics (see Figure 20.17, page 601).

TYPES OF DISINFECTANTS

LEARNING OBJECTIVES

- Identify the methods of action and preferred uses of chemical disinfectants.
- Differentiate between halogens used as antiseptics and as disinfectants.
- Identify the appropriate uses for surface-active agents.
- List the advantages of glutaraldehyde over other chemical disinfectants.
- Identify the method of sterilizing plastic labware.

PHENOL AND PHENOLICS

Lister was the first to use **phenol** (carbolic acid) to control surgical infections in the operating room. Its use had been suggested by its effectiveness in controlling odor in sewage. It is now rarely used as an antiseptic or disinfectant because it irritates the skin and has a disagreeable odor. It is often used in throat lozenges for its local anesthetic effect but has little antimicrobial effect at the low concentrations used. At concentrations above 1% (such as in some throat sprays), however, phenol has a significant

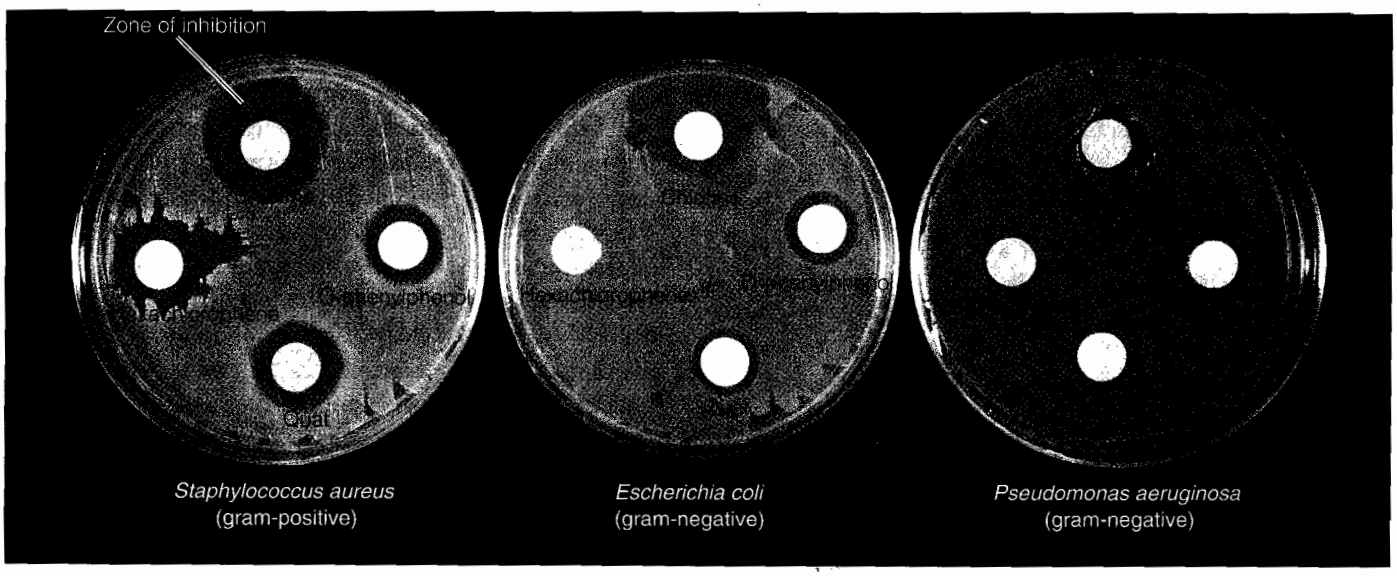


FIGURE 7.6 Evaluation of disinfectants by the disk-diffusion method. In this experiment, paper disks are soaked in a solution of disinfectant and placed on the surface of a nutrient medium on which a culture of test bacteria has been spread to produce uniform growth.

At the top of each plate, the tests show that chlorine (as sodium hypochlorite) was effective against all the test bacteria but was more effective against gram-positive bacteria.

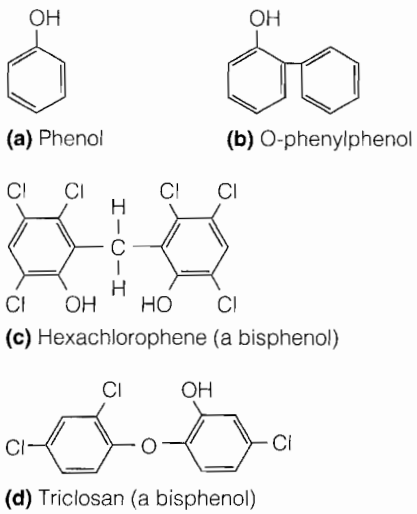
At the bottom row of each plate, the tests show that the quaternary ammonium compound ("quat") was also more effective against the gram-positive bacteria, but it did not affect the pseudomonads at all.

At the left side of each plate, the tests show that hexachlorophene was effective against gram-positive bacteria only.

At the right sides, O-phenylphenol was ineffective against pseudomonads but was almost equally effective against the gram-positive bacteria and the gram-negative bacteria.

All four chemicals worked against the gram-positive test bacteria, but only one of the four chemicals affected pseudomonads.

Which group of bacteria is most resistant to the disinfectants tested?.



(a) Phenol **(b)** O-phenylphenol

(c) Hexachlorophene (a bisphenol)

(d) Triclosan (a bisphenol)

FIGURE 7.7 The structure of phenolics and bisphenols.

Some lozenges intended to alleviate the symptoms of a sore throat contain phenol. Why include this ingredient?

antibacterial effect. The structure of a phenol molecule is shown in Figure 7.7a.

Derivatives of phenol, called **phenolics,** contain a molecule of phenol that has been chemically altered to reduce its irritating qualities or increase its antibacterial activity in combination with a soap or detergent. Phenolics exert antimicrobial activity by injuring lipid-containing plasma membranes, which results in leakage of cellular contents. The cell wall of mycobacteria, the causes of tuberculosis and leprosy, are rich in lipids, which make them susceptible to phenol derivatives. A useful property of phenolics as disinfectants is that they remain active in the presence of organic compounds, they are stable, and they persist for long periods after application. For these reasons, phenolics are suitable agents for disinfecting pus, saliva, and feces.

One of the most frequently used phenolics is derived from coal tar, a group of chemicals called *cresols*. A very important cresol is *O-phenylphenol* (see Figures 7.6 and 7.7b), the main ingredient in most formulations of Lysol. Cresols are very good surface disinfectants.

BISPHENOLS

Bisphenols are derivatives of phenol that contain two phenolic groups connected by a bridge (*bis* indicates two). One bisphenol, *hexachlorophene* (Figures 7.6 and 7.7c), is an ingredient of a prescription lotion, pHisoHex, used for surgical and hospital microbial control procedures. Gram-positive staphylococci and streptococci, which can cause skin infections in newborns, are particularly susceptible to hexachlorophene, so it is often used to control such infections in nurseries. However, excessive use of this bisphenol, such as bathing infants with it several times a day, can lead to neurological damage.

Another widely used bisphenol is *triclosan* (Figure 7.7d), an ingredient in antibacterial soaps and at least one toothpaste. Triclosan has even been incorporated into kitchen cutting boards and the handles of knives and other plastic kitchenware. Its use is now so widespread that resistant bacteria have been reported, and concerns about its effect on microbes' resistance to certain antibiotics have been raised. Triclosan inhibits an enzyme needed for the biosynthesis of fatty acids (lipids), which mainly affects the integrity of the plasma membrane. It is especially effective against gram-positive bacteria but also works well against fungi and gram-negative bacteria. There are certain exceptions, such as *Pseudomonas aeruginosa*, a gram-negative bacterium that is very resistant to triclosan, as well as to many other antibiotics and disinfectants (see the discussion on pages 321, 436, and 622).

BIGUANIDES

Chlorhexidine is a member of the **biguanide** group with a broad spectrum of activity. It is frequently used for microbial control on skin and mucous membranes. Combined with a detergent or alcohol, chlorhexidine is also used for surgical hand scrubs and preoperative skin preparation in patients. A new product, Avagard, which combines chlorhexidine and ethanol, is persistent for about six hours. It is approved by the FDA as a waterless, scrubless presurgical antiseptic. In such applications, chlorhexidine's strong affinity for binding to the skin or mucous membranes is an advantage, as is its low toxicity. However, contact with the eyes can cause damage. Its killing effect is related to the injury it causes to the plasma membrane by blocking an enzyme needed for lipid synthesis. It is biocidal against most vegetative bacteria and yeasts. Mycobacteria are relatively resistant, and endospores and protozoan cysts are not affected. The only viruses affected are certain enveloped (lipophilic) types (see Chapter 13).

HALOGENS

The **halogens,** particularly iodine and chlorine, are effective antimicrobial agents, both alone and as constituents of inorganic or organic compounds. *Iodine* (I_2) is one of the oldest and most effective antiseptics. It is effective against all kinds of bacteria, many endospores, various fungi, and some viruses. Iodine impairs protein synthesis and alters cell membranes, apparently by forming complexes with amino acids and unsaturated fatty acids.

Iodine is available as a **tincture**—that is, in solution in aqueous alcohol—and as an iodophor. An **iodophor** is a combination of iodine and an organic molecule, from which the iodine is released slowly. Iodophors have the antimicrobial activity of iodine, but they do not stain and are less irritating. The most common commercial preparations is Betadine, which is a *povidone-iodine*. Povidone is a surface-active iodophor that improves the wetting action and serves as a reservoir of free iodine. Iodines are used mainly for skin disinfection and wound treatment. Many campers are familiar with iodine for water treatment. To treat water, iodine tablets are added or the water can be passed through iodine-treated resin filters.

Chlorine (Cl_2), as a gas or in combination with other chemicals, is another widely used disinfectant. Its germicidal action is caused by the hypochlorous acid (HOCl) that forms when chlorine is added to water:

(1)
$$\underset{\text{Chlorine}}{Cl_2} + \underset{\text{Water}}{H_2O} \rightleftharpoons \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Chloride ion}}{Cl^-} + \underset{\text{Hypochlorous acid}}{HOCl}$$

(2)
$$\underset{\text{Hypochlorous acid}}{HOCl} \rightleftharpoons \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Hypochlorite ion}}{OCl^-}$$

Exactly how hypochlorous acid exerts its killing power is not known. It is a strong oxidizing agent that prevents much of the cellular enzyme system from functioning. Hypochlorous acid is the most effective form of chlorine because it is neutral in electrical charge and diffuses as rapidly as water through the cell wall. Because of its negative charge, the hypochlorite ion (OCl^-) cannot enter the cell freely.

A liquid form of compressed chlorine gas is used extensively for disinfecting municipal drinking water, water in swimming pools, and sewage. Several compounds of chlorine are also effective disinfectants. For example, solutions of *calcium hypochlorite* [$Ca(OCl)_2$] are used to disinfect dairy equipment and restaurant eating utensils. This compound, once called chloride of lime, was used as early as 1825, long before the concept of a germ theory for disease, to soak hospital dressings in Paris hospitals. It was also the disinfectant used in the 1840s by Semmelweis to control hospital infections during childbirth, as mentioned in Chapter 1, page 11. Another chlorine compound, *sodium hypochlorite* (NaOCl; see Figure 7.6), is used as a household disinfectant and

bleach (Clorox) and as a disinfectant in dairies, food-processing establishments, and hemodialysis systems. When the quality of drinking water is in question, household bleach can provide a rough equivalent of municipal chlorination. After two drops of bleach are added to a liter of water (four drops if the water is cloudy) and the mixture has sat for 30 minutes, the water is considered safe for drinking under emergency conditions. U.S. military forces in the field are issued tablets (Chlor-Floc) that contain *sodium dichloroisocyanurate*, a form of chlorine combined with an agent that flocculates (coagulates) suspended materials in a water sample, causing them to settle out, thus clarifying it.

Chlorine dioxide (ClO_2) is a gaseous form of chlorine, occasionally used for area disinfection, most notably, to kill endospores of the anthrax bacterium.

Another group of chlorine compounds, the *chloramines*, consist of chlorine and ammonia. They are used as disinfectants, antiseptics, or sanitizing agents. Chloramines are very stable compounds that release chlorine over long periods. They are relatively effective in organic matter, but they have the disadvantages of acting more slowly and being less effective purifiers than many other chlorine compounds. Chloramines are used to sanitize glassware and eating utensils and to treat dairy and food-manufacturing equipment. Ammonia is usually mixed with chlorine in municipal water-treatment systems to form chloramines. The chloramines control taste and odor problems caused by the reaction of chlorine with other nitrogenous compounds in the water. Because chloramines are less effective as germicides, sufficient chlorine must be added to ensure a residual of chlorine in the form of HOCl. (Chloramines are toxic to aquarium fish, but pet shops sell chemicals to neutralize them.)

ALCOHOLS

Alcohols effectively kill bacteria and fungi but not endospores and nonenveloped viruses. The mechanism of action of alcohol is usually protein denaturation, but alcohol can also disrupt membranes and dissolve many lipids, including the lipid component of enveloped viruses. Alcohols have the advantage of acting and then evaporating rapidly and leaving no residue. When the skin is swabbed (degermed) before an injection, most of the microbial control activity comes from simply wiping away dirt and microorganisms, along with skin oils. However, alcohols are unsatisfactory antiseptics when applied to wounds. They cause coagulation of a layer of protein under which bacteria continue to grow.

Two of the most commonly used alcohols are ethanol and isopropanol. The recommended optimum concentration of *ethanol* is 70%, but concentrations between 60% and 95% seem to kill as well (Table 7.6). Pure ethanol is less effective than aqueous solutions (ethanol mixed with water) because denaturation requires water. *Isopropanol*, often sold as rubbing alcohol, is slightly superior to ethanol as an antiseptic and disinfectant. Moreover, it is less volatile, less expensive, and more easily obtained than ethanol.

TABLE 7.6 **Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution Against *Streptococcus pyogenes***

	Time (sec)				
Concentration of Ethanol (%)	10	20	30	40	50
100	−	−	−	−	−
95	+	+	+	+	+
90	+	+	+	+	+
80	+	+	+	+	+
70	+	+	+	+	+
60	+	+	+	+	+
50	−	−	+	+	+
40	−	−	−	−	−

NOTE: A minus sign indicates no biocidal action (bacterial growth); a plus sign indicates biocidal action (no bacterial growth). The lighter area represents bacteria killed by biocidal action.

Ethanol and isopropanol are often used to enhance the effectiveness of other chemical agents. For example, an aqueous solution of Zephiran (described on page 203) kills about 40% of the population of a test organism in two minutes, whereas a tincture of Zephiran kills about 85% in the same period. To compare the effectiveness of tinctures and aqueous solutions, see Figure 7.10 on page 204.

HEAVY METALS AND THEIR COMPOUNDS

Several heavy metals can be biocidal or antiseptic, including silver, mercury, and copper. The ability of very small amounts of heavy metals, especially silver and copper, to exert antimicrobial activity is referred to as **oligodynamic action** (*oligo* means few). This action can be seen when we place a coin or other clean piece of metal containing silver or copper on a culture on an inoculated Petri plate. Extremely small amounts of metal diffuse from the coin and inhibit the growth of bacteria for some distance around the coin (Figure 7.8). This effect is produced by the action of heavy metal ions on microbes. When the metal ions combine with the sulfhydryl groups on cellular proteins, denaturation results.

Silver is used as an antiseptic in a 1% *silver nitrate* solution. At one time, many states required that the eyes of

FIGURE 7.8 Oligodynamic action of heavy metals. Clear zones where bacterial growth has been inhibited are seen around the sombrero charm (pushed aside), the dime, and the penny. The charm and the dime contain silver; the penny contains copper.

Q The coins used in this demonstration were minted many years ago; why were current coins not used?

newborns be treated with a few drops of silver nitrate to guard against an infection of the eyes called gonorrheal ophthalmia neonatorum, which the infants might have contracted as they passed through the birth canal. In recent years, antibiotics have replaced silver nitrate for this purpose.

Recently, there has been renewed interest in silver as an antimicrobial agent. Silver-impregnated dressings that slowly release silver ions have proven especially useful when antibiotic-resistant bacteria are a problem. A combination of silver and the drug sulfadiazine, *silver-sulfadiazine*, is the most common formulation. It is available as a topical cream for use on burns. Silver can also be incorporated into indwelling catheters, which are a common source of hospital infections, and in wound dressings. *Surfacine* is a relatively new antimicrobial for application to surfaces, either animate or inanimate. It contains water-insoluble silver iodide in a polymer carrier and is very persistent, lasting at least 13 days. When a bacterium contacts the surface, the cell's outer membrane is recognized, and a lethal amount of silver ions is released.

Inorganic mercury compounds, such as *mercuric chloride*, have a long history of use as disinfectants. They have a very broad spectrum of activity; their effect is primarily bacteriostatic. However, their use is now limited because of their toxicity, corrosiveness, and ineffectiveness in organic matter. At present, the primary use of mercurials is to control mildew in paints.

Copper in the form of *copper sulfate* or other copper-containing additives is used chiefly to destroy green algae (algicide) that grow in reservoirs, stock ponds, swimming pools, and fish tanks. If the water does not contain excessive organic matter, copper compounds are effective in concentrations of one part per million of water. To prevent mildew, copper compounds such as copper 8-hydroxyquinoline are sometimes included in paint.

Another metal used as an antimicrobial is zinc. The effect of trace amounts of zinc can be seen on weathered roofs of buildings down-slope from galvanized (zinc-coated) fittings. The roof is lighter-colored where biological growth is impeded. Copper- and zinc-treated shingles are available. *Zinc chloride* is a common ingredient in mouthwashes, and *zinc oxide* is probably the most widely used antifungal agent in paints, mainly because it is often part of the pigment formulation.

SURFACE-ACTIVE AGENTS

Surface-active agents, or **surfactants,** can decrease surface tension among molecules of a liquid. Such agents include soaps and detergents.

Soaps and Detergents Soap has little value as an antiseptic, but it does have an important function in the mechanical removal of microbes through scrubbing. The skin normally contains dead cells, dust, dried sweat, microbes, and oily secretions from oil glands. Soap breaks the oily film into tiny droplets, a process called *emulsification*, and the water and soap together lift up the emulsified oil and debris and float them away as the lather is washed off. In this sense, soaps are good degerming agents.

Acid-Anionic Sanitizers *Acid-anionic* surface-active sanitizers are very important in the cleaning of dairy utensils and equipment. Their sanitizing ability is related to the negatively charged portion (anion) of the molecule, which reacts with the plasma membrane. These sanitizers, which act on a wide spectrum of microbes, including troublesome thermoduric bacteria, are nontoxic, noncorrosive, and fast acting.

Quaternary Ammonium Compounds (Quats) The most widely used surface-active agents are the cationic detergents, especially the **quaternary ammonium compounds (quats).** Their cleansing ability is related to the positively charged portion—the cation—of the molecule. Their name is derived from the fact that they are modifications of the four-valence ammonium ion, NH_4^+ (Figure 7.9). Quaternary ammonium compounds are strongly bactericidal against gram-positive bacteria and less active against gram-negative bacteria (see Figure 7.6).

CLINICAL PROBLEM SOLVING

A HOSPITAL-ACQUIRED INFECTION FOLLOWING LIPOSUCTION

As you read through this box you will encounter a series of questions that clinicians ask themselves as they formulate a diagnosis and treatment. Try to answer each question before going on to the next one.

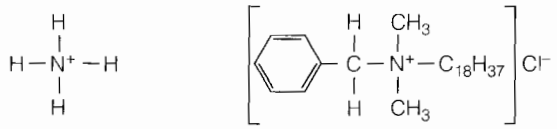
1. During a 17-month period, nine patients in eight hospitals acquired surgical-site infections within 2 months after liposuction. The patients' symptoms included fever, local inflammation, and infected surgical wounds.
   What would you do next?
2. Gram stains and acid-fast stains of pus showed gram-positive, acid-fast rods.
   What are possible organisms?
3. Slow-growing mycobacteria, including *M. tuberculosis* and *M. leprae*, are human pathogens. These infections, however, were caused by rapidly growing mycobacteria (RGM): *Mycobacterium chelonae, M. fortuitum,* and *M. abscessus.*
   Where are other species of mycobacteria normally found?
4. RGM are found in soil and water. The liposuction procedure involves making a small surgical wound and using a cannula (needle) for fat suctioning. The cannulae were cleaned with tap water and soap followed by a quat.
   What was wrong with this procedure?
5. RGM can be found in tap water and soap used to remove dust and microbes. Quats do have some disinfecting properties but are considered low-level disinfectants because they are ineffective against endospores and mycobacteria.
   Why are mycobacteria resistant to some disinfectants?
6. The lipid-rich cell wall prevents entry of most biocides into the cells.
   How could you find the source of the RGM?
7. Nosocomial infections associated with contaminated quats that had been used to disinfect patient-care supplies or equipment have been reported. Nosocomial infections have also been associated with RGM growing in a hospital's hot water system. In this case, cultures of the quat and environmental cultures did not yield bacteria or mycobacteria.
   What changes in procedures would you recommend?
8. Surgical instruments used in liposuction are intended to enter normally sterile tissue and should be sterilized between patient procedures. The hospitals modified their procedures by replacing the quat with either a high-level disinfectant using 2% glutaraldehyde or ethylene oxide gas sterilization.

SOURCE: Adapted from *MMWR* 47(49): 1065–1067 (12/18/98).

Quats are also fungicidal, amoebicidal, and virucidal against enveloped viruses. They do not kill endospores or mycobacteria. (See the box above.) Their chemical mode of action is unknown, but they probably affect the plasma membrane. They change the cell's permeability and cause the loss of essential cytoplasmic constituents, such as potassium.

Two popular quats are Zephiran, a brand name of *benzalkonium chloride* (see Figure 7.9), and Cepacol, a brand name of *cetylpyridinium chloride*. They are strongly antimicrobial, colorless, odorless, tasteless, stable, easily diluted, and nontoxic, except at high concentrations. If your mouthwash bottle fills with foam when shaken, the mouthwash probably contains a quat. However, organic matter interferes with their activity, and they are rapidly neutralized by soaps and anionic detergents.

Anyone associated with medical applications of quats should remember that certain bacteria, such as some species of *Pseudomonas*, not only survive in quaternary ammonium compounds but actively grow in them. This resistance occurs not only to the disinfectant solution but also to moistened gauze and bandages, whose fibers tend to neutralize the quats. Cetylpyridinium chloride, another quat, has recently received FDA (U.S. Food and Drug Administration) approval as an antimicrobial wash during poultry processing.



Ammonium ion Benzalkonium chloride

FIGURE 7.9 The ammonium ion and a quaternary ammonium compound, benzalkonium chloride (Zephiran). Notice how other groups replace the hydrogens of the ammonium ion.

Q **Are quats most effective against gram-positive or gram-negative bacteria?**

Before we move on to the next group of chemical agents, refer to Figure 7.10, which compares the effectiveness of some of the antiseptics we have discussed so far.

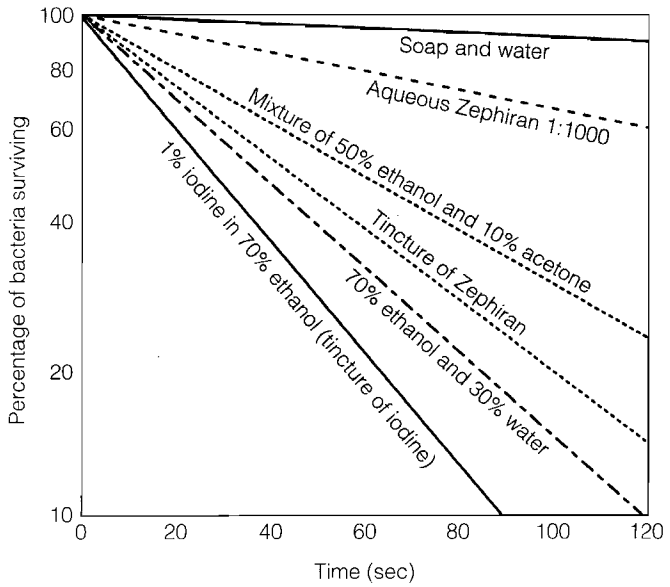


FIGURE 7.10 A comparison of the effectiveness of various antiseptics. The steeper the downward slope of the killing curve of the antiseptic, the more effective it is. A 1% iodine in 70% ethanol solution is the most effective; soap and water are the least effective. Notice that a tincture of Zephiran is more effective than an aqueous solution of the same antiseptic.

Why is the tincture of Zephiran more effective than the aqueous solution?

CHEMICAL FOOD PRESERVATIVES

Chemical preservatives are frequently added to foods to retard spoilage. *Sulfur dioxide* (SO_2) has long been used as a disinfectant, especially in wine-making. Homer's *Odyssey*, written nearly 2800 years ago, mentions its use. Among the more common additives are sodium benzoate, sorbic acid, and calcium propionate. These chemicals are simple organic acids, or salts of organic acids, which the body readily metabolizes and which are generally judged to be safe in foods. *Sorbic acid*, or its more soluble salt *potassium sorbate*, and *sodium benzoate* prevent molds from growing in certain acidic foods, such as cheese and soft drinks. Such foods, usually with a pH of 5.5 or lower, are most susceptible to mold-type spoilage. *Calcium propionate*, an effective fungistat used in bread, prevents the growth of surface molds and the *Bacillus* bacterium that causes ropy bread. These organic acids inhibit mold growth, not by affecting the pH but by interfering with the mold's metabolism or the integrity of the plasma membrane.

Sodium nitrate and *sodium nitrite* are added to many meat products, such as ham, bacon, hot dogs, and sausage. The active ingredient is sodium nitrite, which certain bacteria in the meats can also produce from sodium nitrate. These bacteria use nitrate as a substitute for oxygen under anaerobic conditions. The nitrite has two main functions: to preserve the pleasing red color of the meat by reacting with blood components in the meat, and to prevent the germination and growth of any botulism endospores that might be present. Nitrite selectively inhibits certain iron-containing enzymes of *Clostridium botulinum*. There has been some concern that the reaction of nitrites with amino acids can form certain carcinogenic products known as **nitrosamines,** and the amount of nitrites added to foods has generally been reduced recently for this reason. However, the use of nitrites continues because of their established value in preventing botulism. Because nitrosamines are formed in the body from other sources, the added risk posed by a limited use of nitrates and nitrites in meats is lower than was once thought.

ANTIBIOTICS

The antimicrobials discussed in this chapter are not useful for ingestion or injection to treat disease. Antibiotics are used for this purpose. The use of antibiotics is highly restricted; however, at least two have considerable use in food preservation. Neither is of value for clinical purposes. *Nisin*, which is often added to cheese to inhibit the growth of certain endospore-forming spoilage bacteria, is an example of a bacteriocin, a protein that is produced by one bacterium and inhibits another (see Chapter 8, page 246). Nisin is present naturally in small amounts in many dairy products. It is tasteless, readily digested, and nontoxic. *Natamycin* (pimaricin) is an antifungal antibiotic approved for use in foods, mostly cheese.

ALDEHYDES

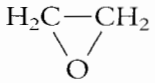
Aldehydes are among the most effective antimicrobials. Two examples are formaldehyde and glutaraldehyde. They inactivate proteins by forming covalent cross-links with several organic functional groups on proteins (—NH_2, —OH, —COOH, and —SH). *Formaldehyde gas* is an excellent disinfectant. However, it is more commonly available as *formalin*, a 37% aqueous solution of formaldehyde gas. Formalin was once used extensively to preserve biological specimens and inactivate bacteria and viruses in vaccines.

Glutaraldehyde is a chemical relative of formaldehyde that is less irritating and more effective than formaldehyde. Glutaraldehyde is used to disinfect hospital instruments, including respiratory-therapy equipment. When used in a 2% solution (Cidex), it is bactericidal, tuberculocidal, and virucidal in 10 minutes and sporicidal in 3 to 10 hours. Glutaraldehyde is one of the few liquid chemical disinfectants that can be considered a sterilizing agent. However, 30 minutes is often considered the maximum time allowed for a sporicide to act, which is a criterion glutaraldehyde cannot meet. Both glutaraldehyde and formalin are used by morticians for embalming.

A possible replacement for glutaraldehyde for many uses is *ortho-phthalaldehyde* (OPA), which is more effective against many microbes and has fewer irritating properties.

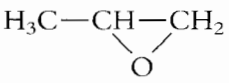
GASEOUS CHEMOSTERILIZERS

Gaseous chemosterilizers are chemicals that sterilize in a closed chamber (similar to an autoclave). A gas suitable for this method is *ethylene oxide:*

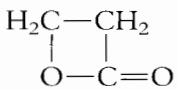


Its activity depends on the denaturation of proteins: the proteins' labile hydrogens, such as —SH, —COOH, or —OH, are replaced by alkyl groups *(alkylation)*, such as $—CH_2CH_2OH$. Ethylene oxide kills all microbes and endospores but requires a lengthy exposure period of 4 to 15 hours. It is toxic and explosive in its pure form, so it is usually mixed with a nonflammable gas, such as carbon dioxide or nitrogen. One of its advantages is that it is highly penetrating, so much so that ethylene oxide was chosen to sterilize spacecraft sent to land on the moon and Mars. Using heat to sterilize the electronic gear on these vehicles was not practical.

Because of their ability to sterilize without heat, gases like ethylene oxide are also widely used on medical supplies and equipment. Many large hospitals have ethylene oxide chambers, some large enough to sterilize mattresses, as part of their sterilizing equipment. Propylene oxide and β-propiolactone are also used for gaseous sterilization:



Propylene oxide



β-propiolactone

A disadvantage of all these gases is that they are suspected carcinogens, especially β-propiolactone. For this reason, there has been concern about the exposure of hospital workers to ethylene oxide from such sterilizers. Because of this hazard, these gases may eventually be replaced by *plasma gas sterilization*. This makes use of vapors of hydrogen peroxide (discussed shortly) subjected to radio frequencies or microwave radiation to produce reactive free radicals. No by-products toxic to humans are produced, and it is an effective sterilant.

PEROXYGENS (OXIDIZING AGENTS)

Peroxygens exert antimicrobial activity by oxidizing cellular components of the treated microbes. Examples are ozone, hydrogen peroxide, and peracetic acid. *Ozone* (O_3) is a highly reactive form of oxygen that is generated by passing oxygen through high-voltage electrical discharges. It is responsible for the air's rather fresh odor after a lightning storm, in the vicinity of electrical sparking, or around an ultraviolet light. Ozone is often used to supplement chlorine in the disinfection of water because it helps neutralize tastes and odors. Although ozone is a more effective killing agent, its residual activity is difficult to maintain in water, and it is more expensive than chlorine.

Hydrogen peroxide is an antiseptic found in many household medicine cabinets and in hospital supply rooms. It is not a good antiseptic for open wounds; in fact, it may slow wound healing. It is quickly broken down to water and gaseous oxygen by the action of the enzyme catalase, which is present in human cells (see Chapter 6, page 167). However, hydrogen peroxide does effectively disinfect inanimate objects, an application in which it is even sporicidal, especially at elevated temperatures. On a nonliving surface, the normally protective enzymes of aerobic bacteria and facultative anaerobes are overwhelmed by the high concentrations of peroxide used. Because of these factors, the food industry is increasing its use of hydrogen peroxide for aseptic packaging (see Chapter 28). The packaging materials pass through a hot solution of the chemical before being assembled into a container. In addition, many wearers of contact lenses are familiar with disinfection by hydrogen peroxide. After disinfection, a platinum catalyst in the lens-disinfecting kit destroys residual hydrogen peroxide so that it does not persist on the lens, where it might be an irritant.

Oxidizing agents are useful for irrigating deep wounds, where the oxygen released makes an environment that inhibits the growth of anaerobic bacteria.

Benzoyl peroxide is another compound useful for treating wounds infected by anaerobic pathogens, but it is probably more familiar as the main ingredient in over-the-counter medications for acne, which is caused by a type of anaerobic bacterium infecting hair follicles.

Peracetic acid is one of the most effective liquid chemical sporicides available and is considered a sterilant. It is generally effective on endospores and viruses within 30 minutes, and kills vegetative bacteria and fungi in less than 5 minutes. Peracetic acid has many applications in the disinfection of food-processing and medical equipment because it leaves no toxic residues and is minimally affected by the presence of organic matter.

MICROBIAL CHARACTERISTICS AND MICROBIAL CONTROL

LEARNING OBJECTIVE

- Explain how the control of microbial control is affected by the type of microbe.

Many biocides tend to be more effective against gram-positive bacteria, as a group, than against gram-negative bacteria. This is illustrated in Figure 7.11, which presents a simplified hierarchy of relative resistance of major microbial

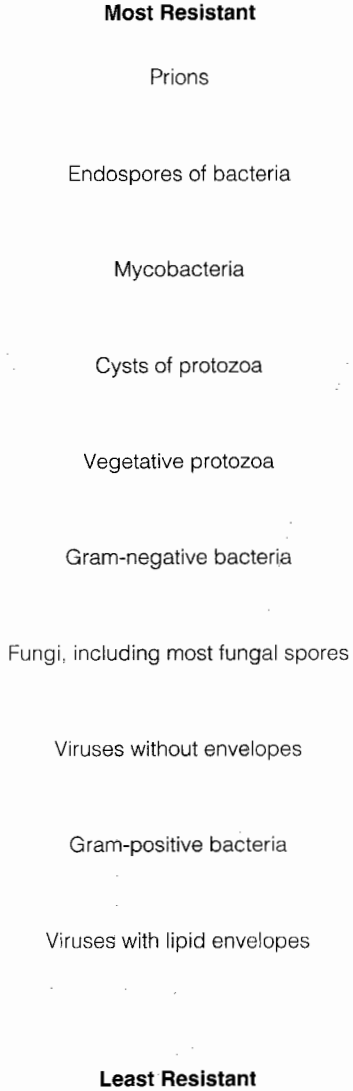


FIGURE 7.11 Decreasing order of resistance of microorganisms to chemical biocides.

Q Why are viruses with lipid-containing envelopes relatively susceptible to certain biocides?

groups to biocides. A principal factor in this relative resistance to biocides is the external lipopolysaccharide layer of gram-negative bacteria. Within gram-negative bacteria, members of the genera *Pseudomonas* and *Burkholderia* are of special interest. These closely related bacteria are unusually resistant to biocides (see Figure 7.6) and will even grow actively in some disinfectants and antiseptics, most notably the quaternary ammonium compounds. In Chapter 20, you will see that these bacteria are also resistant to many antibiotics. This resistance to chemical antimicrobials is mostly related to the characteristics of their *porins* (structural openings in the wall of gram-negative bacteria; see Figure 4.13c, page 86). Porins are highly selective of molecules that they permit to enter the cell.

TABLE 7.7 The Effectiveness of Chemical Antimicrobials Against Endospores and Mycobaceria

Chemical Agent	Endospores	Mycobacteria
Mercury	No activity	No activity
Phenolics	Poor	Good
Bisphenols	No activity	No activity
Quaternary ammonium compounds	No activity	No activity
Chlorines	Fair	Fair
Iodine	Poor	Good
Alcohols	Poor	Good
Glutaraldehyde	Fair	Good
Chlorhexidine	No activity	Fair

The mycobacteria are another group of non–endospore-forming bacteria that exhibit greater than normal resistance to chemical biocides. (See the box on page 203.) This group includes *Mycobacterium tuberculosis*, the pathogen that causes tuberculosis. The cell wall of this organism and other members of this genus have a waxy, lipid-rich component. Instruction labels on disinfectants often state whether they are tuberculocidal, indicating if they are effective against mycobacteria. Special tuberculocidal tests have been developed to evaluate the effectiveness of biocides against this bacterial group.

Bacterial endospores are affected by relatively few biocides. (The activity of the major chemical antimicrobial groups against mycobacteria and endospores is summarized in Table 7.7.) The cysts and oocysts of protozoa are also relatively resistant to chemical disinfection.

Viruses are not especially resistant to biocides, but a distinction must be made between those that possess a lipid-containing envelope and those that do not. Antimicrobials that are lipid-soluble are more likely to be effective against enveloped viruses. If so, this will usually be indicated on the label by a statement that they are effective against lipophilic viruses. Nonenveloped viruses, with only a protein coat, are more resistant—fewer biocides are active against them.

A special problem, not yet completely solved, is the reliable killing of prions (see Chapter 13, page 412). Prions are infectious proteins that are the cause of neurological diseases, the spongiform encephalopathies, such as the popularly named mad cow disease (see Chapter 22, page 664). To destroy prions, infected animal carcasses are incinerated. A

TABLE 7.8 Chemical Agents Used to Control Microbial Growth

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Phenol and Phenolics			
1. Phenol	Disruption of plasma membrane, denaturation of enzymes.	Rarely used, except as a standard of comparison.	Seldom used as a disinfectant or antiseptic because of its irritating qualities and disagreeable odor.
2. Phenolics	Disruption of plasma membrane, denaturation of enzymes.	Environmental surfaces, instruments, skin surfaces, and mucous membranes.	Derivatives of phenol that are reactive even in the presence of organic material; O-phenylphenol is an example.
3. Bisphenols	Probably disruption of plasma membrane.	Disinfectant hand soaps and skin lotions.	Triclosan is an especially common example of a bisphenol. Broad spectrum, but most effective against gram-positives.
Biguanides (Chlorhexidine)	Disruption of plasma membrane.	Skin disinfection, especially for surgical scrubs.	Bactericidal to gram-positives and gram-negatives; nontoxic, persistent.
Halogens	Iodine inhibits protein function and is a strong oxidizing agent; chlorine forms the strong oxidizing agent hypochlorous acid, which alters cellular components.	Iodine is an effective antiseptic available as a tincture and an iodophor; chlorine gas is used to disinfect water; chlorine compounds are used to disinfect dairy equipment, eating utensils, household items, and glassware.	Iodine and chlorine may act alone or as components of inorganic and organic compounds.
Alcohols	Protein denaturation and lipid dissolution.	Thermometers and other instruments; in swabbing the skin with alcohol before an injection, most of the disinfecting action probably comes from a simple wiping away (degerming) of dirt and some microbes.	Bactericidal and fungicidal, but not effective against endospores or nonenveloped viruses; commonly used alcohols are ethanol and isopropanol.

major problem is the disinfection of surgical instruments exposed to prion contamination. Normal autoclaving has proven to be inadequate. The World Health Organization has recommended the combined use of a solution of sodium hydroxide and autoclaving at 134°C. However, a recent report described another technique, whereby instruments were soaked in a strong solution of sodium hydroxide for an hour followed by an hour of autoclaving at 136°C. Nevertheless, the report described the treatment as only "fairly effective." Recent reports indicate that surgical instruments have been successfully treated to inactivate prions, which are proteins, by addition of protease enzymes to cleaning solution. Surgeons sometimes resort to the use of disposable instruments.

In summary, it is important to remember that microbial control methods, especially biocides, are not uniformly effective against all microbes.

* * *

Table 7.8 summarizes chemical agents used to control microbial growth.

The compounds discussed in this chapter are not generally useful in the treatment of diseases. Because antibiotics are used in chemotherapy, antibiotics and the pathogens against which they are active will be discussed together, in Chapter 20.

TABLE 7.8 Chemical Agents Used to Control Microbial Growth *(continued)*

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Heavy Metals and Their Compounds	Denaturation of enzymes and other essential proteins.	Silver nitrate may be used to prevent gonorrheal ophthalmia neonatorum; silver-sulfadiazine used as a topical cream on burns; copper sulfate is an algicide.	Heavy metals such as silver and mercury are biocidal.
Surface-Active Agents			
Soaps and Detergents	Mechanical removal of microbes through scrubbing.	Skin degerming and removal of debris.	Many antibacterial soaps contain antimicrobials.
Acid-Anionic Sanitizers	Not certain; may involve enzyme inactivation or disruption.	Sanitizers in dairy and food-processing industries.	Wide spectrum of activity; nontoxic, noncorrosive, fast-acting.
Quaternary Ammonium Compounds (Cationic Detergents)	Enzyme inhibition, protein denaturation, and disruption of plasma membranes.	Antiseptic for skin, instruments, utensils, rubber goods.	Bactericidal, bacteriostatic, fungicidal, and virucidal against enveloped viruses; examples of quats are Zephiran and Cepacol.
Chemical Food Preservatives			
Organic Acids	Metabolic inhibition, mostly affecting molds; action not related to their acidity.	Sorbic acid and benzoic acid effective at low pH; parabens much used in cosmetics, shampoos; calcium propionate used in bread.	Widely used to control mold and some bacteria in foods and cosmetics.
Nitrates/Nitrites	Active ingredient is nitrite, which is produced by bacterial action on nitrate. Nitrite inhibits certain iron-containing enzymes of anaerobes.	Meat products such as ham, bacon, hot dogs, sausage.	Prevents growth of *Clostridium botulinum* in food; also imparts a red color.
Aldehydes	Protein denaturation.	Glutaraldehyde (Cidex) is less irritating than formaldehyde and is used for disinfection of medical equipment.	Very effective antimicrobials.
Gaseous Chemosterilizers	Protein denaturation.	Excellent sterilizing agent, especially for objects that would be damaged by heat.	Ethylene oxide is the most commonly used.
Peroxygens (Oxidizing Agents)	Oxidation.	Contaminated surfaces; some deep wounds, in which they are very effective against oxygen-sensitive anaerobes.	Ozone is widely used as a supplement for chlorination; hydrogen peroxide is a poor antiseptic but a good disinfectant. Peracetic acid is especially effective.

STUDY OUTLINE

THE TERMINOLOGY OF MICROBIAL CONTROL (pp. 188–189)

1. The control of microbial growth can prevent infections and food spoilage.
2. Sterilization is the process of removing or destroying all microbial life on an object.
3. Commercial sterilization is heat treatment of canned foods to destroy C. *botulinum* endospores.
4. Disinfection is the process of reducing or inhibiting microbial growth on a nonliving surface.
5. Antisepsis is the process of reducing or inhibiting microorganisms on living tissue.
6. The suffix *-cide* means to kill; the suffix *-stat* means to inhibit.
7. Sepsis is bacterial contamination.

THE RATE OF MICROBIAL DEATH (pp. 189–190)

1. Bacterial populations subjected to heat or antimicrobial chemicals usually die at a constant rate.
2. Such a death curve, when plotted logarithmically, shows this constant death rate as a straight line.
3. The time it takes to kill a microbial population is proportional to the number of microbes.
4. Microbial species and life cycle phases (e.g., endospores) have different susceptibilities to physical and chemical controls.
5. Organic matter may interfere with heat treatments and chemical control agents.
6. Longer exposure to lower heat can produce the same effect as shorter time at higher heat.

ACTIONS OF MICROBIAL CONTROL AGENTS (p. 190)

ALTERATION OF MEMBRANE PERMEABILITY (p. 190)

1. The susceptibility of the plasma membrane is due to its lipid and protein components.
2. Certain chemical control agents damage the plasma membrane by altering its permeability.

DAMAGE TO PROTEINS AND NUCLEIC ACIDS (p. 190)

3. Some microbial control agents damage cellular proteins by breaking hydrogen bonds and covalent bonds.
4. Other agents interfere with DNA and RNA replication and protein synthesis.

PHYSICAL METHODS OF MICROBIAL CONTROL (pp. 190–196)

HEAT (pp. 190–194)

1. Heat is frequently used to kill microorganisms.
2. Moist heat kills microbes by denaturing enzymes.
3. Thermal death point (TDP) is the lowest temperature at which all the microbes in a liquid culture will be killed in 10 minutes.
4. Thermal death time (TDT) is the length of time required to kill all bacteria in a liquid culture at a given temperature.
5. Decimal reduction time (DRT) is the length of time in which 90% of a bacterial population will be killed at a given temperature.
6. Boiling (100°C) kills many vegetative cells and viruses within 10 minutes.
7. Autoclaving (steam under pressure) is the most effective method of moist heat sterilization. The steam must directly contact the material to be sterilized.
8. In HTST pasteurization, a high temperature is used for a short time (72°C for 15 seconds) to destroy pathogens without altering the flavor of the food. Ultra-high-temperature (UHT) treatment (140°C for 3 seconds) is used to sterilize dairy products.
9. Methods of dry heat sterilization include direct flaming, incineration, and hot-air sterilization. Dry heat kills by oxidation.
10. Different methods that produce the same effect (reduction in microbial growth) are called equivalent treatments.

FILTRATION (p. 194)

11. Filtration is the passage of a liquid or gas through a filter with pores small enough to retain microbes.
12. Microbes can be removed from air by high-efficiency particulate air filters.
13. Membrane filters composed of cellulose esters are commonly used to filter out bacteria, viruses, and even large proteins.

LOW TEMPERATURES (pp. 194–195)

14. The effectiveness of low temperatures depends on the particular microorganism and the intensity of the application.
15. Most microorganisms do not reproduce at ordinary refrigerator temperatures (0–7°C).
16. Many microbes survive (but do not grow) at the subzero temperatures used to store foods.

HIGH PRESSURE (p. 195)

17. High pressure denatures proteins in vegetative cells.

DESICCATION (p. 195)

18. In the absence of water, microorganisms cannot grow but can remain viable.
19. Viruses and endospores can resist desiccation.

OSMOTIC PRESSURE (p. 195)

20. Microorganisms in high concentrations of salts and sugars undergo plasmolysis.
21. Molds and yeasts are more capable than bacteria of growing in materials with low moisture or high osmotic pressure.

RADIATION (pp. 195–196)

22. The effects of radiation depend on its wavelength, intensity, and duration.
23. Ionizing radiation (gamma rays, X rays, and high-energy electron beams) has a high degree of penetration and exerts its effect primarily by ionizing water and forming highly reactive hydroxyl radicals.
24. Ultraviolet (UV) radiation, a form of nonionizing radiation, has a low degree of penetration and causes cell damage by making thymine dimers in DNA that interfere with DNA replication; the most effective germicidal wavelength is 260 nm.
25. Microwaves can kill microbes indirectly as materials get hot.

CHEMICAL METHODS OF MICROBIAL CONTROL (pp. 196–205)

1. Chemical agents are used on living tissue (as antiseptics) and on inanimate objects (as disinfectants).
2. Few chemical agents achieve sterility.

PRINCIPLES OF EFFECTIVE DISINFECTION (p. 198)

3. Careful attention should be paid to the properties and concentration of the disinfectant to be used.
4. The presence of organic matter, degree of contact with microorganisms, and temperature should also be considered.

EVALUATING A DISINFECTANT (p. 198)

5. In the use-dilution test, bacterial (*S. choleraesuis*, *S. aureus*, and *P. aeruginosa*) survival in the manufacturer's recommended dilution of a disinfectant is determined.
6. Viruses, endospore-forming bacteria, mycobacteria, and fungi can also be used in the use-dilution test.
7. In the disk-diffusion method, a disk of filter paper is soaked with a chemical and placed on an inoculated agar plate; a zone of inhibition indicates effectiveness.

TYPES OF DISINFECTANTS (pp. 198–205)

Phenol and Phenolics (pp. 198–199)

8. Phenolics exert their action by injuring plasma membranes.

Bisphenols (p. 200)

9. Bisphenols such as triclosan (over the counter) and hexachlorophene (prescription) are widely used in household products.

Biguanides (p. 200)

10. Chlorhexidine damages plasma membranes of vegetative cells.

Halogens (pp. 200–201)

11. Some halogens (iodine and chlorine) are used alone or as components of inorganic or organic solutions.
12. Iodine may combine with certain amino acids to inactivate enzymes and other cellular proteins.
13. Iodine is available as a tincture (in solution with alcohol) or as an iodophor (combined with an organic molecule).
14. The germicidal action of chlorine is based on the formation of hypochlorous acid when chlorine is added to water.
15. Chlorine is used as a disinfectant in gaseous form (Cl_2 or ClO_2) or in the form of a compound, such as calcium hypochlorite, sodium hypochlorite, sodium dichloroisocyanurate, and chloramines.

Alcohols (p. 201)

16. Alcohols exert their action by denaturing proteins and dissolving lipids.
17. In tinctures, they enhance the effectiveness of other antimicrobial chemicals.
18. Aqueous ethanol (60–95%) and isopropanol are used as disinfectants.

Heavy Metals and Their Compounds (pp. 201–202)

19. Silver, mercury, copper, and zinc are used as germicides.
20. They exert their antimicrobial action through oligodynamic action. When heavy metal ions combine with sulfhydryl (—SH) groups, proteins are denatured.

Surface-Active Agents (p. 202)

21. Surface-active agents decrease the surface tension among molecules of a liquid; soaps and detergents are examples.
22. Soaps have limited germicidal action but assist in the removal of microorganisms through scrubbing.
23. Acid-anionic detergents are used to clean dairy equipment.
24. Quats are cationic detergents attached to NH_4^+.
25. By disrupting plasma membranes, they allow cytoplasmic constituents to leak out of the cell.

26. Quats are most effective against gram-positive bacteria.

Chemical Food Preservatives (p. 204)

27. SO_2, sorbic acid, benzoic acid, and propionic acid inhibit fungal metabolism and are used as food preservatives.

28. Nitrate and nitrite salts prevent germination of *Clostridium botulinum* endospores in meats.

Antibiotics (p. 204)

29. Nisin and natamycin are anibiotics used to preserve foods, especially cheese.

Aldehydes (p. 204)

30. Aldehydes such as formaldehyde and glutaraldehyde exert their antimicrobial effect by inactivating proteins.

31. They are among the most effective chemical disinfectants.

Gaseous Chemosterilizers (p. 205)

32. Ethylene oxide is the gas most frequently used for sterilization.

33. It penetrates most materials and kills all microorganisms by protein denaturation.

Peroxygens (Oxidizing Agents) (p. 205)

34. Ozone, peroxide, and peracetic acid are used as antimicrobial agents.

35. They exert their effect by oxidizing molecules inside cells.

MICROBIAL CHARACTERISTICS AND MICROBIAL CONTROL (pp. 205–208)

1. Gram-negative bacteria are generally more resistant than gram-positive bacteria to disinfectants and antiseptics.
2. Mycobacteria, endospores, and protozoan cysts and oocysts are very resistant to disinfectants and antiseptics.
3. Nonenveloped viruses are generally more resistant than enveloped viruses to disinfectants and antiseptics.
4. Prions are resistant to disinfection and autoclaving.

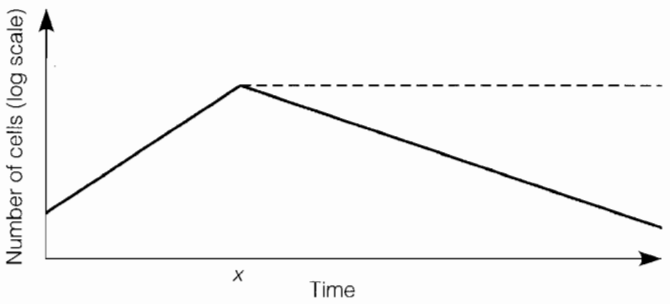
STUDY QUESTIONS

Access more review material either online at **The Microbiology Place** (www.microbiologyplace.com) or with **The Microbiology Place CD-ROM** packaged with your new book. There you'll find activities, practice tests, quizzes, flashcards, case studies, and more to help you succeed.

Answers to the Study Questions can be found in Appendix G.

REVIEW

1. Name the cause of cell death resulting from damage to each of the following:
   a. cell wall
   b. plasma membrane
   c. proteins
   d. nucleic acids
2. The thermal death time for a suspension of *Bacillus subtilis* endospores is 30 minutes in dry heat and less than 10 minutes in an autoclave. Which type of heat is more effective? Why?
3. If pasteurization does not achieve sterilization, why is food treated by pasteurization?
4. Thermal death point is not considered an accurate measure of the effectiveness of heat sterilization. List three factors that can alter thermal death point.
5. The antimicrobial effect of gamma radiation is due to _____. The antimicrobial effect of ultraviolet radiation is due to _____.
6. A bacterial culture was in log phase in the following figure. At time *x*, an antibacterial compound was added to the culture. Which line indicates addition of a bactericidal compound? A bacteriostatic compound? How can you tell? Explain why the viable count does not immediately drop to zero at *x*.



7. Fill in the following table:

Method of Sterilization	Temp.	Time	Type of Heat	Pref'd Use	Types of Action
Autoclaving					
Hot Air					
Pasteurization					

8. How do the examples in question 7 illustrate the concept of equivalent treatments?
9. How do salts and sugars preserve foods? Why are these considered physical rather than chemical methods of microbial control? Name one food that is preserved with sugar and one preserved with salt. How do you account for the occasional growth of *Penicillium* mold in jelly, which is 50% sucrose?
10. List five factors to consider before selecting a disinfectant.

11. Give the method of action and at least one standard use of each of the following types of disinfectants:
    a. phenolics
    b. iodine
    c. chlorine
    d. alcohol
    e. heavy metals
    f. aldehydes
    g. ethylene oxide
    h. oxidizing agents
12. The use-dilution values for two disinfectants tested under the same conditions are: Disinfectant A—1:2; Disinfectant B—1:10,000. If both disinfectants are designed for the same purpose, which would you select?
13. A large hospital washes burn patients in a stainless steel tub. After each patient, the tub is cleaned with a quat. It was noticed that 14 of 20 burn patients acquired *Pseudomonas* infections after being bathed. Provide an explanation for this high rate of infection.

MULTIPLE CHOICE

1. Which of the following does *not* kill endospores?
    a. autoclaving
    b. incineration
    c. hot-air sterilization
    d. pasteurization
    e. All of the above kill endospores.
2. Which of the following is most effective for sterilizing mattresses and plastic Petri dishes?
    a. chlorine
    b. ethylene oxide
    c. glutaraldehyde
    d. autoclaving
    e. nonionizing radiation
3. Which of these disinfectants does *not* act by disrupting the plasma membrane?
    a. phenolics
    b. phenol
    c. quaternary ammonium compounds
    d. halogens
    e. biguanides
4. Which of the following *cannot* be used to sterilize a heat-labile solution stored in a plastic container?
    a. gamma radiation
    b. ethylene oxide
    c. nonionizing radiation
    d. autoclaving
    e. short-wavelength radiation
5. Which of the following is *not* a characteristic of quaternary ammonium compounds?
    a. bactericidal against gram-positive bacteria
    b. sporicidal
    c. amoebicidal
    d. fungicidal
    e. kills enveloped viruses
6. A classmate is trying to determine how a disinfectant might kill cells. You observed that when he spilled the disinfectant in your reduced litmus milk, the litmus turned blue again. You suggest to your classmate that
    a. the disinfectant might inhibit cell wall synthesis.
    b. the disinfectant might oxidize molecules.
    c. the disinfectant might inhibit protein synthesis.
    d. the disinfectant might denature proteins.
    e. he take his work away from yours.
7. Which of the following is most likely to be bactericidal?
    a. membrane filtration
    b. ionizing radiation
    c. lyophilization (freeze-drying)
    d. deep-freezing
    e. all of the above
8. Which of the following is used to control microbial growth in foods?
    a. organic acids
    b. alcohols
    c. aldehydes
    d. heavy metals
    e. all of the above

Use the following information to answer questions 9 and 10. The data were obtained from a use-dilution test comparing four disinfectants against *Salmonella choleraesuis*.

	Bacterial Growth After Exposure to			
Dilution	**Disinfectant A**	**Disinfectant B**	**Disinfectant C**	**Disinfectant D**
1:2	−	+	−	−
1:4	−	+	−	+
1:8	−	+	+	+
1:16	+	+	+	+

9. Which disinfectant is the most effective?
10. Which disinfectant(s) is (are) bactericidal?
    a. A, B, C, and D
    b. A, C, and D
    c. A only
    d. B only
    e. none of the above

CRITICAL THINKING

1. The disk-diffusion method was used to evaluate three disinfectants. The results were as follows:

Disinfectant	Zone of Inhibition
X	0 mm
Y	5 mm
Z	10 mm

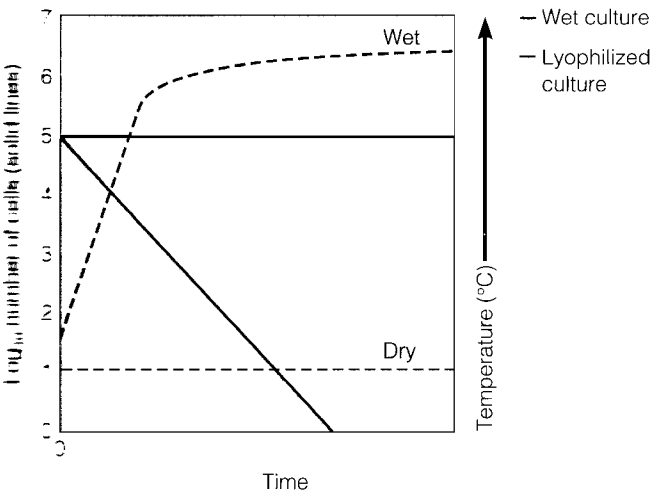
    a. Which disinfectant was the most effective against the organism?
    b. Can you determine whether compound Y was bactericidal or bacteriostatic?
2. Why is each of the following bacteria often resistant to disinfectants?
    a. *Mycobacterium*
    b. *Pseudomonas*
    c. *Bacillus*

3. A use-dilution test was used to evaluate two disinfectants against *Salmonella choleraesuis*. The results were as follows:

	Bacterial Growth After Exposures		
Time of Exposure (min)	**Disinfectant A**	**Disinfectant B Diluted with Distilled Water**	**Disinfectant B Diluted with Tap Water**
10	+	−	+
20	+	−	−
30	−	−	−

   a. Which disinfectant was the most effective?
   b. Which disinfectant should be used against *Staphylococcus*?

4. To determine the lethal action of microwave radiation, two 10^5 suspensions of *E. coli* were prepared. One cell suspension was exposed to microwave radiation while wet, whereas the other was lyophilized (freeze-dried) and then exposed to radiation. The results are shown in the following figure. Dashed lines indicate the temperature of the samples. What is the most likely method of lethal action of microwave radiation? How do you suppose these data might differ for *Clostridium*?



CLINICAL APPLICATIONS

1. *Entamoeba histolytica* and *Giardia lamblia* were isolated from the stool sample of a 45-year-old man, and *Shigella sonnei* was isolated from the stool sample of an 18-year-old woman. Both patients experienced diarrhea and severe abdominal cramps, and prior to onset of digestive symptoms both had been treated by the same chiropractor. The chiropractor had administered colonic irrigations (enemas) to these patients. The device used for this treatment was a gravity-dependent apparatus using 12 liters of tap water. There were no check valves to prevent backflow, so all parts of the apparatus could have become contaminated with feces during each colonic treatment. The chiropractor provided colonic treatment to four or five patients per day. Between patients, the adaptor piece that is inserted into the rectum was placed in a "hot-water sterilizer."

   What two errors were made by the chiropractor?

2. Between March 9 and April 12, five chronic peritoneal dialysis patients at one hospital became infected with *Pseudomonas aeruginosa*. Four patients developed peritonitis (inflammation of the abdominal cavity), and one developed a skin infection at the catheter insertion site. All patients with peritonitis had low-grade fever, cloudy peritoneal fluid, and abdominal pain. All patients had permanent indwelling peritoneal catheters, which the nurse wiped with gauze that had been soaked with an iodophor solution each time the catheter was connected to or disconnected from the machine tubing. Aliquots of the iodophor were transferred from stock bottles to small in-use bottles. Cultures from the dialysate concentrate and the internal areas of the dialysis machines were negative; iodophor from a small in-use plastic container yielded a pure culture of *P. aeruginosa*.

   What improper technique led to this infection?

3. Eleven patients received injections of methylprednisolone and lidocaine to relieve the pain and inflammation of arthritis at the same orthopedic surgery office. All of them developed septic arthritis caused by *Serratia marcescens*. Unopened bottles of methylprednisolone from the same lot numbers tested sterile; the methylprednisolone was preserved with a quat. Cotton balls were used to wipe multiple-use injection vials before the medication was drawn into a disposable syringe. The site of injection on each patient was also wiped with a cotton ball. The cotton balls were soaked in benzalkonium chloride, and fresh cotton balls were added as the jar was emptied. Opened methylprednisolone containers and the jar of cotton balls contained *S. marcescens*.

   How was the infection transmitted? What part of the routine procedure caused the contamination?