7 The Control of Microbial Growth

THE TERMINOLOGY OF MICROBIAL CONTROL

Sterilization is the removal or destruction of all forms of microbial life (although it usually assumes the absence of prions which are rare but exceptionally resistant). **Commercial sterilization** subjects canned food to only enough heat to destroy the endospores of *Clostridium botulinum.* **Disinfection** is the destruction of vegetative pathogens on a surface, usually with chemicals. Spores and viruses are not necessarily destroyed. **Antisepsis** is the chemical disinfection of living tissue, such as skin or mucous membrane. **Asepsis** is the absence of pathogens on an object or area, as in **aseptic surgery. Degerming** (degermation) is the removal of transient microbes from skin by mechanical cleansing or by an antiseptic. **Sanitization** is the reduction of microbial populations on objects to safe public health levels. In general, the suffix *-cide* indicates the killer of a specified organism. A **biocide** or **germicide** kills microorganisms. **Fungicides** kill fungi, **virucides** kill viruses, and so on. The suffix *-stat* or *-stasis* used in this way indicates only that the substance inhibits—for example, **bacteriostasis.**

THE RATE OF MICROBIAL DEATH

Bacterial populations killed by heat or chemicals tend to die at constant rates—for example, 90% every 10 minutes. Plotted logarithmically, these figures form straight descending lines.

ACTIONS OF MICROBIAL CONTROL AGENTS

Alteration of Membrane Permeability

The plasma membrane controls the passage of nutrients and wastes into and out of the cell. Damage to the plasma membrane causes leakage of cellular contents and interferes with cell growth.

Damage to Proteins and Nucleic Acids

Chemicals may denature proteins by reacting, for example, with disulfide bonds (or disulfide bridges), which give proteins their three-dimensional active shape. Chemicals and radiation may prevent proper replication or functioning of DNA or RNA.

PHYSICAL METHODS OF MICROBIAL CONTROL

Heat

Thermal death point is the lowest temperature required to kill a liquid culture of a certain species of bacteria in 10 minutes. **Thermal death time** is the length of time required to kill all bacteria in a liquid culture at a given temperature. **Decimal reduction time** (*D value*) is the length of time, in minutes, required to kill 90% of the population of bacteria at a given temperature.

Moist Heat and Pasteurization. Boiling (100°C) kills vegetative forms of bacterial pathogens, many viruses, and fungi within 10 minutes. Endospores and some viruses survive boiling for longer times. **Steam under pressure** allows temperatures above boiling to be reached. **Autoclaves,** retorts, and pressure cookers are vessels in which high steam pressures can be contained. A typical operating condition for sterilization is 15 psi (pounds per square inch) at 121°C for 15 minutes. Moisture must touch all surfaces in order to bring about sterilization. Air must be completely exhausted from the container. An autoclave is shown in Figure 7.1.

Pasteurization is mild heating that is sufficient to kill particular spoilage or disease organisms without seriously damaging the taste of the product. Dairies use the *phosphatase test* to determine whether products have been properly pasteurized. **High-temperature, short-time pasteurization** uses temperatures of at least 72°C for about 15 seconds to pasteurize milk. **Equivalent treatments** are illustrated by the following example: the heat of 115°C acting on an organism for 70 minutes is equivalent to heat of 125°C acting on an organism for only 7 minutes; that is, applying a higher temperature for a shorter time may kill the same number of microbes as a lower temperature for a longer time. Milk can be sterilized and stored without refrigeration when given **ultra-high-treatment (UHT).**

Dry Heat Sterilization. **Incineration,** as in direct **flaming,** is efficient for limited purposes. **Hot-air sterilization,** as in an oven, requires higher temperatures (such as 170°C) and longer times (such as 2 hours) to ensure sterilization. Moist heat is generally more efficient.

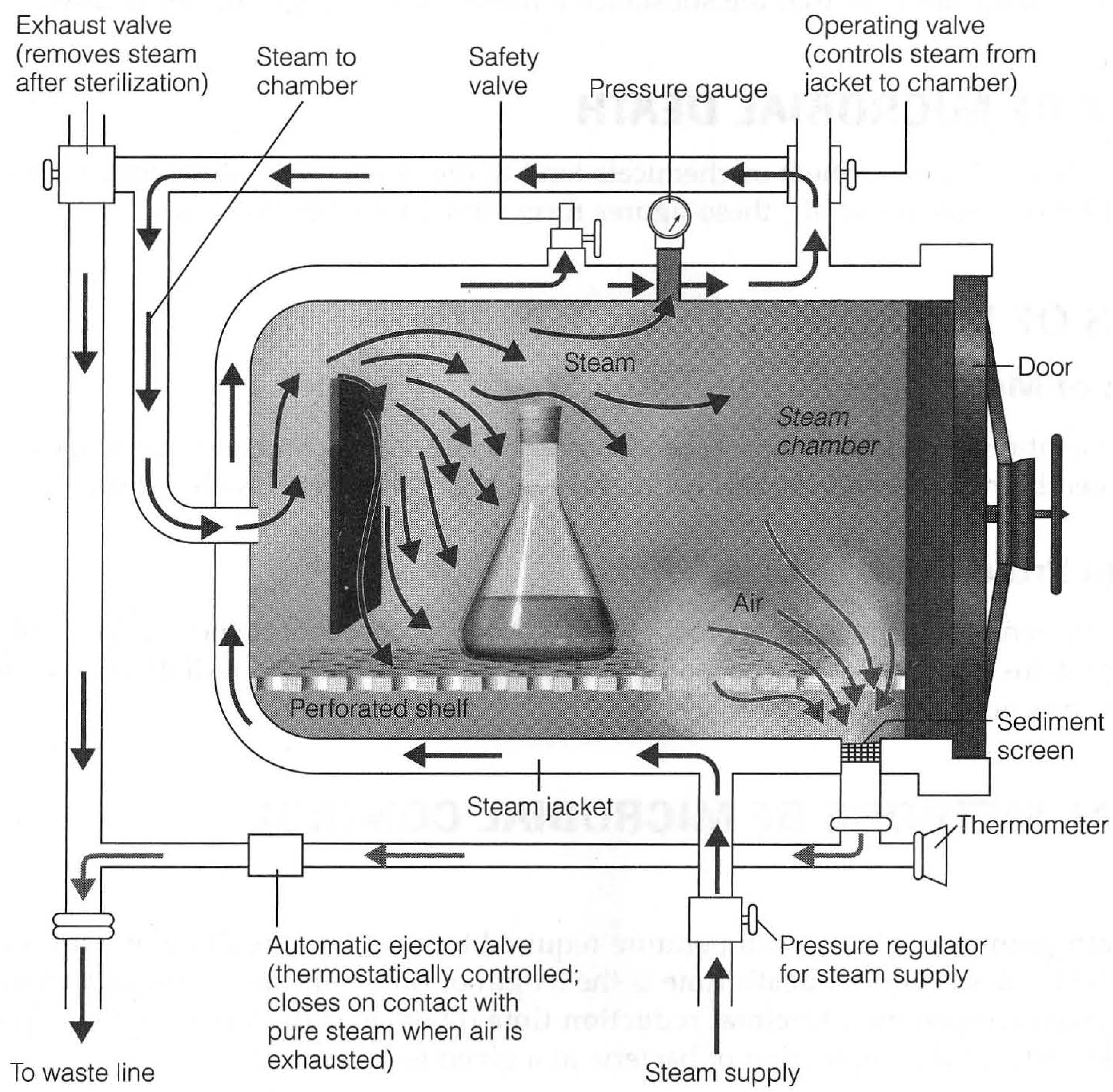

Figure 7.1 Autoclave. The entering steam forces the air out of the bottom (gray arrows). The automatic ejector valve remains open as long as an air-steam mixture is passing out of the waste line. When all the air has been ejected, the higher temperature of the pure steam closes the valve, and the pressure in the chamber increases.

Filtration

Liquids sensitive to heat can be passed through a thin **membrane filter** that has carefully controlled pore sizes to retain microorganisms. Operating theaters and special clean rooms receive air passed through **high-efficiency particulate air (HEPA) filters.**

Low Temperatures

Refrigerator temperatures (0–7°C) slow the metabolic rate of microbes; however, psychrotrophic species still grow slowly. Some organisms grow at temperatures slightly below freezing, but microbes at the usual temperatures of freezer compartments are completely dormant.

High Pressure

High pressure applied to liquid suspensions such as fruit juices can kill vegetative bacterial cells while preserving flavors, colors, and nutrient values. Endospores are relatively resistant.

Desiccation

Microbes require water for growth, and adequately dried (**desiccated**) foods will not support their growth.

Osmotic Pressure

High salt or sugar concentrations cause water to leave the cell; this is an example of **osmosis** (see *plasmolysis* in Chapter 6). Generally, molds and yeasts resist osmotic pressures better than bacteria.

Radiation

Ionizing radiation such as *X rays, gamma rays,* and *high-energy electron beams* carry high energy and break DNA strands. Ionizing radiation forms reactive hydroxyl radicals. Such radiation is used to sterilize pharmaceuticals. **Nonionizing radiation** such as ultraviolet (UV) light has a longer wavelength and less energy. UV light causes bonds to form between adjacent thymines (*thymine dimers*) in DNA chains. Penetration is low. Sunlight has some biocidal activity, mainly due to formation of singlet oxygen (see Chapter 6) in the cytoplasm.

CHEMICAL METHODS OF MICROBIAL CONTROL

Evaluating a Disinfectant

Bacteria are tested under standard conditions against concentrations of phenol and the test disinfectant.

Use-Dilution Test. In the **American Official Analytic Chemist's use-dilution test,** a series of tubes containing increasing concentrations of the test disinfectants is inoculated and incubated. The more the chemical can be diluted and still be effective, the higher its rating.

The Disk-Diffusion Method. A disk of filter paper is soaked in a chemical agent, which is placed on an inoculated surface of an agar plate. A clear zone around the disk indicates inhibition.

Types of Disinfectants

Phenol and Phenolics. **Phenol** (carbolic acid) is seldom used today. Derivatives of the phenol molecule, however, are widely used. **Phenolics** injure plasma membranes, inactivate enzymes, or denature proteins. They are stable, persistent, and are not sensitive to organic matter. **O-phenylphenol,** a *cresol,* is the main ingredient in most formulations of Lysol.

Bisphenols. Phenol derivatives called **bisphenols** contain two phenolic groups connected by a bridge. **Hexachlorophene** is the main ingredient in pHisoHex and is used in nurseries to control gram-positive skin bacteria such as staphylococci and streptococci. Excessive use can cause neurological damage. **Triclosan** is a widely used bisphenol found in many household products. It has a broad spectrum of activity, especially against gram-positive bacteria. It is also effective against gram-negative bacteria and fungi.

Biguanides. **Chlorhexidine,** a member of the biguanide group, is not a phenol, but its structure and applications resemble those of hexachlorophene. It is frequently used for surgical skin preparation and surgical hand scrubs. *Alexidine* is similar but more rapid in action.

Halogens. The **halogens,** especially iodine and chlorine, are effective antimicrobial agents. **Iodine** impairs protein synthesis. A **tincture** is a solution of iodine in water, and an **iodophore** is a combination of iodine and an organic molecule from which the iodine is slowly released. An example of *povidone-iodine* is Betadine. **Chlorine** is a widely used disinfectant, either as a gas or in a chemical combination. When added to water, it forms *hypochlorous acid,* which is the germicidal form. It is a strong oxidizing agent that inhibits enzymatic function. *Calcium hypochlorite* (chloride of lime) is used to disinfect utensils. *Sodium hypochlorite* (household bleach) is a widely used disinfectant. *Sodium dichloroisocyanurate* (Chlor-Floc) is a water disinfectant issued by the U.S. military. *Chlorine dioxide* is a gas used for area disinfection, most notably to kill endospores of anthrax bacteria. It is also used in solution as a surface disinfectant. *Chloramines* are a combination of chlorine and ammonia. They are more stable than other forms of chlorine and are used as a sanitizer and for disinfection in municipal water systems.

Alcohols. Both **ethanol** and **isopropanol** (rubbing alcohol) are widely used, normally at a concentration of about 70%. Concentrations of 60–95% are effective. They are bactericidal and fungicidal but are not effective against endospores or nonenveloped viruses. Alcohols enhance the effectiveness of other chemical agents. Purell, a widely used hand cleaner, contains 62–65% ethanol.

Heavy Metals and Their Compounds. The fact that tiny amounts of heavy metals are effective antimicrobials can be illustrated by **oligodynamic action.** A silver coin on an inoculated nutrient medium will inhibit growth for some distance. A 1% *silver nitrate* solution has been used to prevent gonorrheal eye infections in newborns. *Silver-sulfadiazine* is used in wound dressings. Silver combines with sulfhydryl groups on proteins, denaturing them. *Surfacine* is a water-insoluble silver iodide in a carrier that is a persistent disinfectant for surfaces. *Mercuric chloride* is highly bactericidal, but it is toxic and corrosive and is inactivated by organic matter. *Copper sulfate* is often used to destroy green algae in reservoirs or other waters. *Zinc chloride* is used in mouthwashes. *Zinc pyrithione* is an ingredient in antidandruff shampoo.

Surface-Active Agents. **Surface-active agents,** or **surfactants,** decrease the surface tension of a liquid. Soaps and detergents are examples. They emulsify oils and are good degerming agents. *Acid-anionic* sanitizers are important for cleaning dairy equipment.

Quaternary Ammonium Compounds. The **quaternary ammonium compounds (quats)** (Figure 7.2) are most effective against gram-positive bacteria, less so against gram-negative bacteria. These *cationic* detergents have good fungicidal, amoebicidal, and virucidal (enveloped virus) activity, but they are not sporicidal. They are colorless, odorless, tasteless, nontoxic, and stable, but they are inactivated by organic matter, soaps, detergents, and surfaces such as gauze. They may even support the growth of *Pseudomonas* bacteria. They act by disrupting the plasma membranes and by denaturing enzymes. Widely used examples of quats are *benzalkonium chloride* (Zephiran) and *cetylpyridinium chloride* (Cepacol).

Chemical Food Preservatives. *Sorbic acid (potassium sorbate)* inhibits mold spoilage in foods such as cheese. *Benzoic acid (sodium benzoate)* is an antifungal used in soft drinks and other acidic foods. Methylparaben and propylparaben, which are derivatives of benzoic acid, work at a neutral pH. They inhibit molds in liquid cosmetics and shampoos. *Calcium propionate* prevents mold growth in bread. All of these organic acids inhibit enzymatic or metabolic activity; their activity is not related to their acidity. *Sodium nitrate* and *sodium nitrite* are added to meats to produce a red color and to inhibit outgrowth of botulism endospores. (The active principle is the nitrite ion; bacteria in the meat reduce the nitrate to

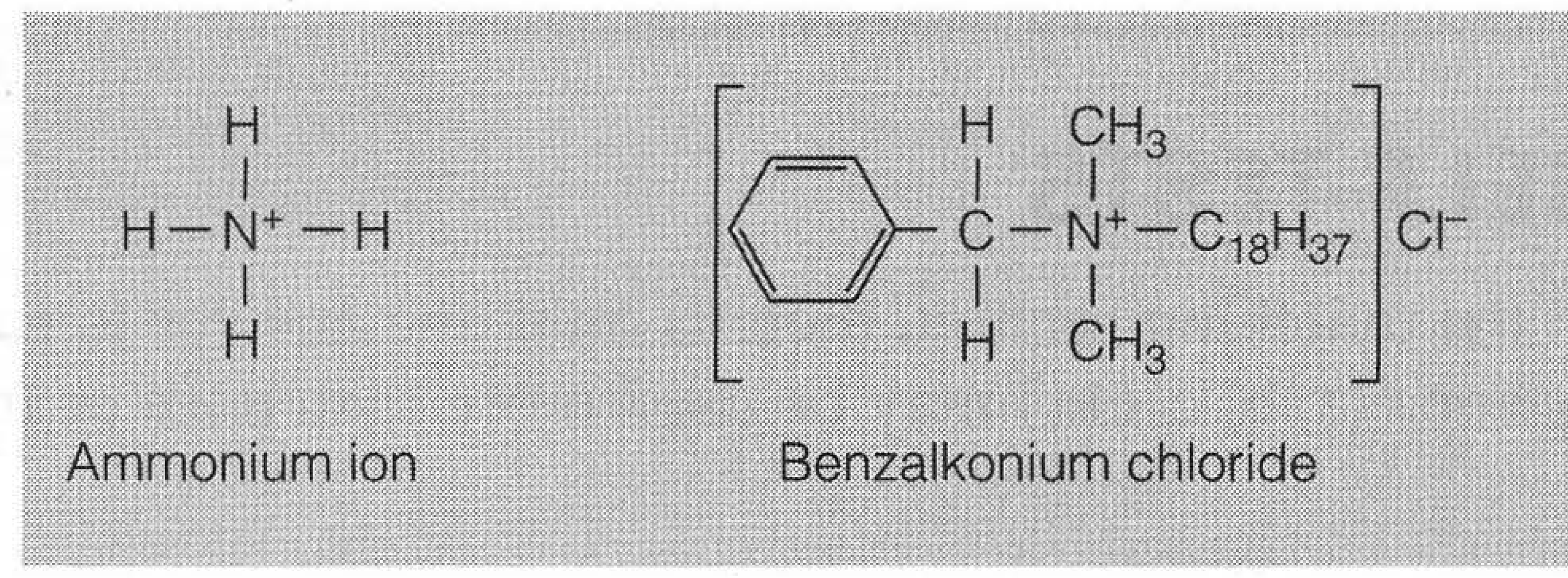

Figure 7.2 The ammonium ion and a quaternary ammonium compound, benzalkonium chloride (Zephiran). Note how other groups replace the hydrogens of the ammonium ion.

nitrite.) **Nitrosamines,** formed by a reaction between certain amino acids and nitrite, are possibly carcinogenic (cancer causing).

Antibiotics. A few antibiotics, not medically useful, are used as food preservatives. These include *nisin,* a bacteriocin (a protein produced by a bacterium that inhibits another), which is added to cheese to inhibit endospore formers. Another is *Natamycin (pimaricin),* an antifungal mostly used in cheese.

Aldehydes. Among the most effective antimicrobials are the aldehydes such as *formaldehyde.* In the form of an aqueous solution, this gas is called *formalin* and is used to preserve biological specimens. *Glutaraldehyde* is a less irritating form; in a 2% solution (Cidex), it is bactericidal, tuberculocidal, and virucidal in 10 minutes. It is sporicidal after about 3 to 10 hours of contact. Both glutaraldehyde and formaldehyde are used for embalming. A possible replacement for glutaraldehyde is *ortho-phthalaldehyde* (OPA).

Chemical Sterilization. *Ethylene oxide* gas, which requires a closed chamber similar to an autoclave, is the most familiar example of a chemical sterilizer. Its activity depends on *alkylation,* which leads to a cross-linking of nucleic acids and proteins. Heated *hydrogen peroxide* can also be used as a gaseous sterilant. *Chlorine dioxide,* usually manufactured at the site, is a gas used to fumigate enclosed building ereas.

Plasmas. **Plasma** represents a state of matter in which a gas is excited to make a mixture of nuclei with assorted electrical charges and free electrons. *Plasma sterilization* is a method available for the difficult task of sterilizing surgical devices with small interior diameters. Such plasmas have many radicals that destroy even endospores at relatively low temperatures.

Supercritical Fluids. When carbon dioxide is compressed into a "supercritical" state, it has properties of both a liquid and a gas. *Supercritical carbon dioxide* is used for decontamination of foods and medical implants. It inactivates even endospores at temperatures of only about 45°C.

Peroxygens and Other Forms of Oxygen. *Hydrogen peroxide* effectively disinfects inanimate objects; at high concentrations it is sporicidal. *Peracetic acid (peroxyacetic acid,* or *PAA)* is one of the most effective liquid chemical sporicides available. The U.S. Food and Drug Adminstration (FDA) has approved PAA for washing fruits and vegetables, and it is used to disinfect medical equipment. Other oxidizing agents include *benzoyl peroxide* (used to treat acne) and *ozone,* a reactive form of oxygen used to supplement chlorine for water treatment. It is produced on site by electrical discharges.

MICROBIAL CHARACTERISTICS AND MICROBIAL CONTROL

Chemical antimicrobials are not uniformly effective against all microorganisms. *Gram-positive bacteria* are relatively more resistant than *gram-negative. Viruses* with an envelope (lipophilic) are more susceptible than those with only a protein coat. *Endospores,* and *cysts* and *oocysts* of protozoa, are affected by few liquid chemical agents. *Mycobacteria* have a waxy cell wall that makes them relatively resistant. **Prions,** infectious proteins that cause neurological diseases such as mad cow disease (see Chapter 22), are difficult to render noninfectious. Normal autoclaving is inadequate, and at least autoclaving at a higher temperature, 134°C in a solution of sodium hydroxide, is recommended. Incineration can be used in some cases.

SELF-TESTS

In the matching section, there is only one answer to each question; however, the lettered options (a, b, c, etc.) may be used more than once or not at all.

I. Matching

___ 1. A suffix meaning "to kill."

___ 2. Destroying or removing *all* forms of microbial life.

___ 3. The absence of pathogens on an object or area.

___ 4. The reduction of microbial populations to safe public health levels.

___ 5. The chemical disinfection of living tissue, such as skin or a mucous membrane.

___ 6. The removal of transient microbes from skin by mechanical cleansing or by an antiseptic.

___ 7. Heat sufficient only to kill endospores of the botulism bacterium.

a. Disinfection
b. Sterilization
c. Antisepsis
d. Asepsis
e. Sanitization
f. Degerming
g. *-cide*
h. *-stat*
i. Commercial sterilization

II. Matching

___ 1. The lowest temperature required to kill a liquid culture of a certain species of bacteria in 10 minutes.

___ 2. The time in minutes required to kill 90% of a bacterial population.

___ 3. Mild heating to destroy particular spoilage organisms or disease organisms in milk or similar products.

___ 4. A test for the effectiveness of a chemical disinfectant.

___ 5. The absence of water, resulting in a condition of dryness.

a. Thermal death time
b. Decimal reduction time
c. Thermal death point
d. Phenol coefficient
e. Pasteurization
f. Desiccation
g. Incineration

III. Matching

___ 1. Ethylene oxide.

___ 2. Sodium hypochlorite.

___ 3. Copper sulfate.

___ 4. Silver nitrate.

___ 5. Benzalkonium chloride.

___ 6. Acid-anionic detergents.

___ 7. Sorbic acid.

___ 8. Benzoyl peroxide.

___ 9. Hexachlorophene.

___ 10. Isopropanol.

a. Bisphenol

b. Halogen

c. Alcohol

d. Heavy metal

e. Quaternary ammonium compound

f. Surface-active agents

g. Organic acid

h. Aldehydes

i. Gaseous chemosterilizer

j. Oxidizing agent

IV. Matching

___ 1. An effective liquid sporicide.

___ 2. A bacteriocin classified as an antibiotic.

___ 3. Pimaricin.

___ 4. A biguanide.

___ 5. A bisphenol found in many household products.

___ 6. An antibiotic antifungal.

a. Peracetic acid

b. Chlorhexidine

c. Triclosan

d. Natamycin

e. Nisin

V. Matching

___ 1. Added to chlorine to form chloramines.

___ 2. An antibacterial effect of ultraviolet radiation on DNA.

___ 3. Formaldehyde in an aqueous solution.

___ 4. An example would be iodine in an aqueous-alcohol solution.

___ 5. For example, povidone-iodine solution.

a. Iodophore

b. Formalin

c. Thymine dimers

d. Ammonia

e. Tincture

VI. Matching

___ 1. Chlorine in tablet form issued as a water purifier by the U.S. military.

___ 2. Name of a test that determines if milk has been properly pasteurized.

___ 3. Used as an antiseptic in certain mouthwashes.

___ 4. Used in many water treatment plants as a disinfectant; produced by electrical discharges at the site.

___ 5. Antifungal organic compound used in food.

___ 6. Ingredient in antidandruff shampoo.

a. Sodium dichloroisocyanurate

b. Phosphatase

c. Zinc chloride

d. Sodium benzoate

e. Ozone

f. Zinc pyrithione

Fill in the Blanks

1. Ultraviolet light is an example of ____________ radiation.
2. Sunlight owes its biocidal activity mainly to the formation of ____________ oxygen.
3. A good example of ionizing radiation is ____________.
4. Ethanol is usually used in a concentration of about ____________.
5. A less irritating form of formaldehyde is ____________.
6. A compound that would only inhibit the growth of a fungus would be a fungi____________ (supply the suffix).
7. Steam ____________ allows temperatures above boiling to be reached.
8. Steam under pressure is obtained in retorts, pressure cookers, and ____________.
9. Supercritical ____________ is used for decontaminating foods and medical implants.
10. Generally speaking, the group of organisms that is more resistant to osmotic pressure than bacteria is ____________.

Critical Thinking

1. What physical method of control would be most effective in each of the following situations?
   a. To eliminate endospore-forming pathogens.

   b. To sterilize milk for storage at room temperature.

   c. To sterilize vaccines.

   d. To sterilize microbiological media.

2. What chemical agent would be most effective in each of the following situations?
   a. A puncture wound acquired while gardening.
   b. For presurgical scrubbing.
   c. To sterilize packaged bandages.
   d. To prevent the growth of molds in liquid cosmetics.
3. Compare and contrast sterilization and sanitation.
4. Discuss the advantages and disadvantages associated with each of the following physical methods of control.
   a. Osmotic pressure
   b. Desiccation
   c. Refrigeration
   d. Filtration
5. Discuss the advantages and disadvantages of UV light as a method to control microbial growth.

ANSWERS

Matching

I.	1. g	2. b	3. d	4. e	5. c	6. f	7. i			
II.	1. c	2. b	3. e	4. d	5. f					
III.	1. i	2. b	3. d	4. d	5. e	6. f	7. g	8. j	9. a	10. c
IV.	1. a	2. e	3. d	4. b	5. c	6. d				
V.	1. d	2. c	3. b	4. e	5. a					
VI.	1. a	2. b	3. c	4. e	5. d 6. f					

Fill in the Blanks

1. nonionizing 2. singlet 3. X rays, gamma rays, high-energy electrons 4. 70%
5. glutaraldehyde 6. -stat 7. under pressure (as in an autoclave) 8. autoclaves
9. carbon dioxide 10. fungi, such as molds and yeasts

Critical Thinking

1. a. Autoclaving at 121°C, 15 psi for 15 minutes will kill all organisms and their endospores.

   b. The milk should be sterilized by ultra-high-temperature (UHT) treatment.

   c. Vaccines are heat-sensitive and must be filter-sterilized.

   d. Most media can be safely autoclaved. Heat-sensitive media can be filter-sterilized.

2. a. An oxidizing agent such as hydrogen peroxide would be a good choice. Oxidizing agents are especially effective against anaerobic bacteria.

   b. Chlorhexidine is useful for surgical scrubbing because it is bactericidal against both gram-positive and gram-negative organisms.

   c. Ethylene oxide would be appropriate because it is 100% effective and can penetrate the wrapping material covering the bandage.

   d. The addition of a compound such as methylparaben would inhibit mold growth.

3. *Sterilization* refers to the destruction or the removal of *all* microbial life, including endospores. There are many ways to achieve sterilization, including the use of heat, chemical agents, or filtration. *Sanitation* is the *reduction* of pathogens on inanimate objects (such as eating utensils) to "safe" levels. This may be achieved by mechanical cleaning or with chemical agents.

4. a. The use of high concentrations of salt or sugar creates a hypertonic environment that results in the osmotic loss of water from microbial cells. The advantage is that this is a simple way to preserve meat and fruit. Applications include jams and jellies. Disadvantages are that molds may grow on foods prepared this way and that it isn't a practical way to preserve many foods.

   b. Desiccation involves drying food (for example, meat and fruit). The lack of water retards the growth and reproduction of microbes. The advantage is that it is an easy way to preserve some foods. The disadvantages are that many microorganisms are able to survive desiccation for long periods of time and are revived upon the addition of moisture. Applications are beef jerky and sun-dried tomatoes.

   c. Refrigeration is a simple and relatively effective way to retard the spoilage of food. Although many bacteria can survive and even reproduce at refrigerator temperatures, the rate of chemical reactions is slowed.

   d. Filtration is the passage of gas or liquid through a screenlike material with pores small enough to retain microbes. There are many applications of filtration, such as sterilizing heat-sensitive materials. It is difficult to filter-sterilize viscous materials such as some media.

5. Nonionizing radiation (for example, UV light) damages the DNA of exposed cells and is used to control microbes in air and to sterilize vaccines, serums, and toxins. A serious disadvantage of nonionizing radiation is that because of its relatively low energy content, it penetrates poorly. Organisms protected by practically anything are not affected.