Chapter 9

Control of Microorganisms by Physical and Chemical Agents

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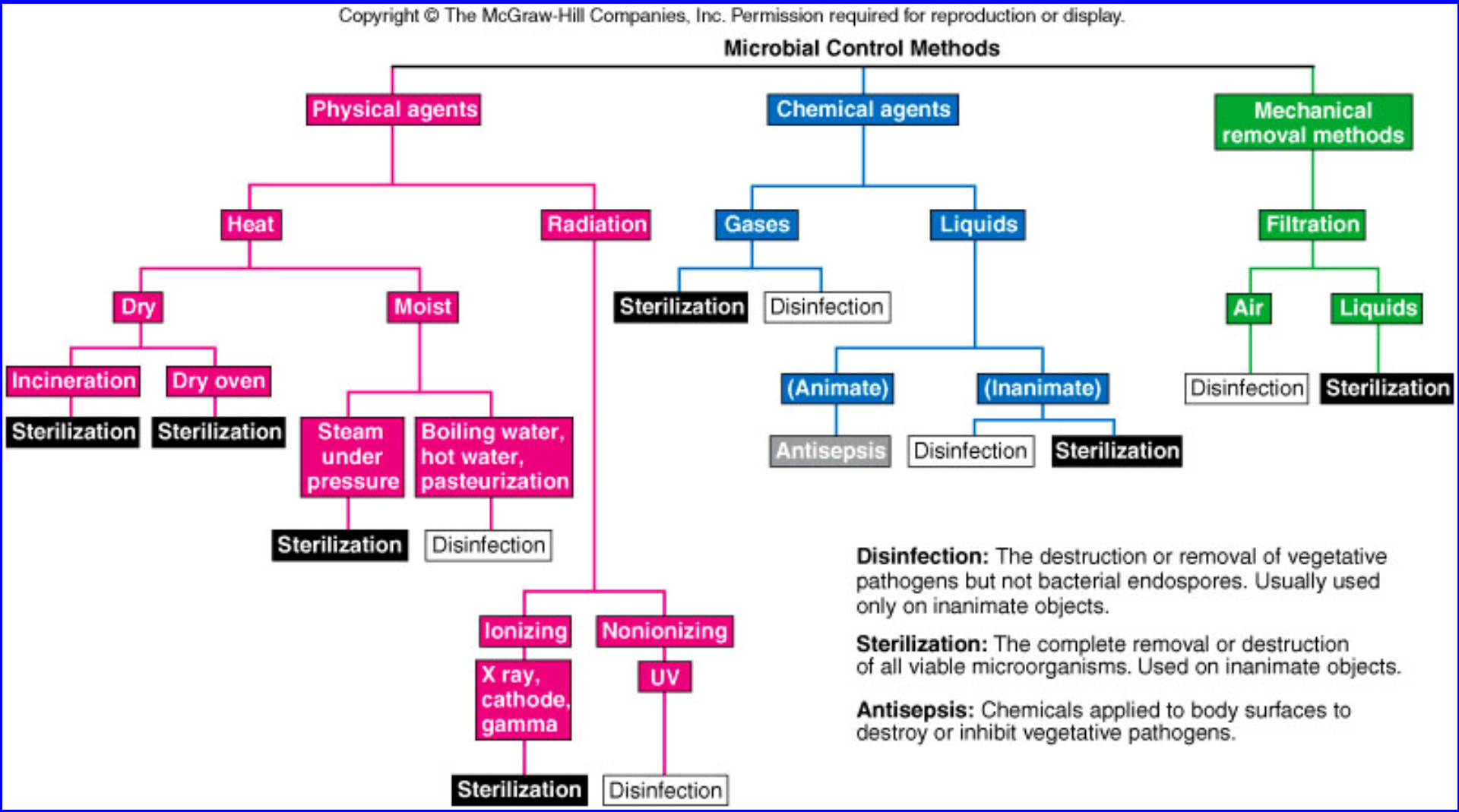


Definition of Frequently Used Terms

- **sterilization**
  - **destruction or removal of all viable organisms**
- **disinfection**
  - **killing, inhibition, or removal of pathogenic organisms**
  - **disinfectants**
    - **agents, usually chemical, used for disinfection**
    - **usually used on inanimate objects**

More definitions…

- **sanitization**
  - **reduction of microbial population to levels deemed safe (based on public health standards)**
- **antisepsis**
  - **prevention of infection of living tissue by microorganisms**
  - **antiseptics**
    - **chemical agents that kill or inhibit growth of microorganisms when applied to tissue**

Antimicrobial agents

- agents that kill microorganisms or inhibit their growth
- -cidal agents kill
- -static agents inhibit growth

-cidal agents

-cide

- **suffix indicating that agent kills**

- **germicide**
  - **kills pathogens and many nonpathogens but not necessarily endospores**
- **include bactericides, fungicides, algicides, and viricides**

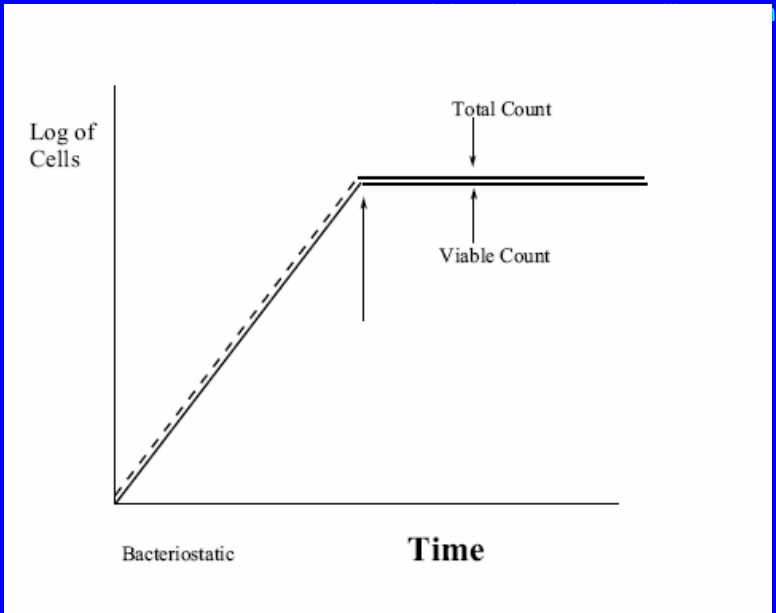
-static agents

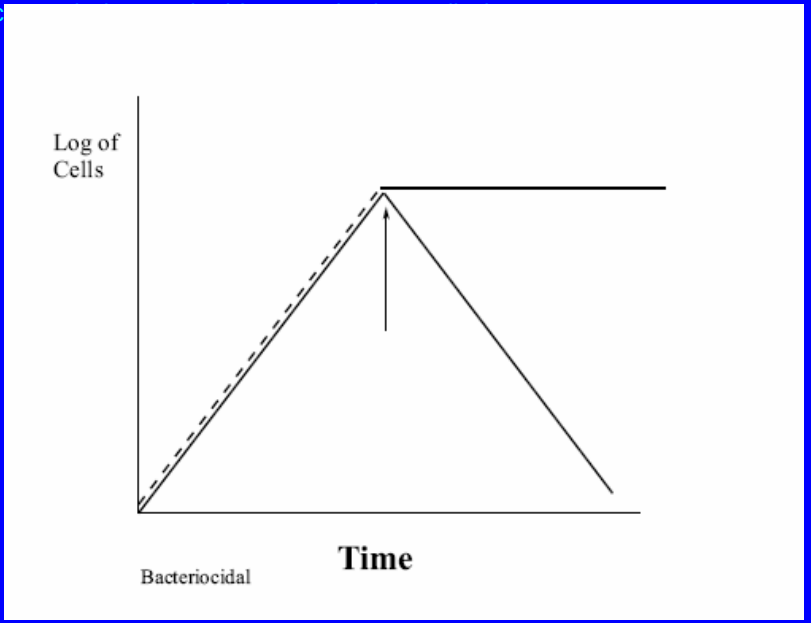
-static

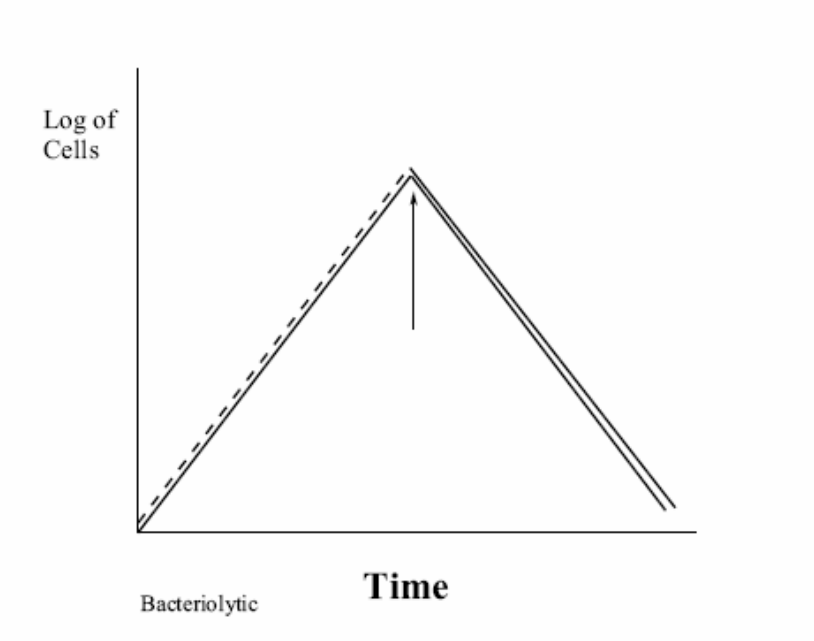
- **suffix indicating that agent inhibits growth**
- **include bacteriostatic and fungistatic**

The Pattern of Microbial Death

- microorganisms are not killed instantly
- population death usually occurs exponentially
- microorganisms are considered to be dead when they are unable to reproduce in conditions that normally support their reproduction


Log of Cells
Total Count
Viable Count
Bacteriostatic
Time


Log of Cells
Bacteriocidal
Time


Log of Cells
Bacteriolytic
Time

Conditions Influencing the Effectiveness of Antimicrobial Agent Activity

- **population size**
  - **larger populations take longer to kill than smaller populations**
- **population composition**
  - **microorganisms differ markedly in their sensitivity to antimicrobial agents**

More conditions…

- concentration or intensity of an antimicrobial agent
  - usually higher concentrations or intensities kill more rapidly
  - relationship is not linear
- duration of exposure

  longer exposure ⇒ more organisms killed

More conditions…

- temperature
  - higher temperatures usually increase amount of killing
- local environment
  - many factors (e.g., pH, viscosity and concentration of organic matter) can profoundly impact effectiveness

The Use of Physical Methods in Control

- heat
- low temperatures
- filtration
- radiation

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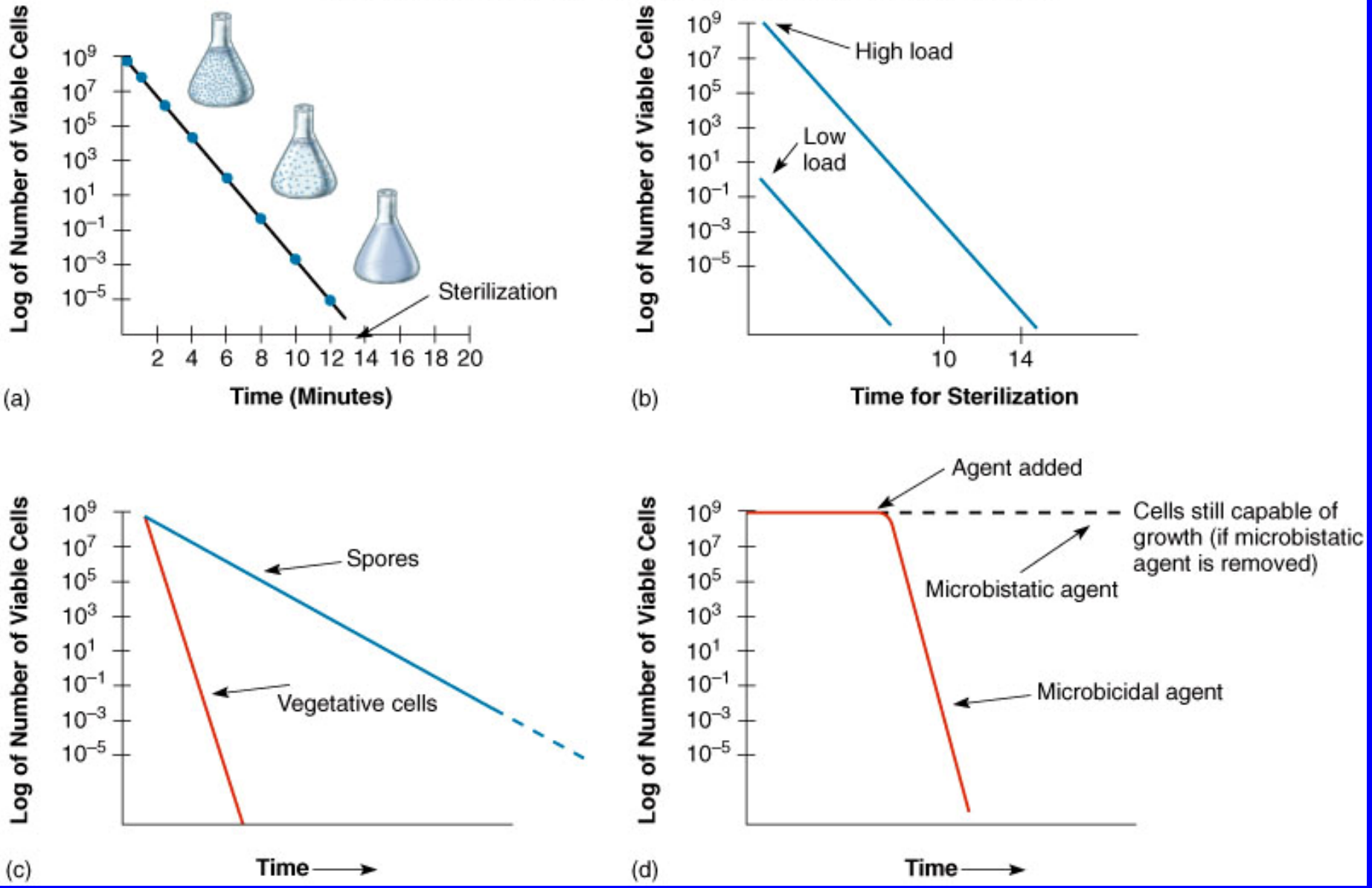
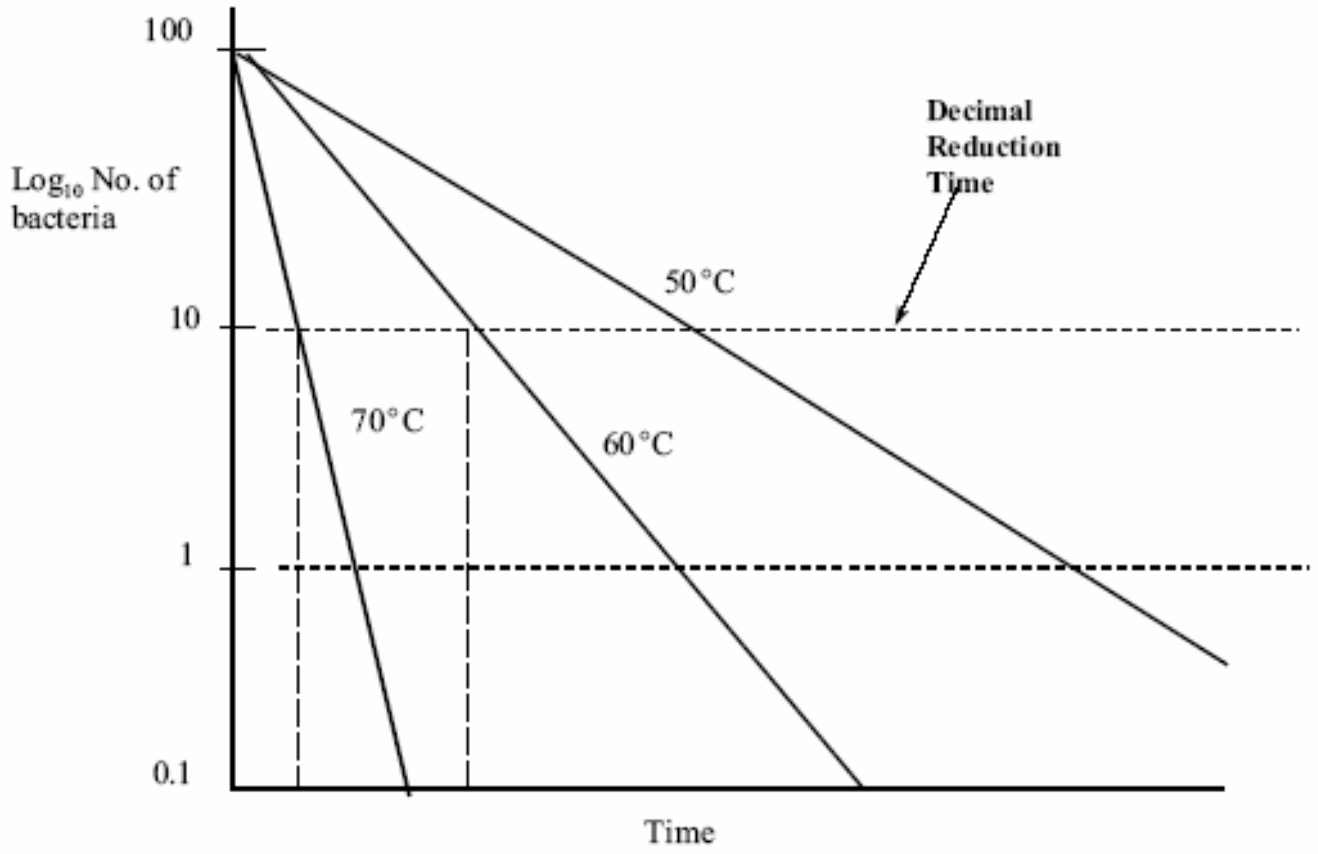


Heat

- **moist heat**
  - **effective against all types of microorganisms**
  - **degrades nucleic acids, denatures proteins, and disrupts membranes**
- **dry heat sterilization**
  - **less effective, requiring higher temperatures and longer exposure times**
  - **oxidizes cell constituents and denatures proteins**

Measuring heat-killing efficiency

- **thermal death time (TDT)**
  - **shortest time needed to kill all microorganisms in a suspension at a specific temperature and under defined conditions**
- **decimal reduction time (*D* or *D value*)**
  - **time required to kill 90% of microorganisms or spores in a sample at a specific temperature**



The Effect of temperature on viability of mesophilic bacterium

Moist/Dry Heat

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TABLE 11.2 Comparison of Times and Temperatures to Achieve Sterilization with Moist and Dry Heat

	Temperature	Time to Sterilize
Moist heat	121°C	15 min
	125°C	10 min
	134°C	3 min
Dry heat	121°C	600 min
	140°C	180 min
	160°C	120 min
	170°C	60 min

Moist heat

Table 7.2 Approximate Conditions for Moist Heat Killing

Organism	Vegetative Cells	Spores
Yeasts	5 minutes at 50–60°C	5 minutes at 70–80°C
Molds	30 minutes at 62°C	30 minutes at 80°C
Bacteria[a]	10 minutes at 60–70°C	2 to over 800 minutes at 100°C 0.5–12 minutes at 121°C
Viruses	30 minutes at 60°C	

[a]Conditions for mesophilic bacteria.

autoclaves

– used to kill endospores efficiently

– use saturated steam under pressure to reach temperatures above boiling

TABLE 11.3 Thermal Death Times of Various Endospores

Organism	Temperature	Time of Exposure to Kill Spores
Moist heat		
Bacillus subtilis	121°C	1 min
B. stearothermophilis	121°C	12 min
Clostridium botulinum	120°C	10 min
C. tetani	105°C	10 min
Dry heat		
Bacillus subtilis	121°C	120 min
B. stearothermophilis	140°C	5 min
Clostridium botulinum	120°C	120 min
C. tetani	100°C	60 min

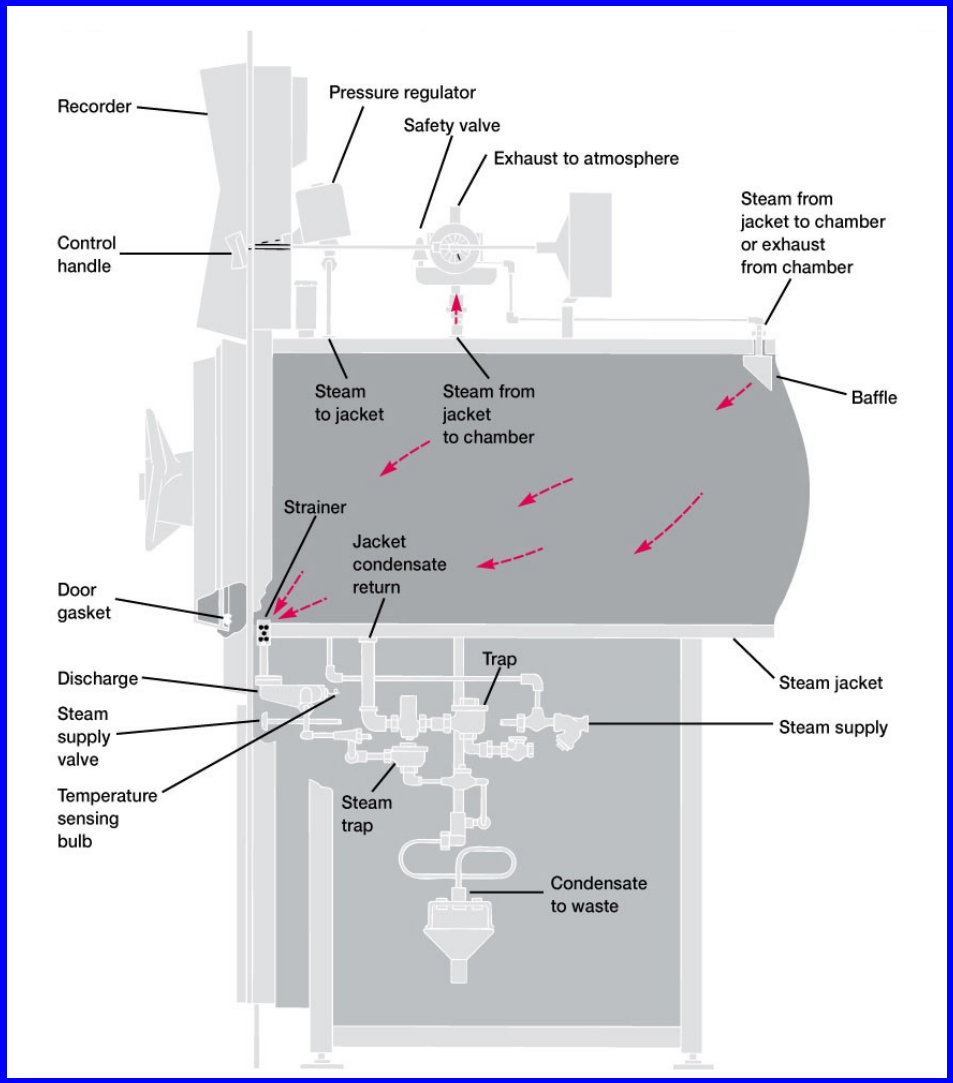

Figure 7.3

Moist heat…

- **pasteurization**
  - **controlled heating at temperatures well below boiling**
  - **reduces total microbial population and thereby increases shelf life of treated material**

Pasteurization of milk

- **flash pasteurization (high temperature short-term – HTST)**
  - **72°C for 15 seconds then rapid cooling**
- **ultrahigh-temperature (UHT) sterilization**
  - **140 to 150°C for 1 to 3 seconds**

Low Temperatures

- freezing
  - stops microbial reproduction due to lack of liquid water
  - some microorganisms killed by ice crystal disruption of cell membranes
- refrigeration
  - slows microbial growth and reproduction

Filtration

- reduces microbial population or sterilizes solutions of heat-sensitive materials by removing microorganisms
- also used to reduce microbial populations in air

Filtering liquids

- **depth filters**
  - **thick fibrous or granular filters that remove microorganisms by physical screening, entrapment, and/or adsorption**
- **membrane filters**
  - **porous membranes with defined pore sizes that remove microorganisms primarily by physical screening**

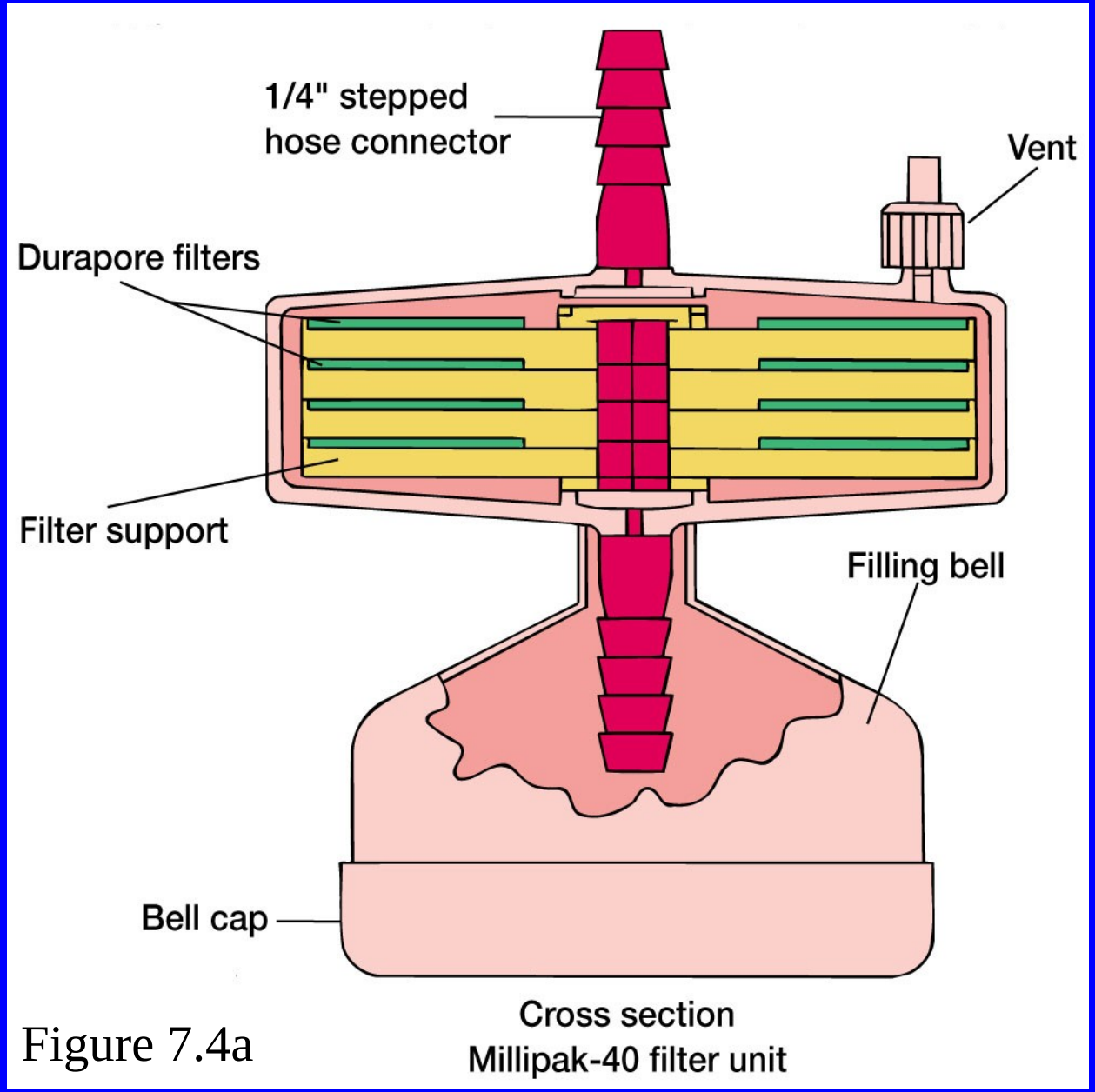

Figure 7.4a

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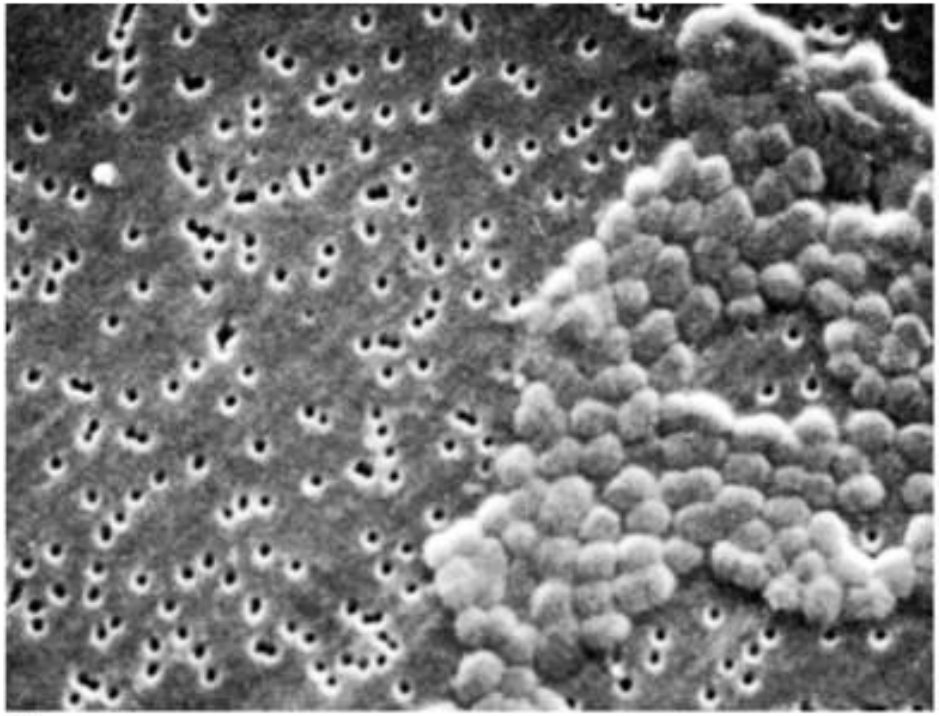
(a)



(b)

nylon membrane with bacterial removal rating of 0.2 µm

polycarbonate membrane 0.4 µm pores

Filtering air

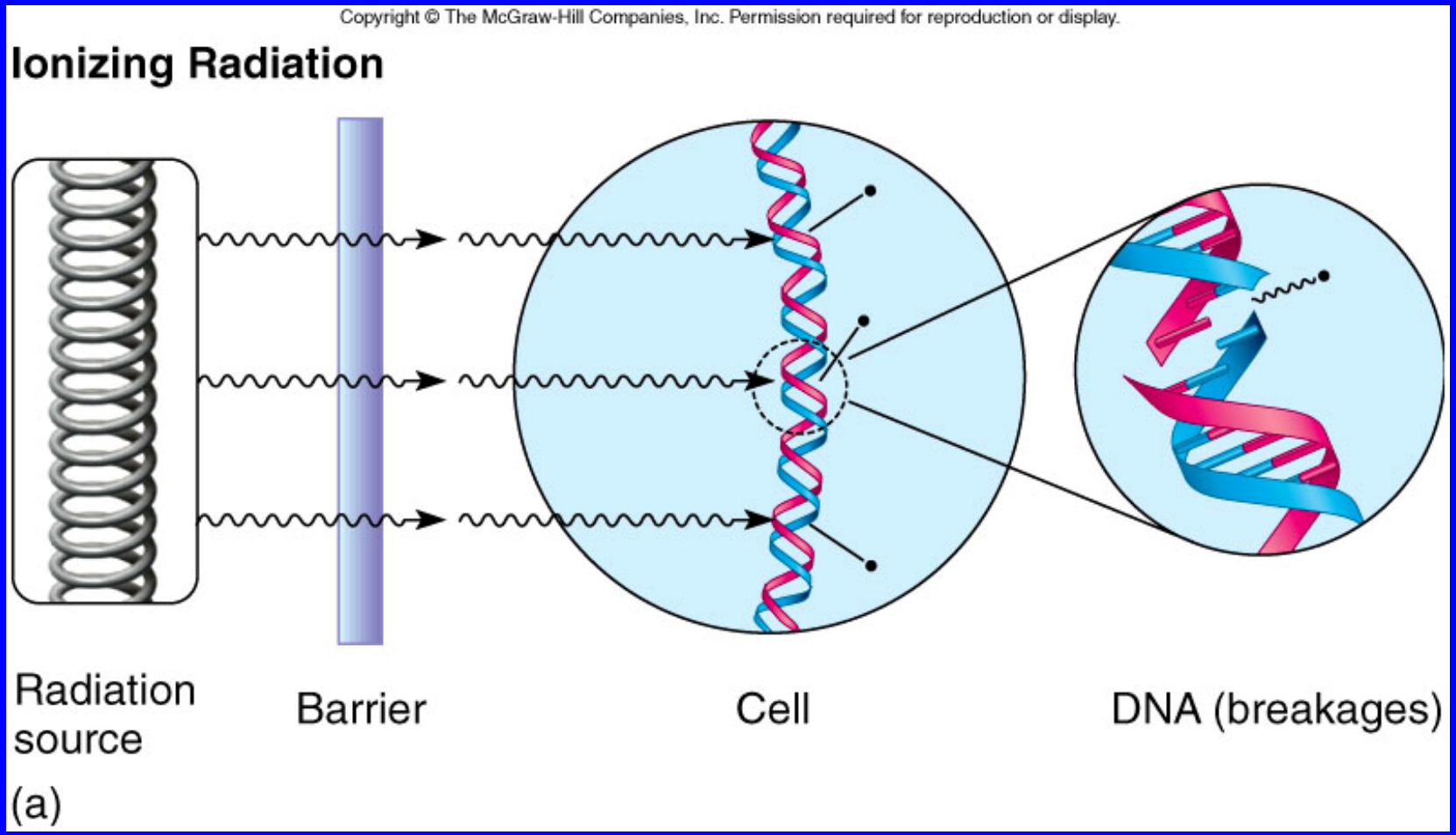
- surgical masks
- cotton plugs on culture vessels
- high-efficiency particulate air (HEPA) filters
  - used in laminar flow biological safety cabinets

Figure 7.6a

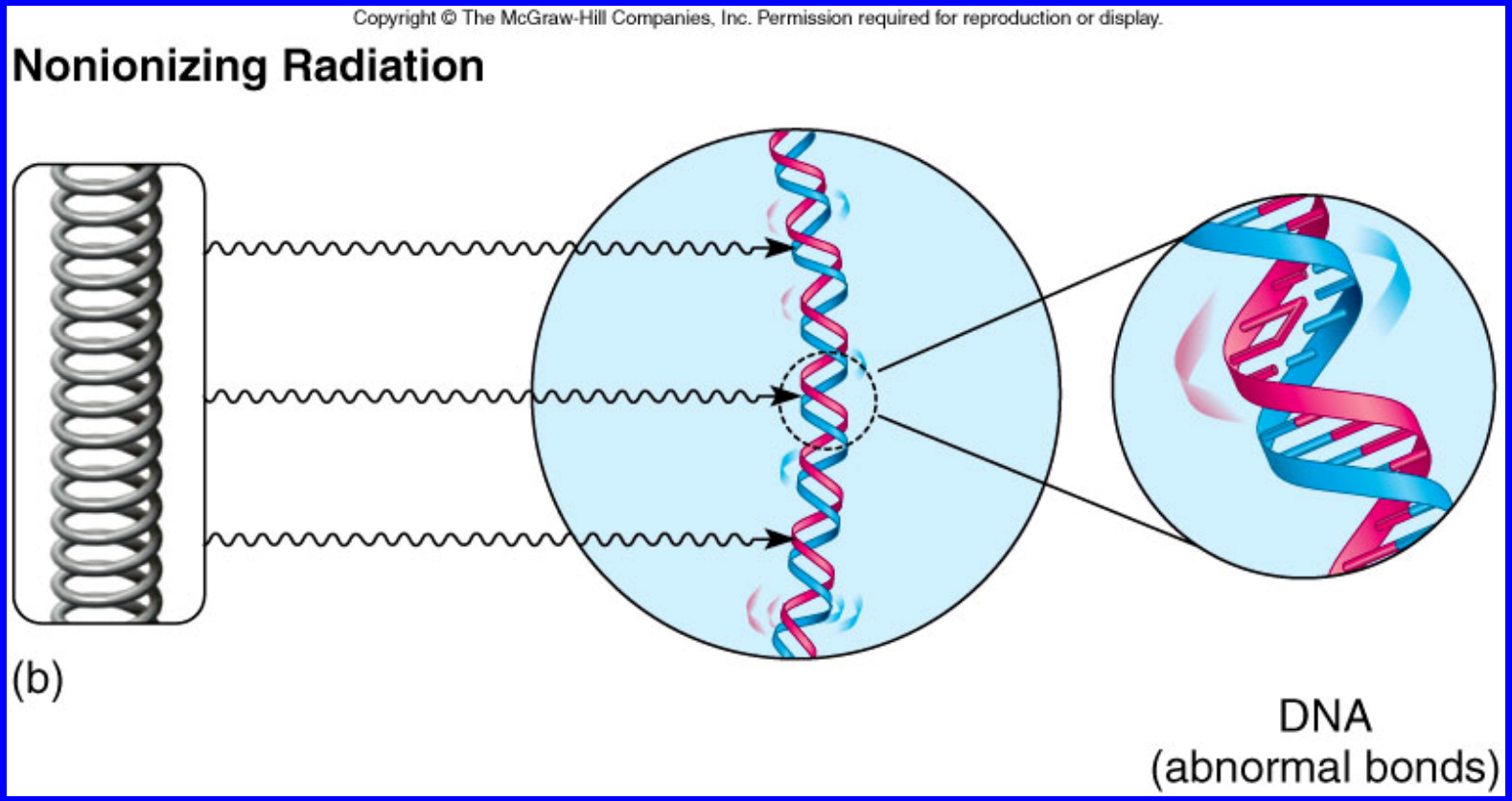
Radiation

- **ionizing radiation**
  - **penetrates deep into objects**
  - **destroys bacterial endospores; not always effective against viruses**
  - **used for sterilization and pasteurization of antibiotics, hormones, sutures, plastic disposable supplies, and food**
- **ultraviolet (UV) radiation (Non-Ionizing)**
  - **limited to surface sterilization because UV radiation does not penetrate glass, dirt films, water, and other substances**

Ionizing Radiation



Ultra Violet (UV) Non-Ionizing Radiation



The Use of Chemical Control Agents

TABLE 11.5 Qualities of Chemical Agents Used in Health Care

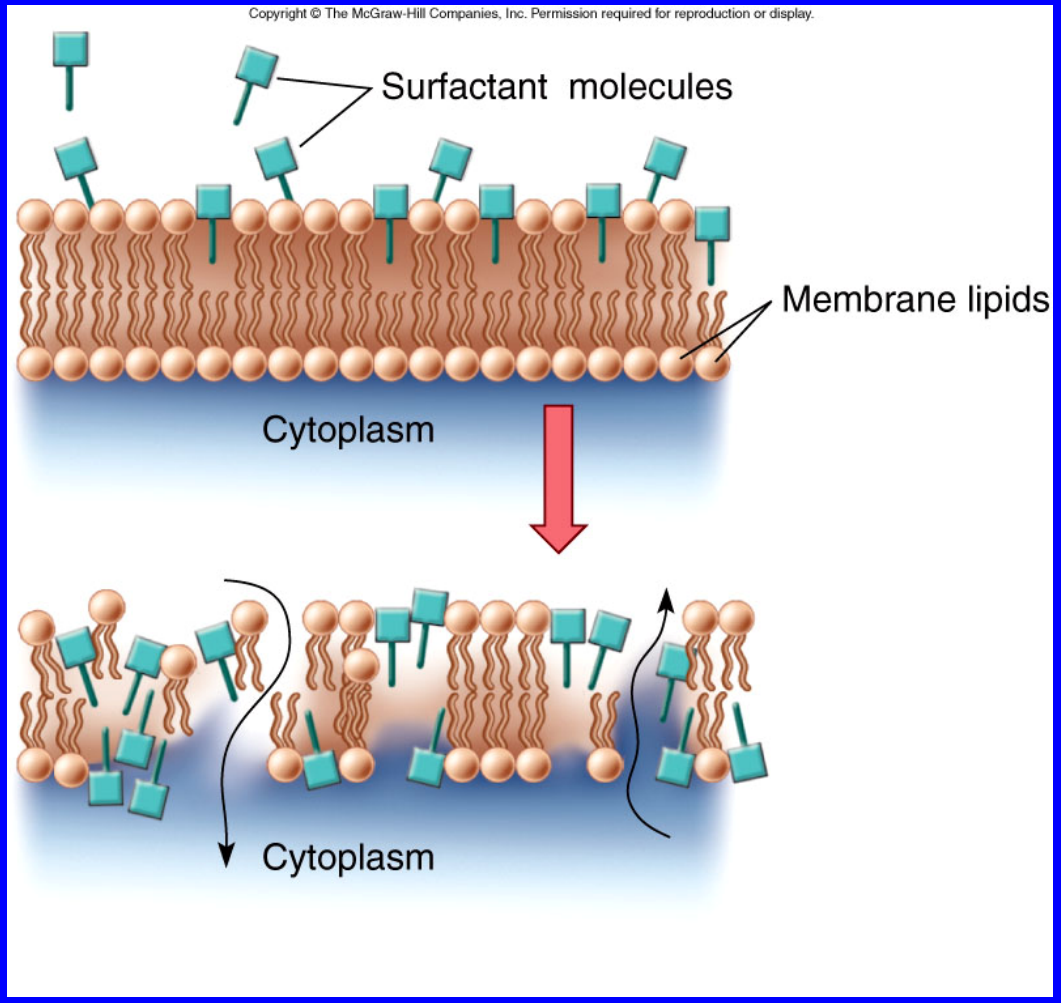
Agent	Target Microbes	Level of Activity	Toxicity	Comments
Chlorine	Sporicidal (slowly)	Intermediate	Gas is highly toxic; solution irritates skin	Inactivated by organics; unstable in sunlight
Iodine	Sporicidal (slowly)	Intermediate	Can irritate tissue; toxic if ingested	Iodophors* are milder forms
Phenolics	Some bacteria, viruses, fungi	Low to intermediate	Can be absorbed by skin; can cause CNS damage	Poor solubility; expensive
Alcohols	Most bacteria, viruses, fungi	Intermediate	Toxic if ingested; a mild irritant; dries skin	Flammable, fast-acting
Hydrogen peroxide,* stabilized	Sporicidal	High	Toxic to eyes; toxic if ingested	Improved stability; works well in organic matter
Quaternary ammonium compounds	Some bactericidal, virucidal, fungicidal activity	Low	Irritating to mucous membranes; poisonous if taken internally	Weak solutions can support microbial growth; easily inactivated
Soaps	Certain very sensitive species	Very low	Nontoxic; few if any toxic effects	Used for removing soil, oils, debris
Mercurials	Weakly microbistatic	Low	Highly toxic if ingested, inhaled, absorbed	Easily inactivated
Silver nitrate	Bactericidal	Low	Toxic, irritating	Discolors skin
Glutaraldehyde*	Sporicidal	High	Can irritate skin; toxic if absorbed	Not inactivated by organic matter; unstable
Formaldehyde	Sporicidal	Intermediate to high	Very irritating; fumes damaging, carcinogenic	Slow rate of action; limited applications
Ethylene oxide gas*	Sporicidal	High	Very dangerous to eyes, lungs; carcinogenic	Explosive in pure state; good penetration; materials must be aerated
Dyes	Weakly bactericidal, fungicidal	Low	Low toxicity	Stains materials, skin
Chlorhexidine*	Most bacteria, some viruses, fungi	Low to intermediate	Low toxicity	Fast-acting, mild, has residual effects

*These chemicals approach the ideal by having many of the following characteristics: broad spectrum, low toxicity, fast action, penetrating abilities, residual effects, stability, potency in organic matter, and solubility.

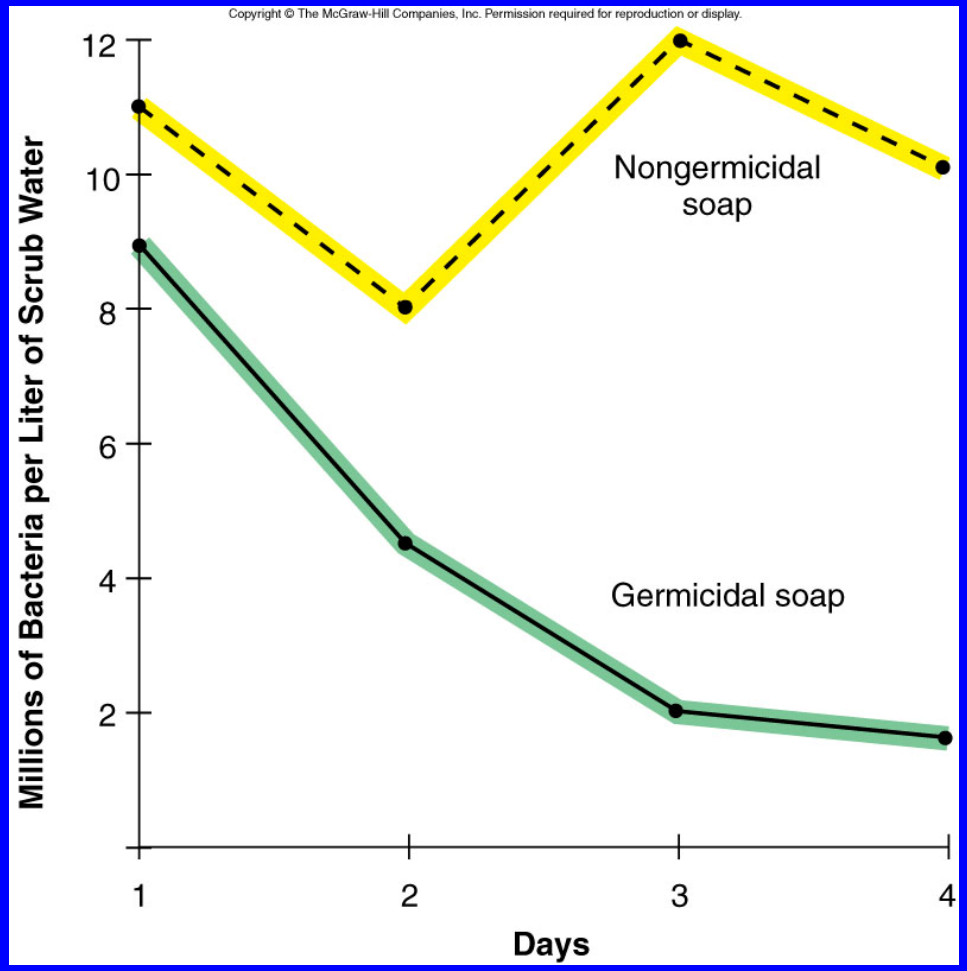
Targets of Physical and Chemical Agents

- 1. The Cell Wall
- 2. The Cell Membrane
- 3. Cellular synthetic process
  - (DNA, RNA)
- 4. Proteins

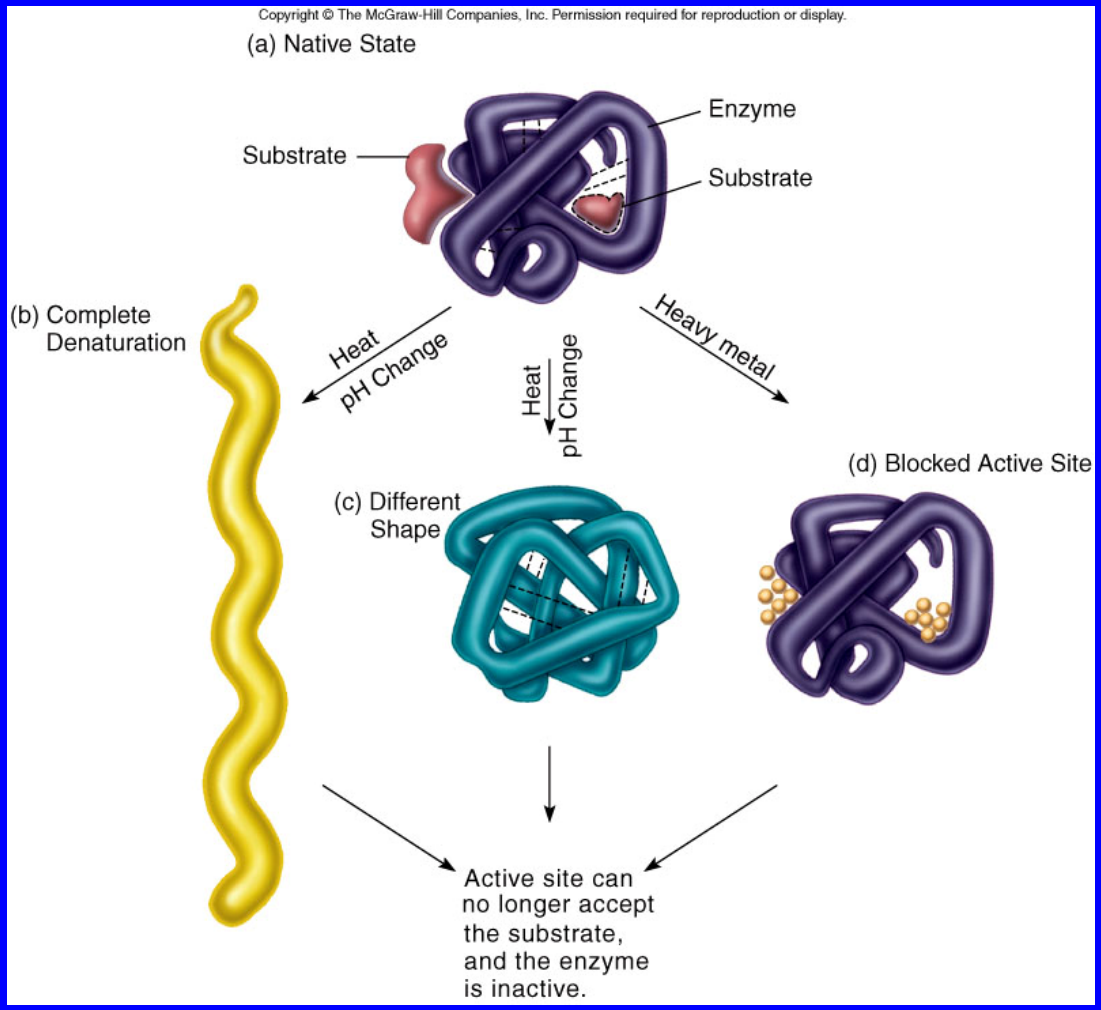
Surfactant Molecules



Efficacy of Soap



Mode Action up Protein



The Use of Chemical Agents in Control

Phenolics

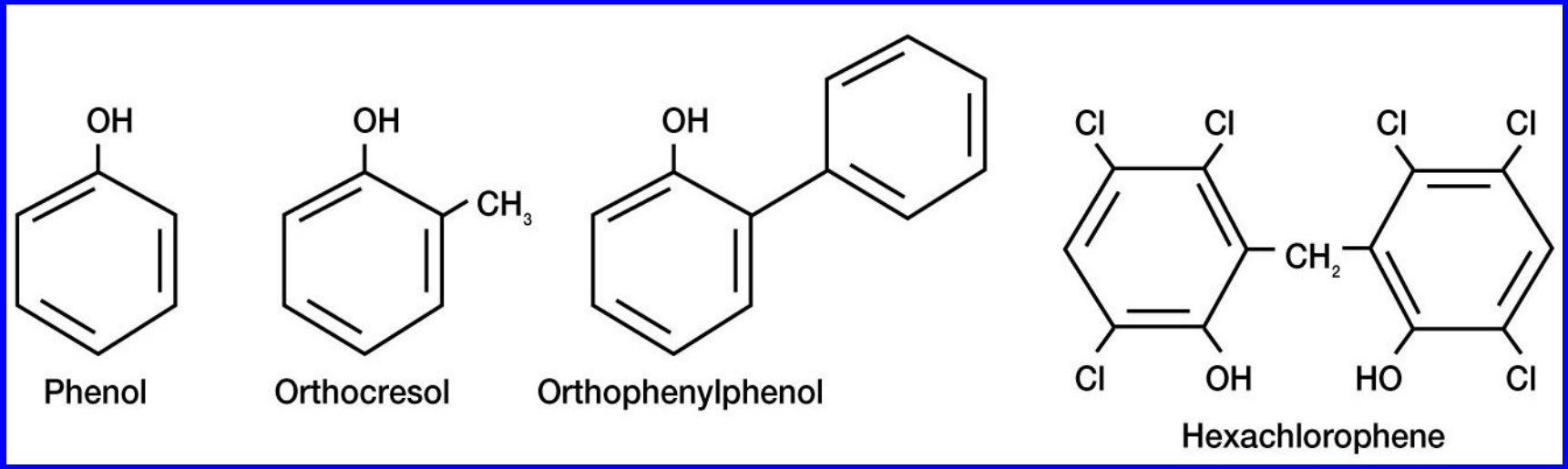


Figure 7.7

Phenolics

- commonly used as laboratory and hospital disinfectants
- act by denaturing proteins and disrupting cell membranes
- tuberculocidal, effective in presence of organic material, and long lasting
- disagreeable odor and can cause skin irritation

Alcohols

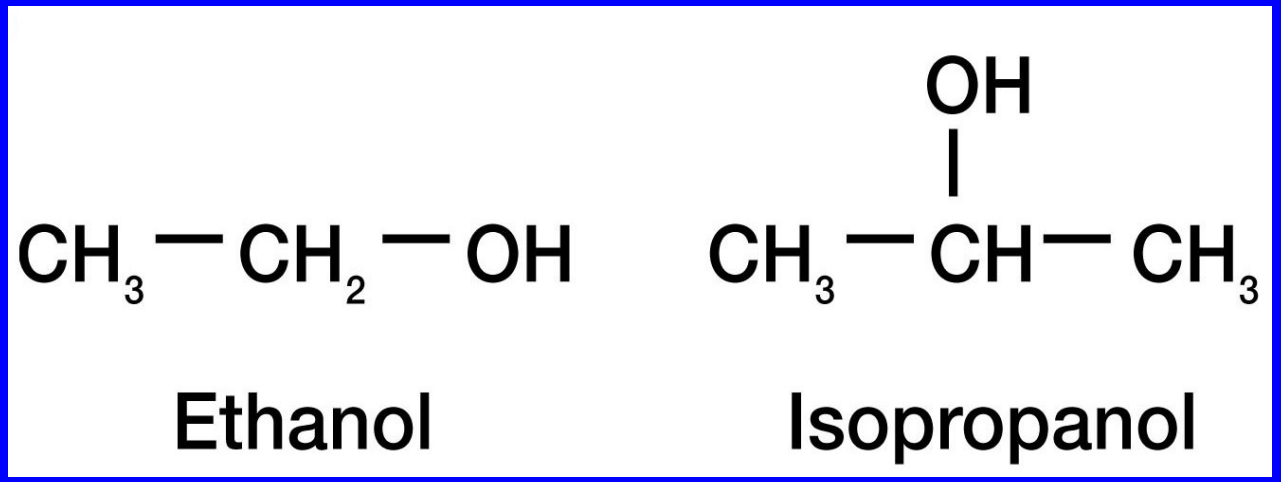


Figure 7.7

- **bactericidal, fungicidal, but not sporicidal**
- **inactivate some viruses**
- **denature proteins and possibly dissolve membrane lipids**

Halogens

- e.g., iodine
  - skin antiseptic
  - oxidizes cell constituents and iodinates proteins
  - at high concentrations may kill spores
  - skin damage, staining, and allergies can be a problem
  - **iodophore**
    - iodine complexed with organic carrier

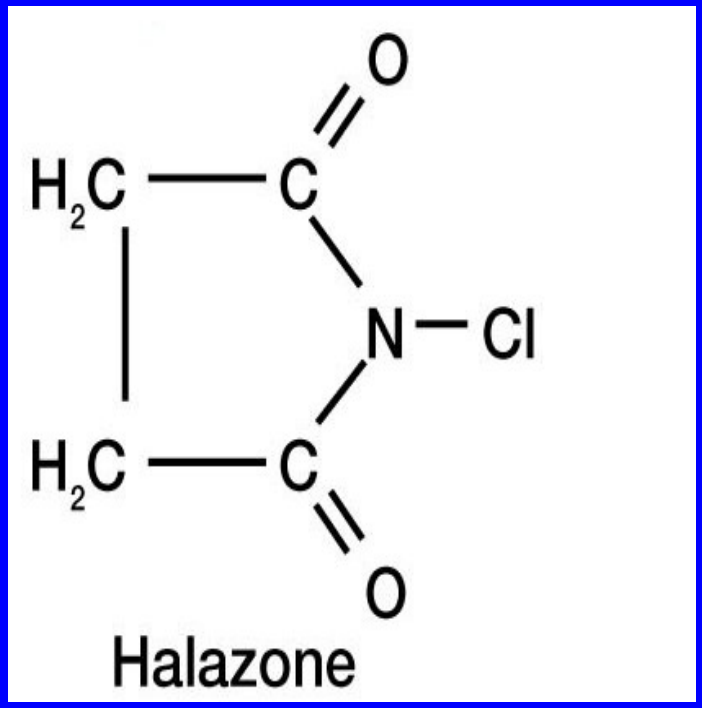


Figure 7.7

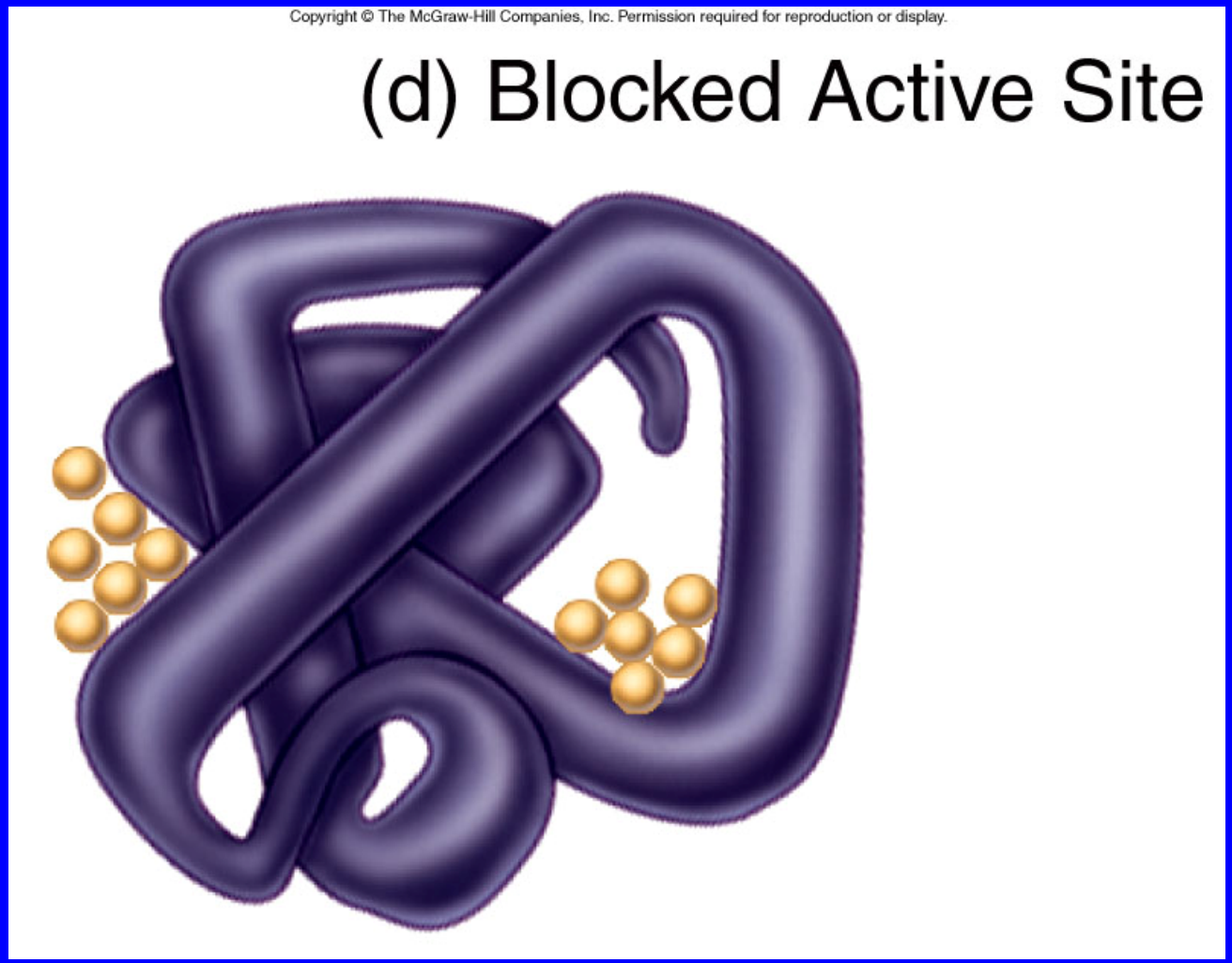
Halogens…

- e.g., chlorine
  - oxidizes cell constituents
  - important in disinfection of water supplies and swimming pools, used in dairy and food industries, effective household disinfectant
  - destroys vegetative bacteria and fungi, but not spores
  - can react with organic matter to form carcinogenic compounds

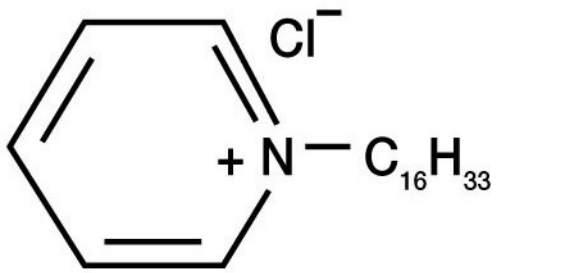
Heavy Metals

- e.g., ions of mercury, silver, arsenic, zinc, and copper
- effective but usually toxic
- combine with and inactivate proteins; may also precipitate proteins

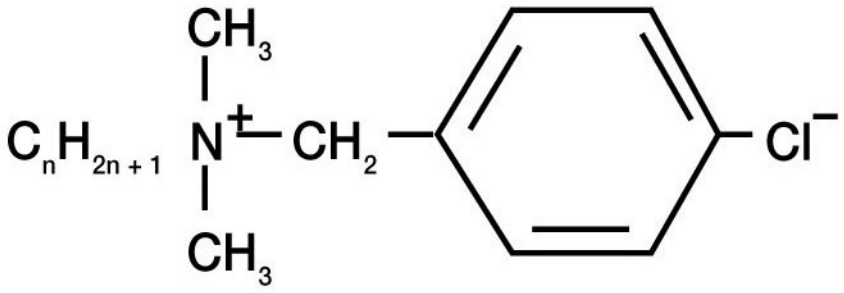
Heavy Metal Mode of Action



Quaternary Ammonium Compounds



Cetylpyridinium chloride



Benzalkonium chloride

Figure 7.7

Quaternary Ammonium Compounds

- **detergents**
  - **organic molecules with hydrophilic and hydrophobic ends**
  - **act as wetting agents and emulsifiers**
- **cationic detergents are effective disinfectants**
  - **kill most bacteria, but not *Mycobacterium tuberculosis* or endospores**
  - **safe and easy to use, but inactivated by hard water and soap**

Aldehydes

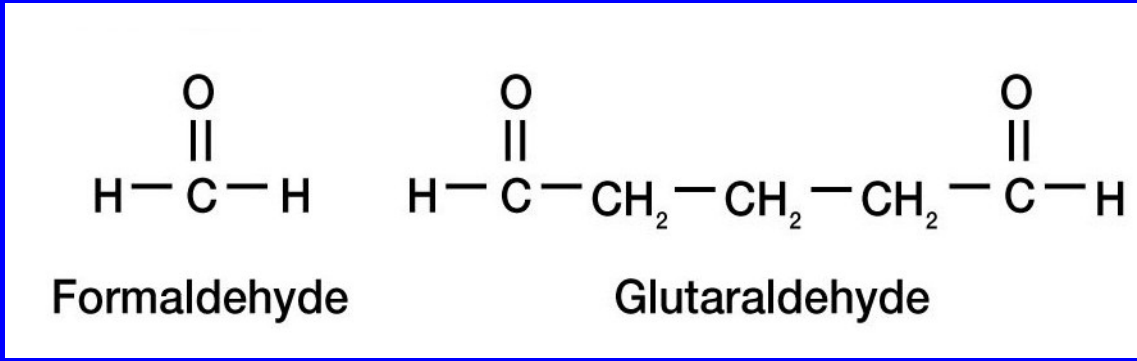


Figure 7.7

- highly reactive molecules
- sporicidal and can be used as chemical sterilants
- combine with and inactivate nucleic acids and proteins

Alkylating Agents React with Amino Acids.