Chapter 11

Topics: Controlling Microorganisms

- Physical Control

- Chemical control

An overview of the microbial control methods.

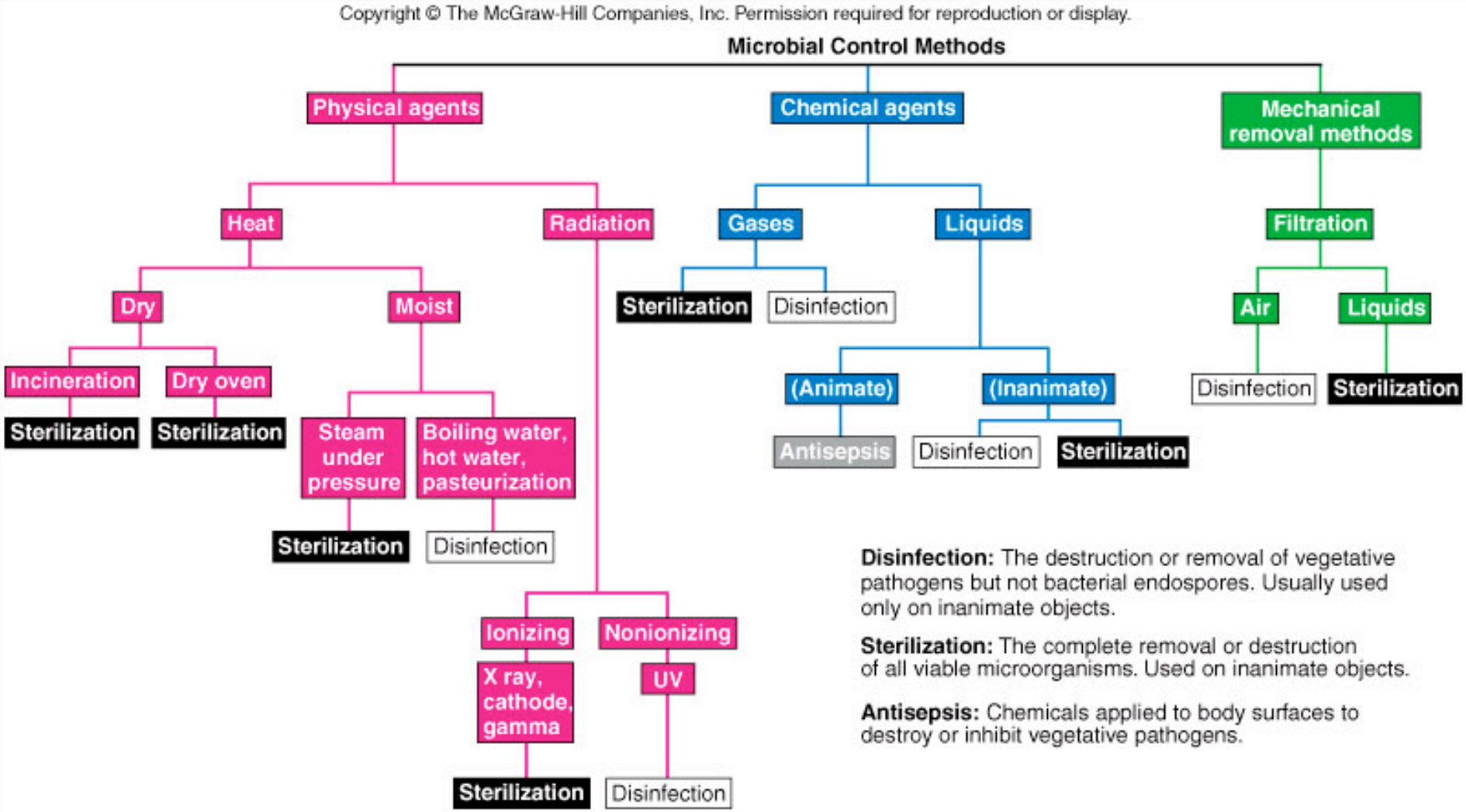


Fig. 11.1 Microbial control methods

Controlling Microorganisms

- **Microbial agents**

- Terms
- Effectiveness
- Mode of action

Microbial agents

- -static agents
- -cidal agents

~ static agents

- Temporarily preventing the growth of microbes

Stasis and *static*: to stand still

  - Bacteri(o)static: prevent the growth of bacteria
  - Fungistatic: inhibit fungal growth
  - Microbistatic: materials used to control microorganisms in the body, for example

~ cidal agents

- Killing or destroying a microorganism

cide: to kill

  - Bacteri(o)cide: chemical that destroys bacteria (not endospores)
  - Fungicide: a chemical that kills fungal spores, hyphae and yeast cells
  - Virucide: a chemical that destroys viruses
  - Sporicide: can destroy bacterial endospores
  - Germicide and microbicide: chemical agents that kill microorganisms

Controlling Microorganisms

- Microbial agents

- **Terms**

- Effectiveness
- Mode of action

Terms

- Resistance - to destruction by agents
- Sterilization - the destruction of all life
- Disinfection - removal of live from inanimate surfaces
- Antisepsis - reducing contamination on a living surface
- Decontamination - reducing microbial count
  - De-germination
  - Sanitation

Resistance against **agents**

- Highest resistance - bacterial spores and prions
- Moderate resistance - some bacteria, protozoan cysts, fungal sexual spores, naked viruses
- Least resistance - most bacteria, fungal nonsexual spores and hyphae, enveloped viruses, yeast, protozoan trophozoites

Comparing the resistance between endospores and vegetative cells to control agents.

TABLE 11.1 Relative Resistance of Bacterial Endospores and Vegetative Cells to Control Agents

Method	Endospores*	Vegetative Forms*	Relative Resistance**
Heat (moist)	120°C	80°C	1.5×
Radiation (X-ray) dosage	4000 Grays	1000 Grays	4×
Sterilizing gas (ethylene oxide)	1,200 mg/1	700 mg/1	1.7×
Sporicidal liquid (2% glutaraldehyde)	3 h	10 min	18×

*Values are based on methods (concentration, exposure time, intensity) that are required to destroy the most resistant pathogens in each group.

**The greater resistance of spores versus vegetative cells given as an average figure.

Table 11.1 Relative resistance of bacterial endospores and vegetative cells

Sterilization

- A process that destroys or removes all forms of live (usually from inanimate objects) including viable microorganisms (including spores) and viruses
- Physical (usually heat) or chemical agents (sterilants)
- Inanimate objects
  - Surgical instruments,
  - commercially packaged foods

Disinfection

- Use of physical process or chemical agent (disinfectant) to destroy vegetative pathogens.
- Usually used only on inanimate objects
- Also removes toxins

- Does not eliminate bacterial endospores

Antisepsis

- When chemical agents (antiseptics) destroy or inhibit vegetative pathogens from animate surfaces
  - Skin and mucous membranes
- **Sepsis**: the growth of microorganisms in the blood and other tissues
- **Asepsis**: any practice that prevents the entry of infectious agents into sterile tissues

Decontamination

- Used when actual sterilization isn't needed but need to decrease the risk of infection or spoilage (ex. food industry)
  - **Sanitization**
  - **Degermation**

De-germination

- Reduces the numbers of microbes on the human skin (ex. alcohol wipes)
- Physical and chemical agents

Sanitation

- Any cleansing technique that mechanically removes microorganisms (or substratum that could support their growth) to reduce contamination to safe levels
- **Sanitizer**: compound such as soap or detergent that sanitizes
- **Sanitary**: may not be free from microbes but are safe for normal use

- Physical chemical agents

Practical Concerns in Microbial Control

- Does the application require sterilization, or is disinfection adequate?
- Is the item to be reused or permanently discarded?
- If it will be reused, can it withstand heat, pressure, radiation, or chemicals?
- Is the control method suitable for a given application?
- Will the agent penetrate to the necessary extent?
- Is the method cost- and labor-efficient, and is it safe?

Controlling Microorganisms

- Microbial agents
- Terms

- **Effectiveness**

- Mode of action

What is Microbial Death?

- When various cell structures become dysfunctional and the entire cell sustains irreversible damage
- If a cell can no longer reproduce under ideal environmental conditions
- Death begins when a certain threshold of microbicidal agent is met, and continues in a logarithmic manner

Effectiveness

- Number of microorganisms
- Targets (bacteria, fungi, spores, viruses)
- Temperature and pH
- Concentration of agent
- Mode of action
- Interfering agents (solvents, debris, saliva, blood, feces)

Factors that influence the effectiveness of antimicrobial agents.

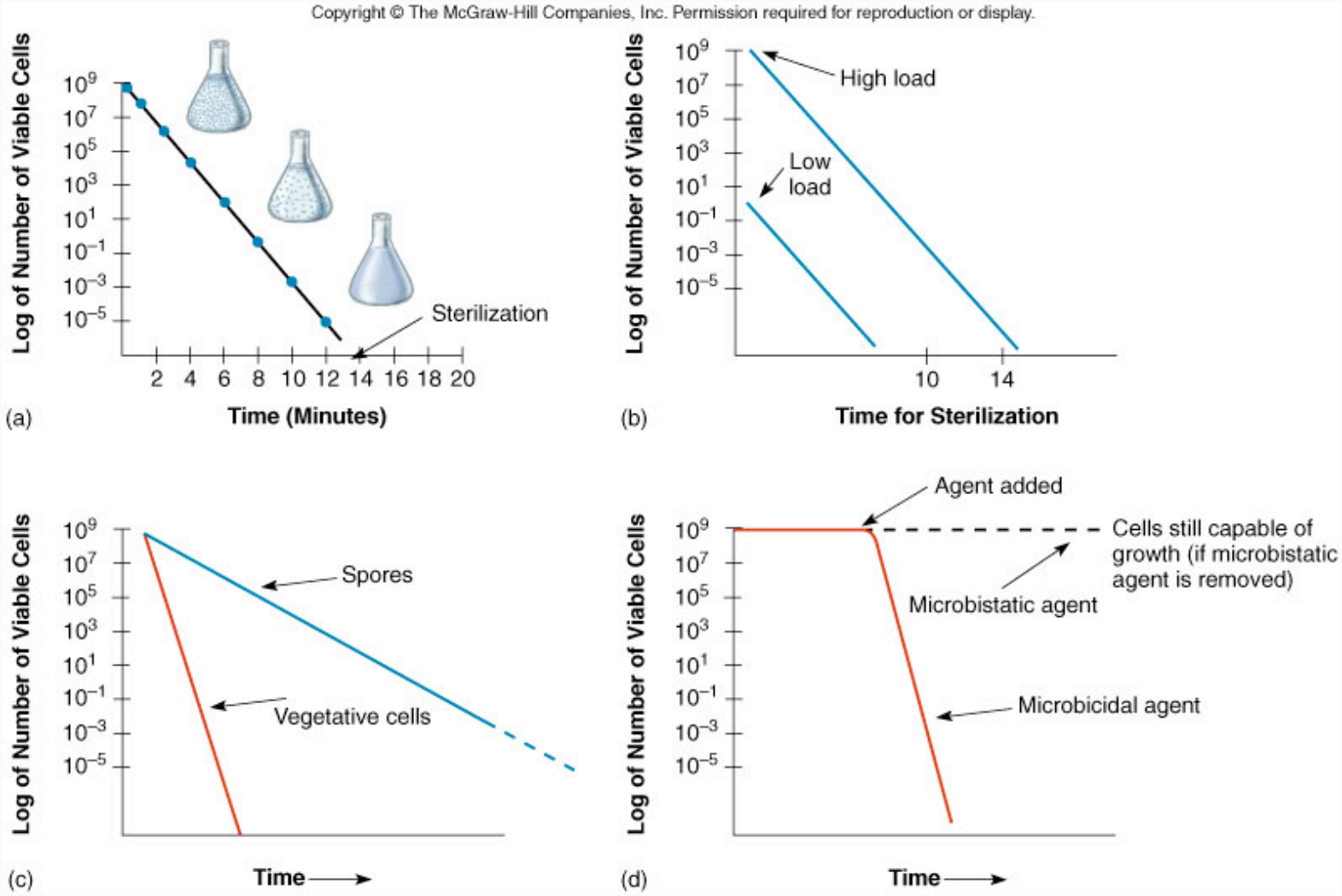


Fig. 11.2 Factors that influence the rate at which Agents are killed by antimicrobial agents (picture does not address virus).

Mode of action

- **Cell wall**
- **Cell membrane**

- Nucleic acid synthesis
- Protein synthesis
- Protein function

Cell wall

- Agent
  - Penicillin (Cephalosporin), Vancomycin, Bacitracin,
  - detergents, alcohols

- Bacteria and fungi
  - Block synthesis
  - Degrade cellular components
  - Destroy or reduce stability

Membrane

- Agents:
  - Nisin, Gramicidin, Polymyxin
  - Surfactants
- All microbes and enveloped viruses
  - Disruption of membrane function (pores)
  - Bind and penetrate lipids
  - Lose selective permeability (leakage)

The effect of surfactants on the cell membrane.

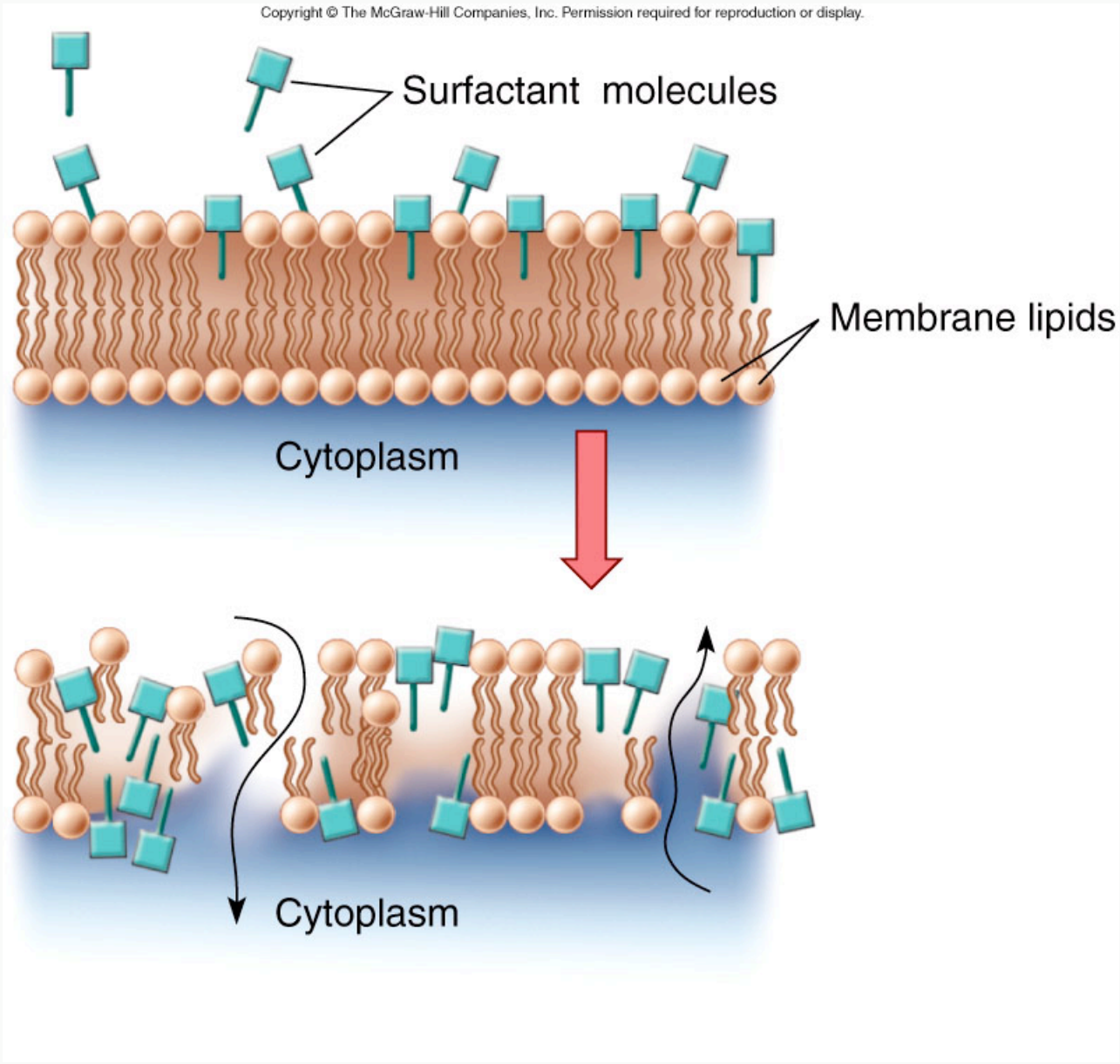


Fig. 11.3 Mode of action of surfactants

Mode of action

- Cell wall
- Cell membrane

- **Nucleic acid synthesis**

- Protein synthesis
- Protein function

Nucleic acid synthesis

- Agent:
  - Chemical agent
    - formaldehyde; Trimethoprim, Novobiocin, Nalidixic Acid, Rifamp(ic)in
  - Physical agent
    - radiation
- Action:
    - Mutations: Irreversible injury to DNA
    - Inhibition of nucleotide biosynthesis
    - Inhibition of DNA-gyrase
    - Inhibition of DNA-polymerase
    - Inhibition of RNA-polymerase
- Consequence: Stop of replication, transcription and (eventually) translation

Mode of action

- Cell wall
- Cell membrane
- Nucleic acid synthesis

- **Protein synthesis**
- **Protein function**

Protein synthesis

- Agents:
  - Chloramphenicol, Tetracyclines*, Aminoglycosides; Macrolides, Puromycin
- bind to ribosomes (30S subunit, 50S subunit)
  - Stops initiation, elongation* or termination
  - Prevents peptide bond formation

Protein function

- Agent
  - Physical – Heat, pH change
  - Chemical – alcohols, acids, phenolics, metallic ions
- Block protein active sites
- Prevent binding to substrate
- Denature protein

The effects of heat, pH, and blocking agents on the function of proteins.

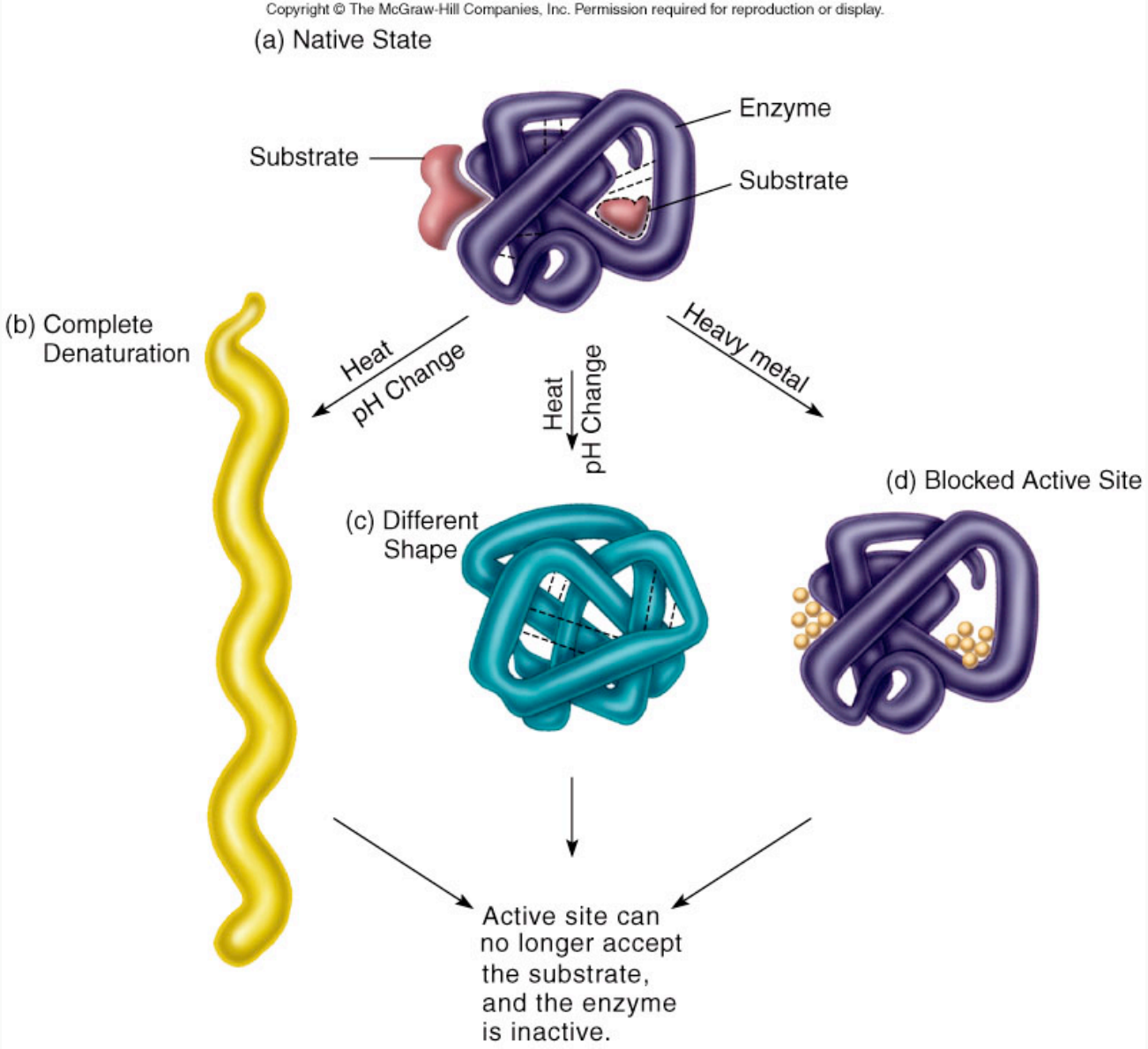


Fig. 11.4 Modes of action affecting protein function

Physical Control

- **Heat**
- Radiation
- Filtration

Heat - Mode of action

- Moist heat
  - Coagulation of proteins
  - Denaturation of proteins
- Dry heat
  - Dehydration
  - Denaturation
  - Oxidation (burning to ashes)
- Thermal death time

Moist heat

- Steam and pressure
- Tyndallization
- Pasteurization
- Boiling water

Steam and pressure

- Pressure above normal atmospheric pressure will result in temperatures above 100˚C
- Effectively destroys spores
- Sterilizes inanimate objects (glassware)
- Ex. Autoclave and home pressure cooker

A diagram of an autoclave.

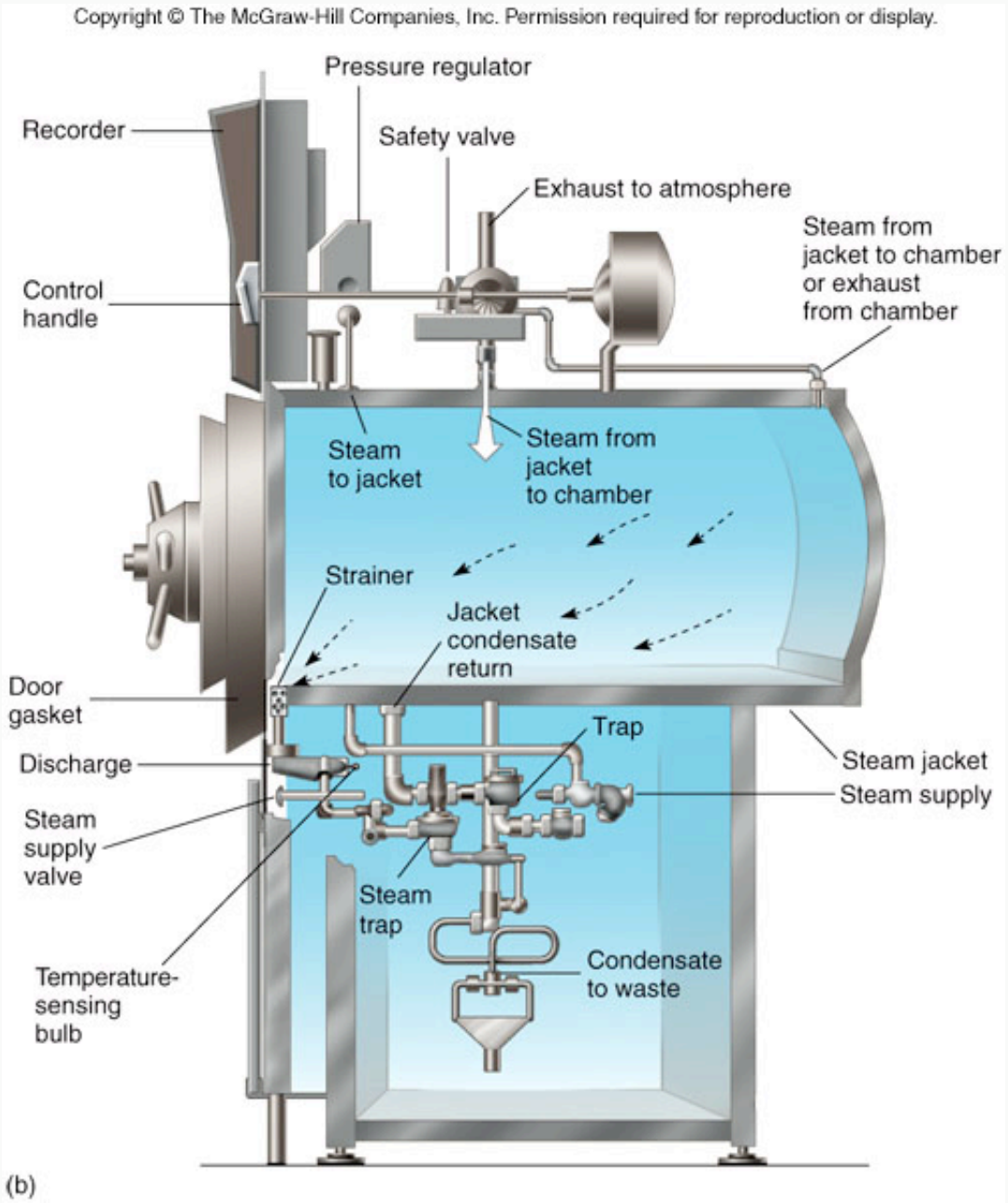


Fig. 11.5 Steam sterilization with the autoclave

Tyndallization

- Intermittent sterilization
- Used for heat-sensitive media, canned foods
- Will not destroy spores
- Ex. Exposure to free-flowing steam for 30 to 60 minutes

Pasteurization

- Disinfection of beverages
- Exposes beverages to 71.6 ˚C for 15 seconds
  - Stops fermentation
- Prevents the transmission of milk-borne diseases
  - *Salmonella, Campylobacter, Listeria, Mycobacteria*
- Examples: Milk industry, wineries, breweries

Boiling water

- Decontaminates at 100 ˚C for 30 minutes
- Kills most non-spore forming pathogens
- Examples: home sanitizing and disinfecting, disinfecting unsafe water

Dry heat

- Hot air
- Incineration
- Temperature and time of exposure is greater than moist heat

Hot air

- Hot air
  - Oven
  - Effective at 150˚C to 180˚C for 2-4 hrs
  - Effective for inanimate objects and oils

Incineration

- Destroys microbes to ashes or gas
  - Flame - 1870˚C
  - Furnace - 800˚C to 6500˚C

An infrared incinerator uses flame to burn or oxidize materials into ashes.

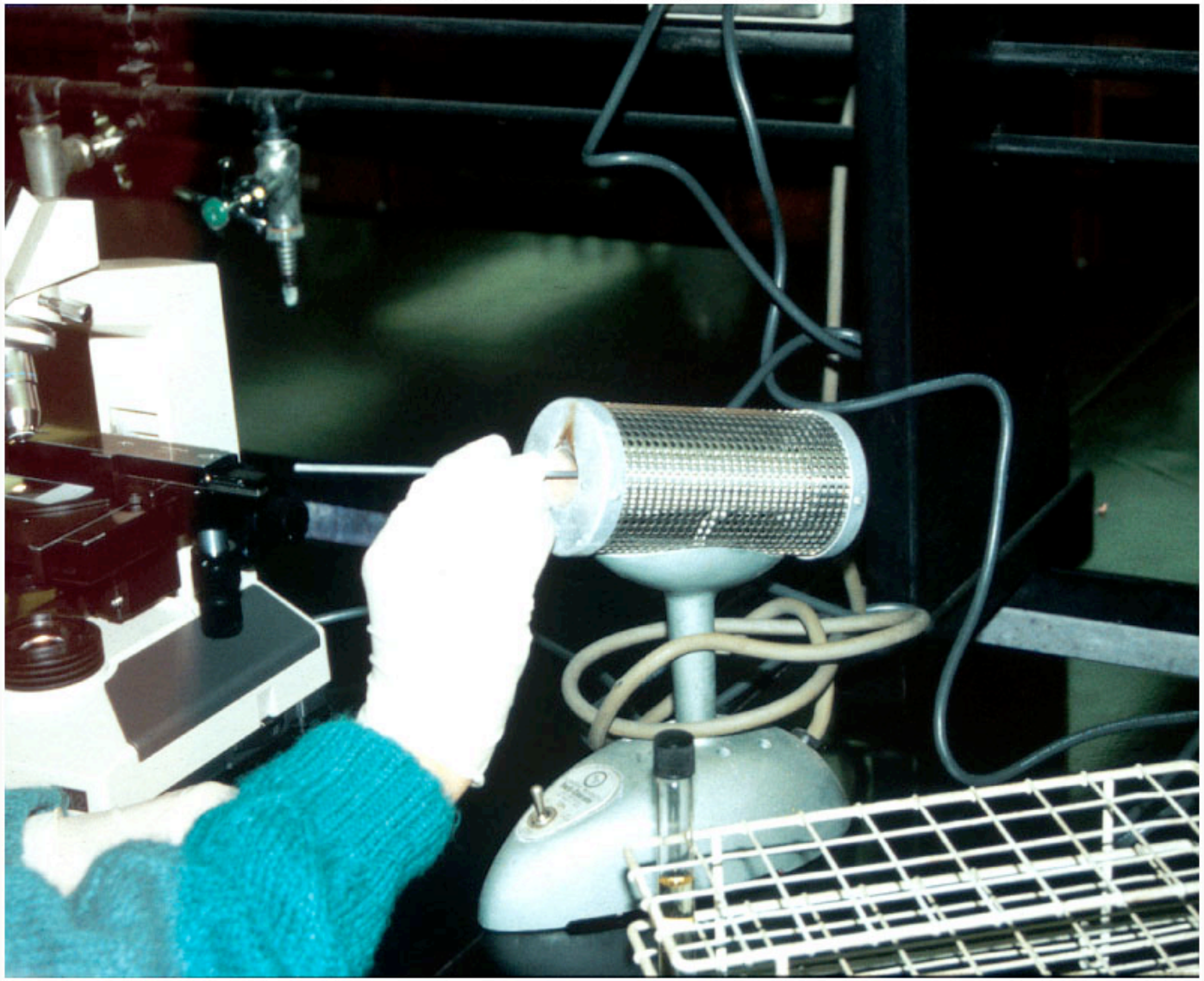


Fig. 11.6 Dry heat incineration

Dry Oven

- Usually an electric oven
- Coils radiate heat within an enclosed compartment
- Exposure to 150°C to 180°C for 2 to 4 hours
- Used for heat-resistant items that do not sterilize well with moist heat

Effects of cold and desiccation

- Cold temperatures reduce the activity of some microbes, but NOT psychrophiles
  - Not a disinfection or sterilization method
- Desiccation or dehydration kill some microorganisms
  - Lyophilization – freezing and drying method used to preserve microbes

Physical Control

- Heat
- **Radiation**
- Filtration

Radiation

- Types of radiation
- Modes of action
- Applications

Types of radiation

- Ionizing
  - Gamma rays (High energy)
  - X-rays (Intermediate energy)
  - Cathode rays (least energy)
- Non-ionizing
  - Ultraviolet

Mode of actions

- Ionizing radiation ejects orbital electrons from an atom
  - High energy
    - Penetrates liquids and solids effectively
- Non-ionizing radiation raises atoms to a higher energy state
  - Low energy
    - Less penetration capability
    - Pyrimidine dimers

The effects of ionizing and non-ionizing radiation on DNA.

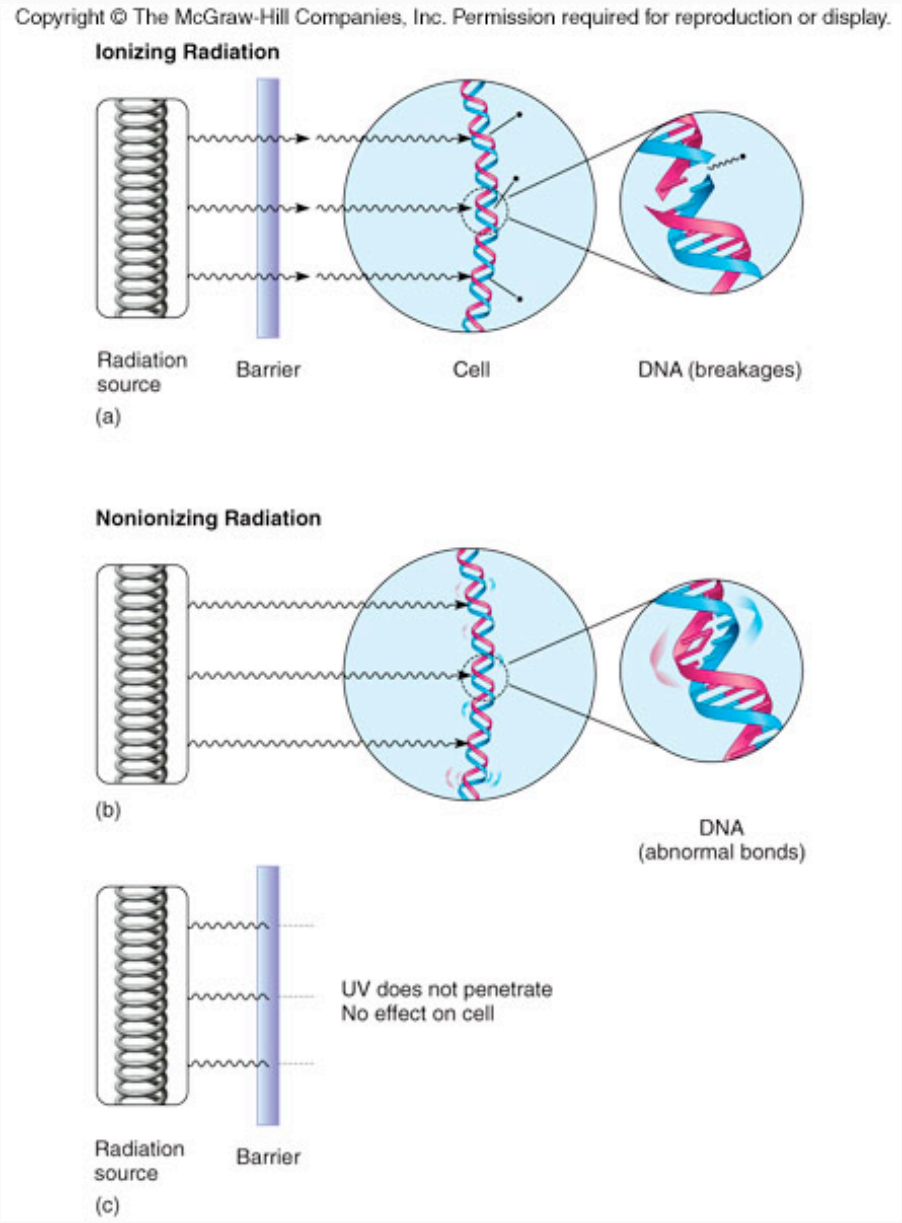


Fig. 11.7 Cellular effects of irradiation

Ultraviolet (UV) radiation can cause the formation of pyrimidine dimers on DNA.

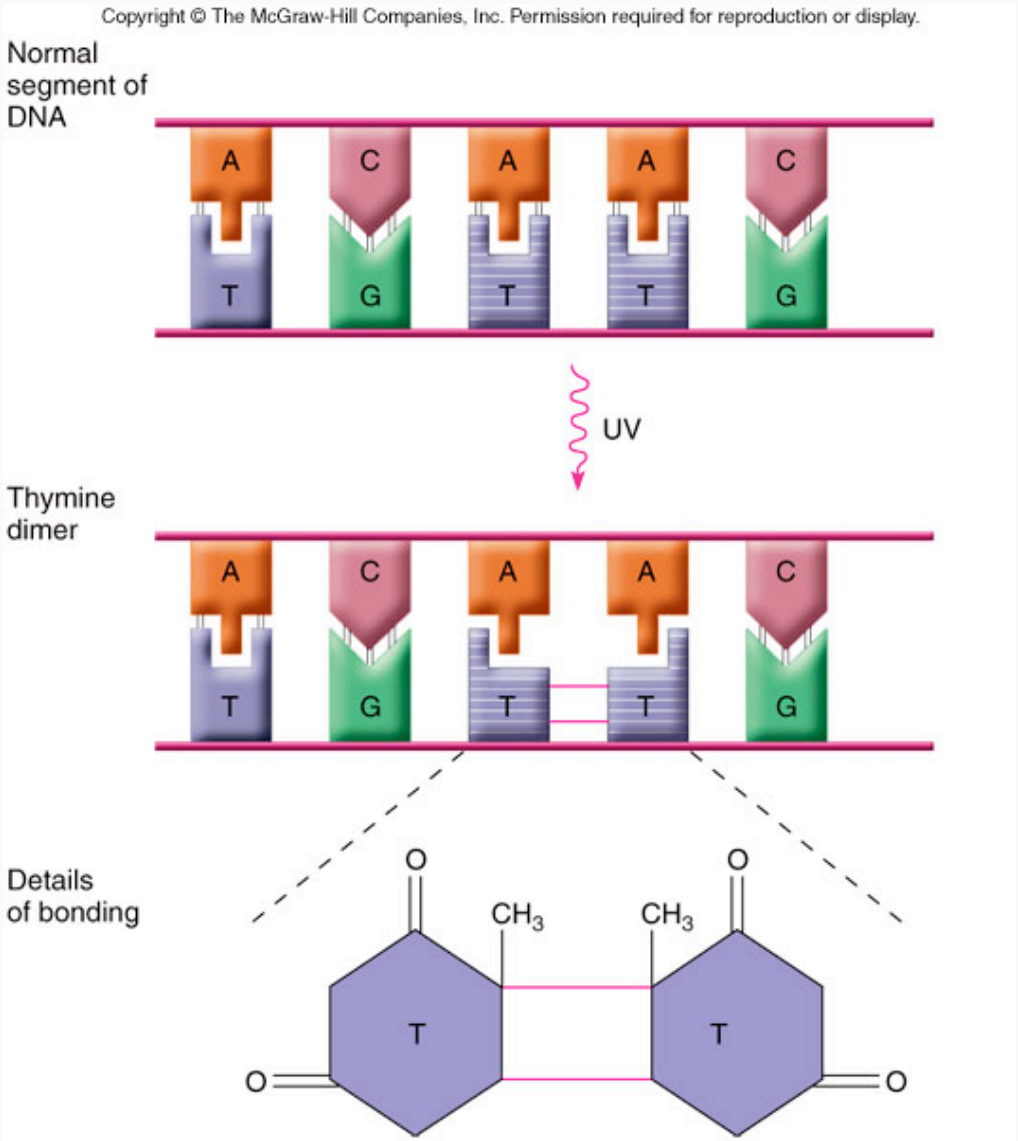


Fig. 11.9 Formation of pyrimidine dimers by the action of UV radiation.

Applications

- Ionizing radiation
  - Alternative sterilization method
  - Materials sensitive to heat or chemicals
  - Some foods (fruits, vegetables, meats)

- Non-ionizing radiation
  - Alternative disinfectant
  - Germicidal lamp in hospitals, schools, food preparation areas (inanimate objects, air, water)

A gamma radiation machine (ionizing radiation) used to sterilize fruits, vegetables, meats, fish, and spices.

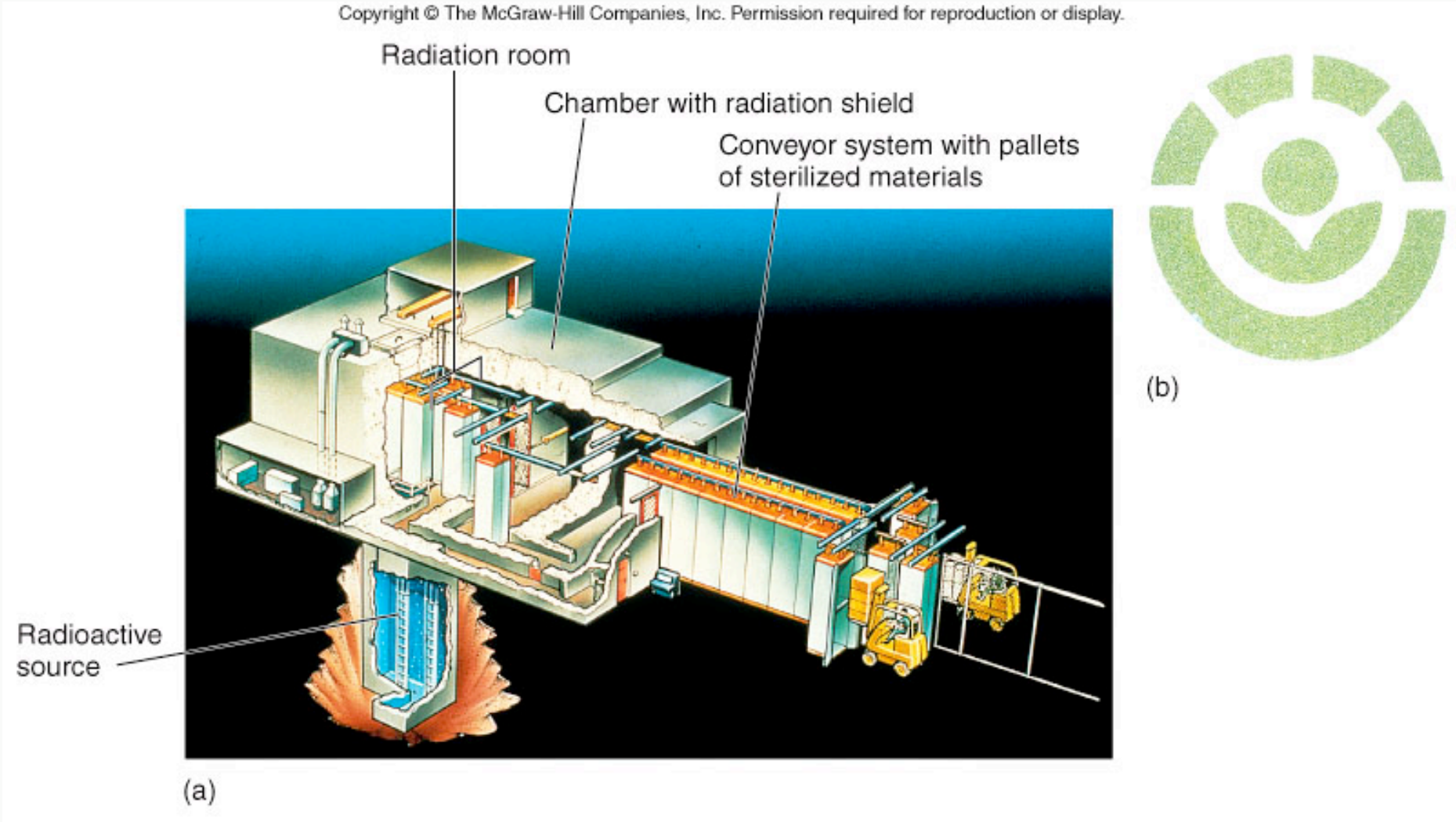


Fig. 11.8 Sterilization with Ionizing Radiation

A UV treatment system can be used to disinfect water.

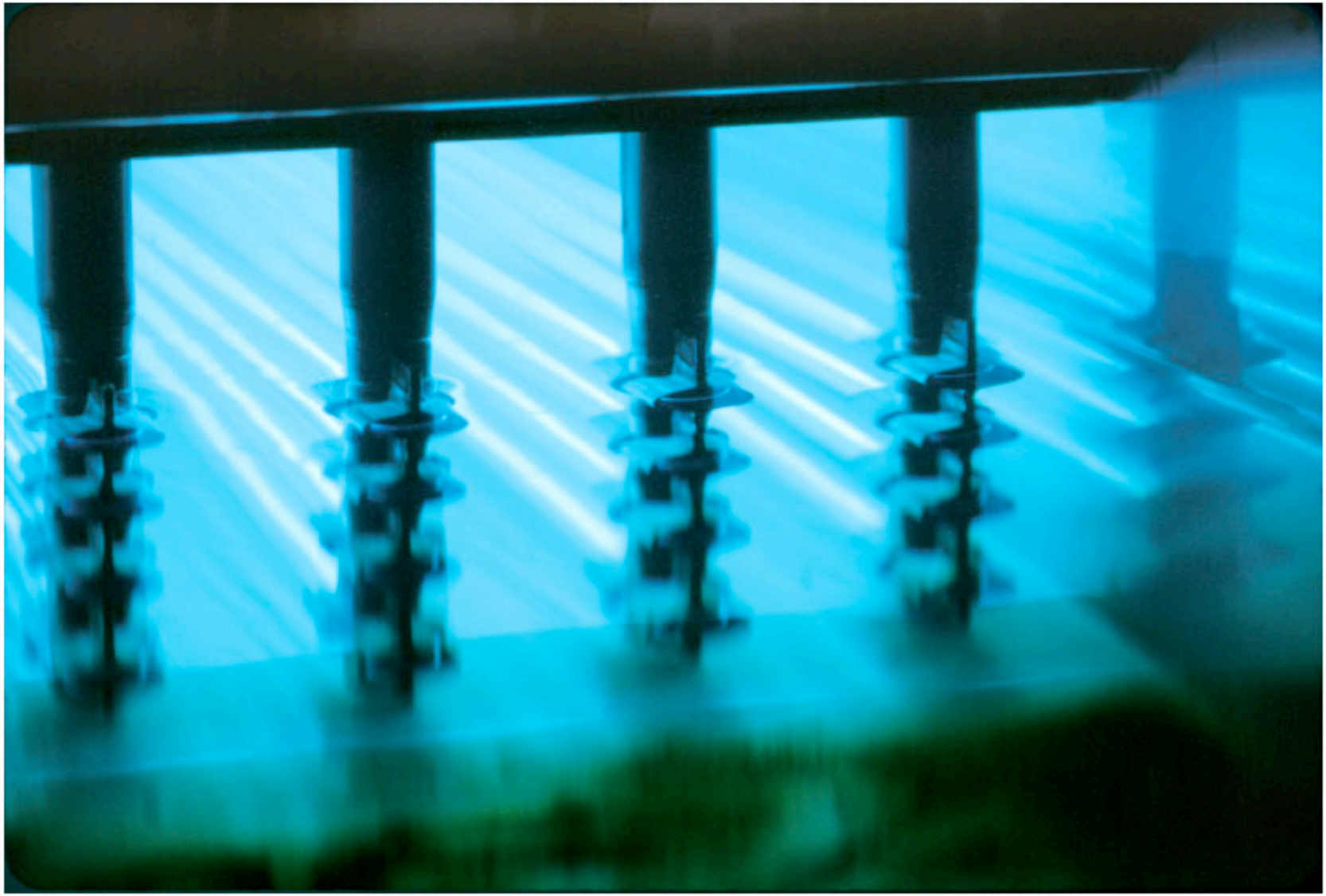


Fig. 11.10 A UV treatment system for disinfection of water

Physical Control

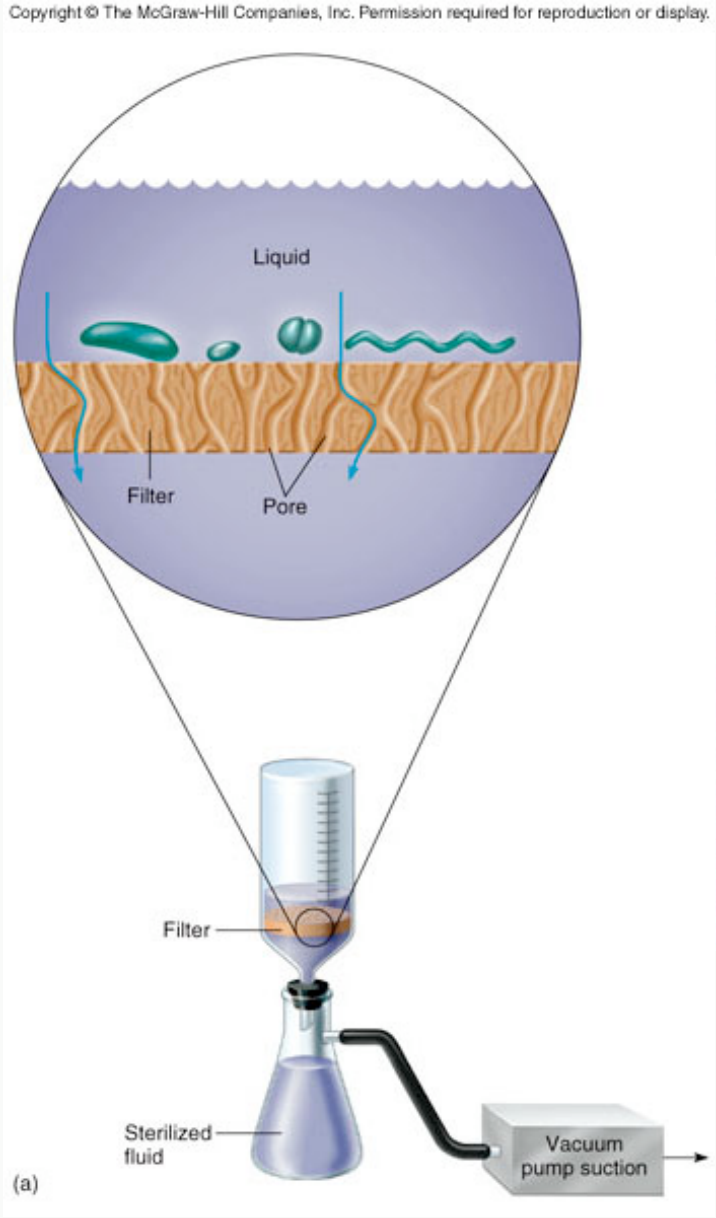
- Heat
- Radiation

- **Filtration**

Filtration

- Removes microbes and spores from liquids and air
- Perforated membrane
  - Pore sizes vary
- Applications
  - Liquids that are sensitive to heat
    - Serum, vaccines, media

An example of a filtration system.



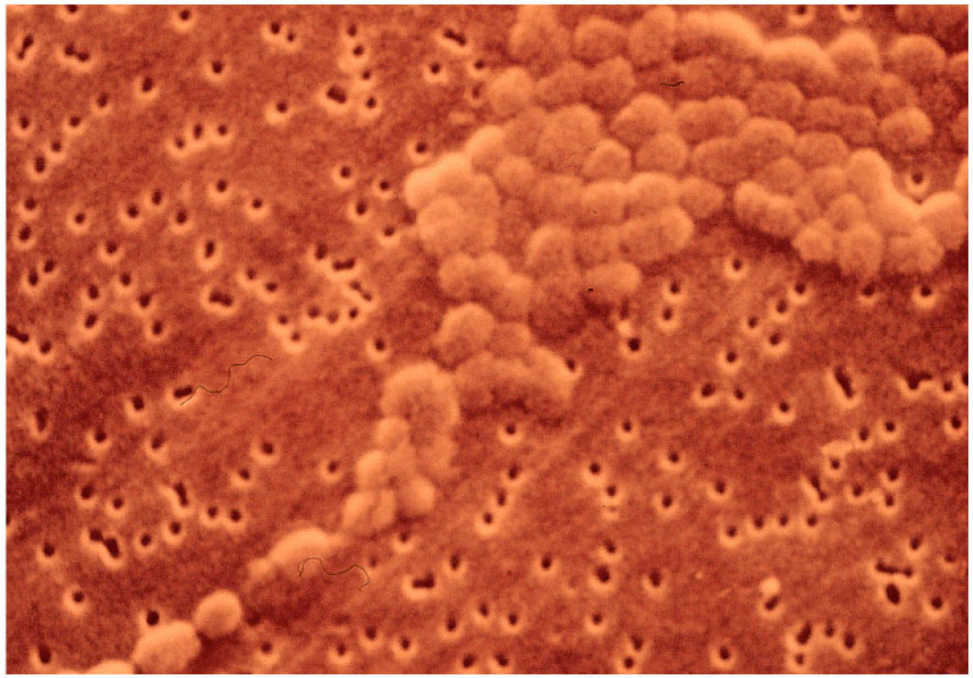


Fig. 11.11 Membrane filtration

Chemical control

- **Widely used agents and their Applications**

Example of chemical agents, their target microbe, level of activity, and toxicity.

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.

Table 11.5 Qualities of chemical agents used in health care

Applications

- Halogens
- Phenolics
- Surfactants
- Hydrogen peroxide
- Detergents and soaps
- Heavy metals
- Aldehydes
- Gases
- Dyes, acids, and alkalis

Halogens

- Chlorine
  - Disinfectant and antiseptic
    - Disrupt sulfhydryl groups in amino acids
- Iodine
  - Topical antiseptic
    - Disruption is similar to chlorines

Phenolics

- Vary based on functional groups attached to the aromatic ring

- Examples: Hexachlorophene, Triclorsan
  - Microcidal
  - Ingredient in soaps to kitty litter
    - Disrupts cell walls and membranes,

Phenolics contain a basic phenolic aromatic ring with different functional groups.

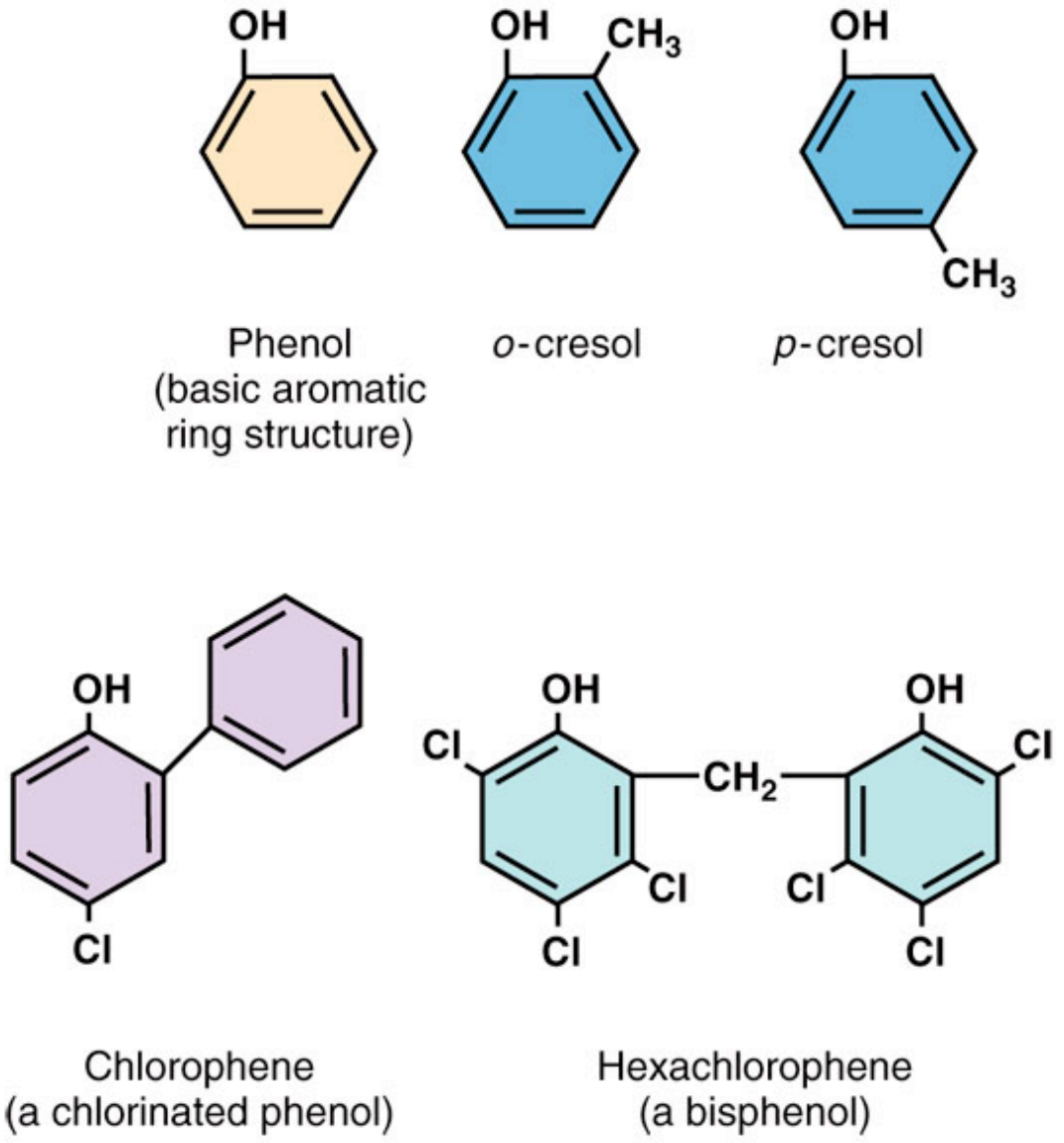


Fig. 11.12 Some phenolics

Alcohols

- Ethyl alcohol, isopropyl (rubber alcohol)
  - 70% concentration dissolve membrane lipids, disrupt cell surface tension, denatures proteins
- Germicidal and skin de-germing

Hydrogen peroxide

- Colorless and caustic liquid
- Form hydroxyl free radicals
  - Effective against anaerobes
- Skin and wound cleaner
- Quick method for sterilizing medical equipment

Examples of different devices used to sterilize medical equipment.

Fig. 11.13 Sterile processing of invasive equipment protects patients.

Detergents and soaps

- Quaternary ammonium (quats)
  - Cationic
  - Bind and disrupt cell membrane
  - Low-level disinfectant in the clinical setting
- Soaps
  - Fatty acids, oils, sodium or potassium salts
  - Cleaning agents
  - More effective if mixed with germicides

For detergents, the positive charge region binds bacteria and the uncharged region integrates into the cell membrane.

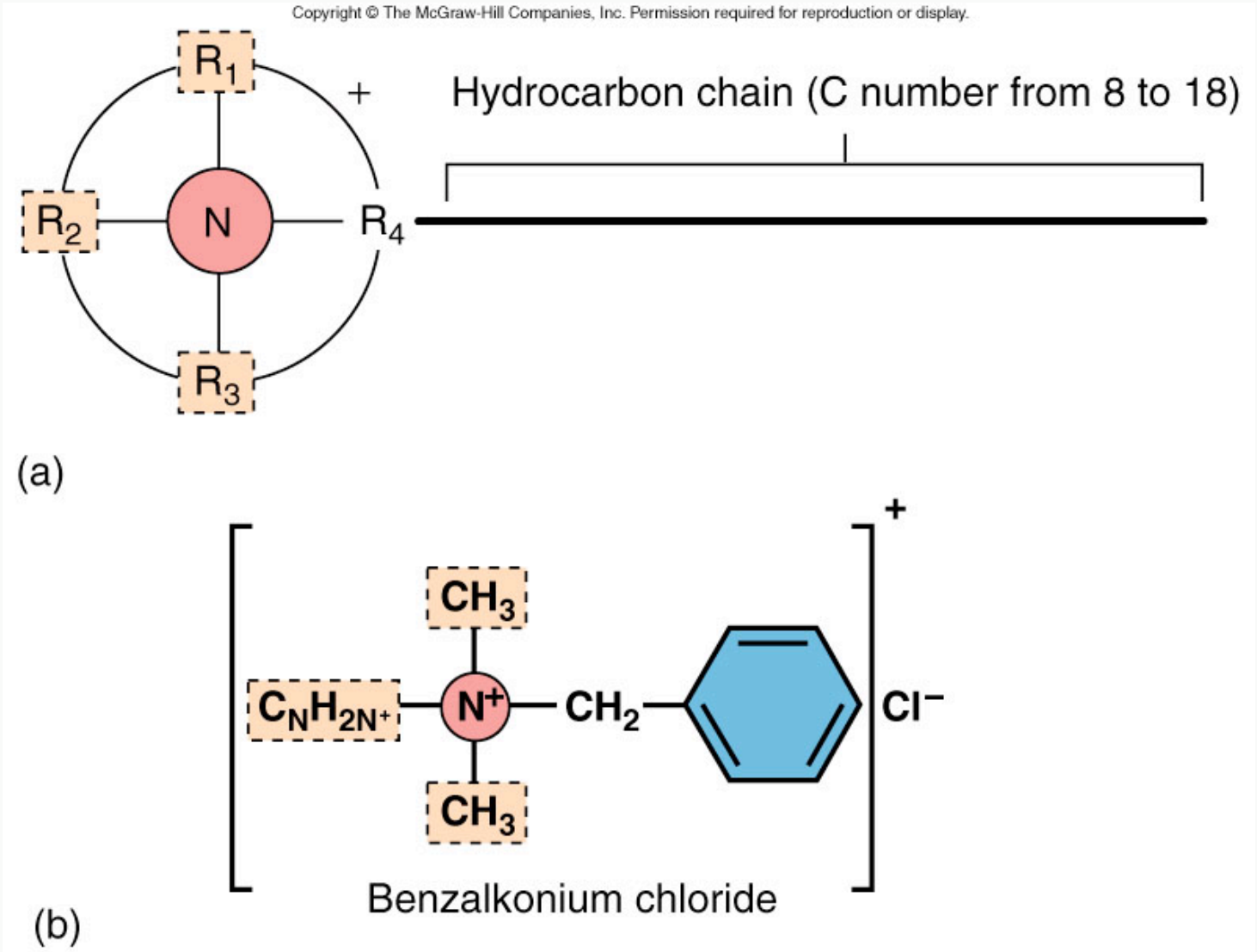


Fig. 11.14 The structure of detergents

Comparison between non-germicidal and germicidal soaps.

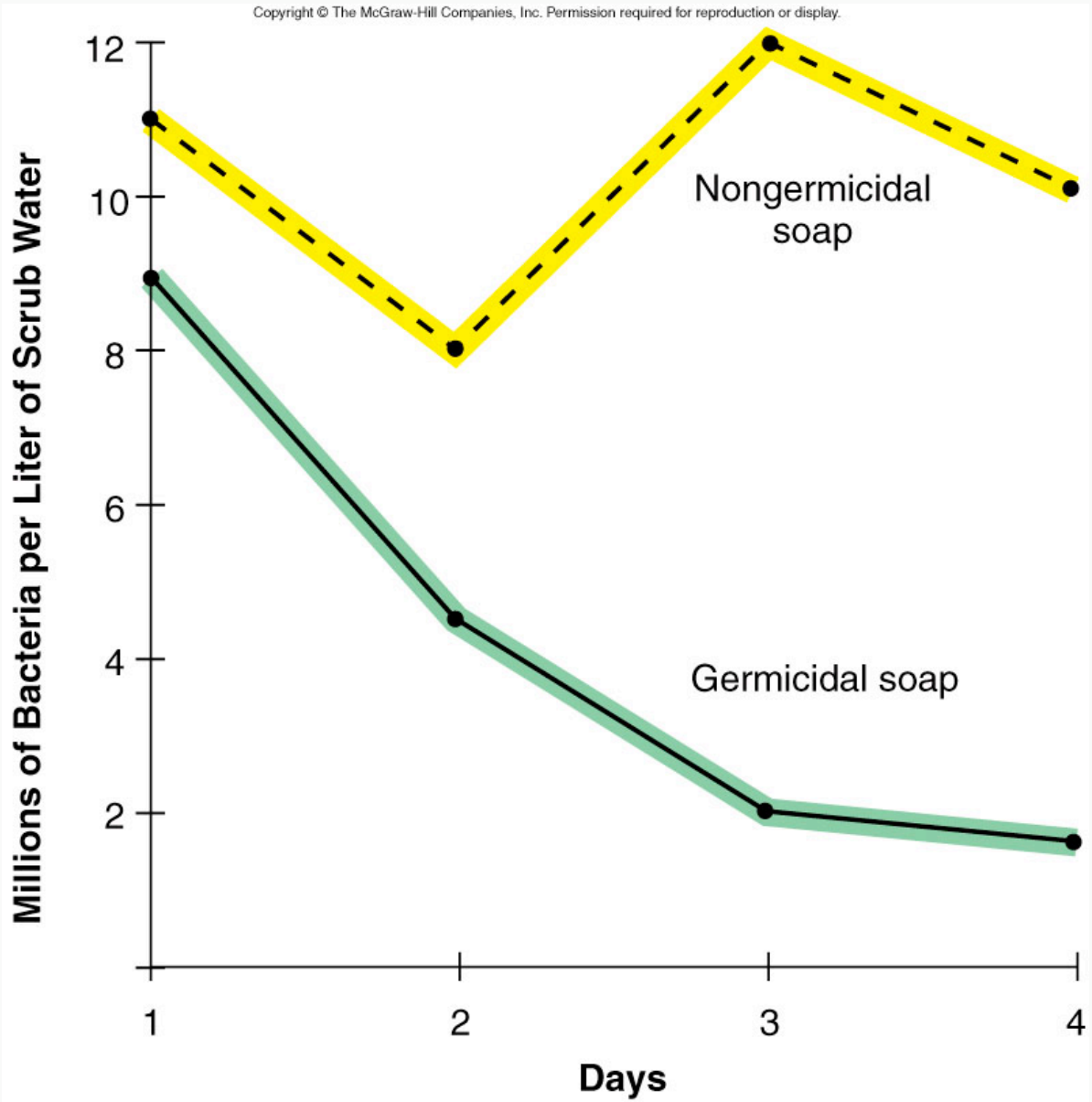


Fig. 11.15 Graph showing effects of handscrubbing

Heavy metals

- Mercury, silver,
  - Inactivate proteins
  - Preservatives in cosmetics and ophthalmic solutions

Demonstration of the effects silver and gold have on microbial growth.

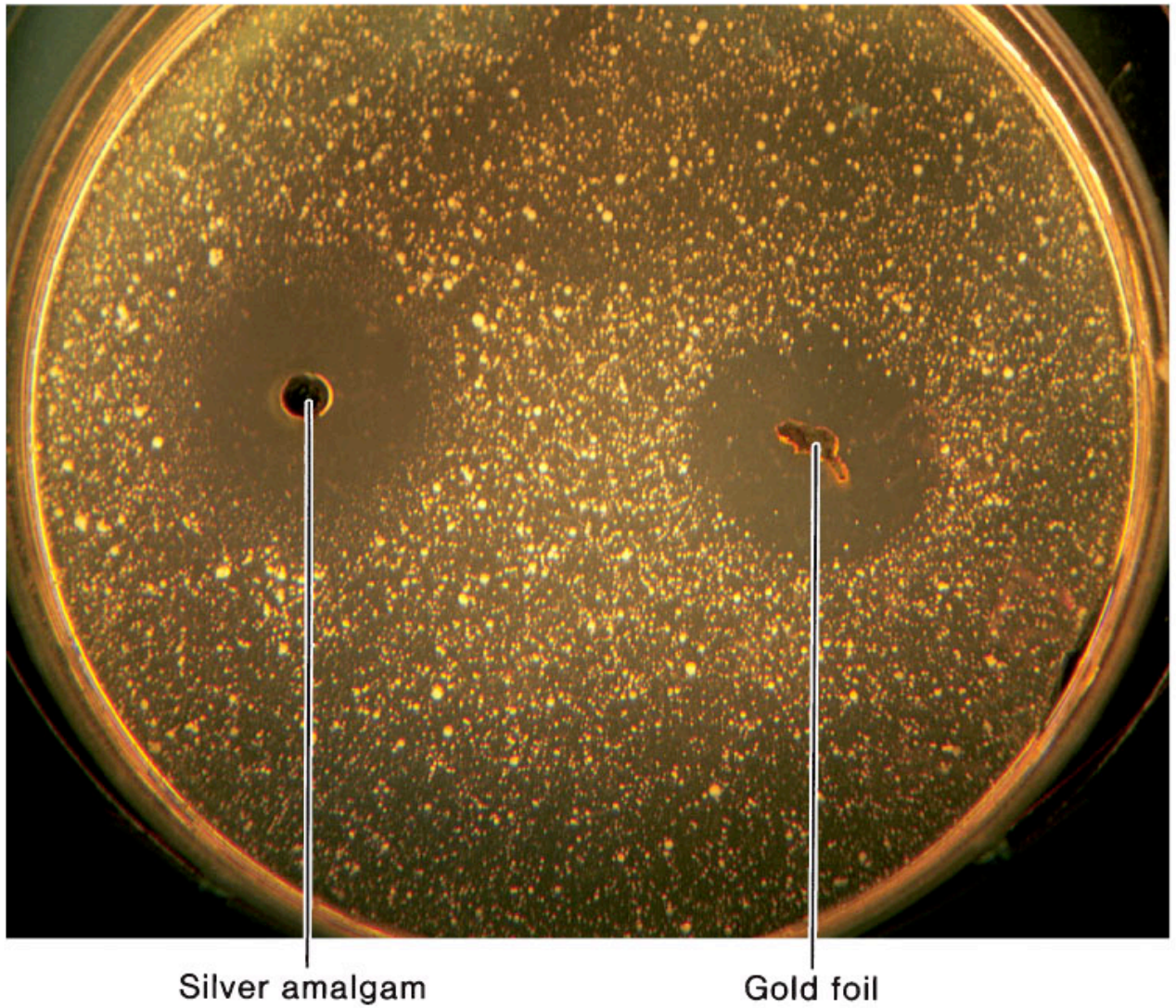


Fig. 11.16 demonstration of the oligodynamic action of heavy metals.

Aldehydes

- Glutaraldehyde
  - Crosslink with proteins on the cell surface
  - Disinfectant for surgical instruments

Representation of the action of glutaraldehyde.

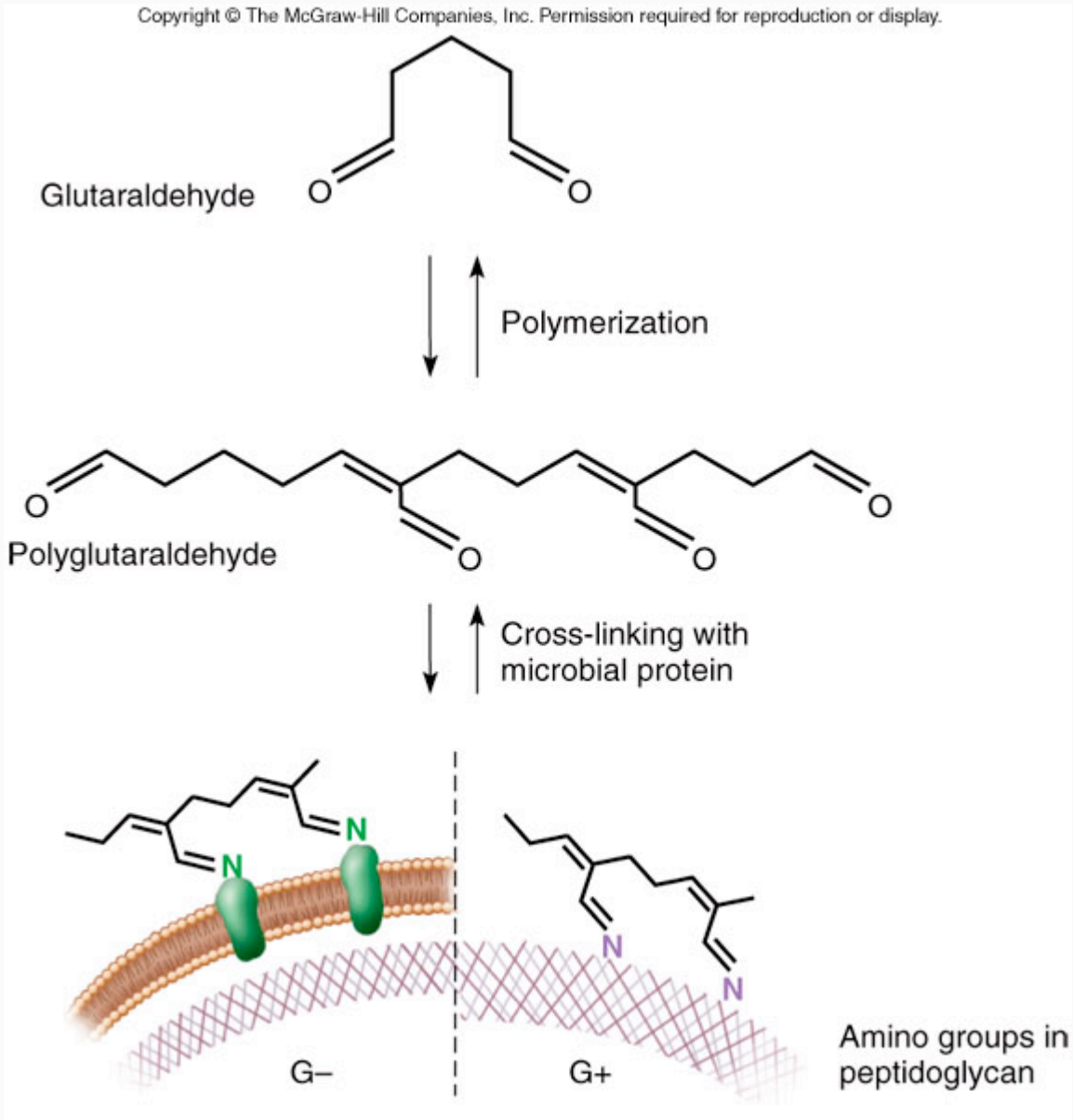


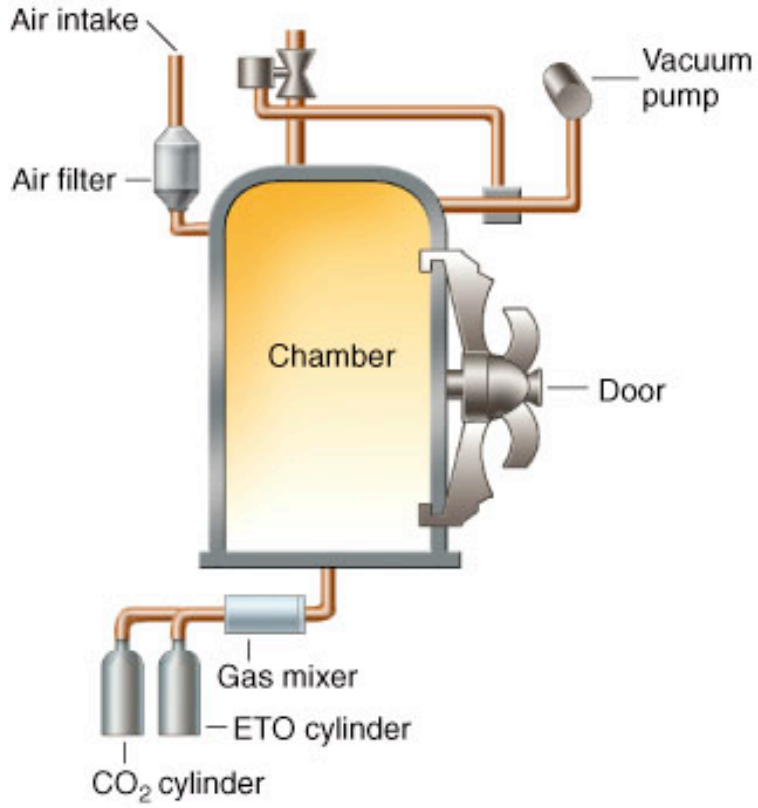
Fig. 11.17 Actions of glutaraldehyde

Gases

- Ethylene oxide
  - Reacts with functional groups of DNA and proteins
  - Sterilizes and disinfects plastic materials

Examples of different devices that use gas to sterilize equipment.

(a)



(b)

Fig. 11.18 Sterilization using gas

Dyes

- Crystal violet

- Effective against Gram positive bacteria
- Ointments

Acids and alkalis

- Acetic acid
- Ammonium hydroxide
- Prevents spore germination and vegetative growth
- Food preservative

TABLE 11.5 Active Ingredients of Various Commercial Antimicrobial Products

Product	Specific Chemical Agent	Antimicrobial Category
Lysol Sanitizing Wipes	Dimethyl benzyl ammonium chloride	Detergent (quat)
Clorox Disinfecting Wipes	Dimethyl benzyl ammonium chloride	Detergent (quat)
Tilex Mildew Remover	Sodium hypochlorites	Halogen
Lysol Mildew Remover	Sodium hypochlorites	Halogen
Ajax Antibacterial Hand Soap	Triclosan	Phenolic
Dawn Antibacterial Hand Soap	Triclosan	Phenolic
Dial Antibacterial Hand Soap	Triclosan	Phenolic
Lysol Disinfecting Spray	Alkyl dimethyl benzyl ammonium saccharinate/ethanol	Detergent (quats)/alcohol
ReNu Contact Lens Solution	Polyaminopropyl biguanide	Chlorhexidine
Wet Ones Antibacterial Moist Towelettes	Benzethonium chloride	Detergents (quat)
Noxzema Triple Clean	Triclosan	Phenolic
Scope Mouthwash	Ethanol	Alcohol
Purell Instant Hand Sanitizer	Ethanol	Alcohol
Pine-Sol	Phenolics and surfactant	Mixed
Allergan Eye Drops	Sodium chlorite	Halogen