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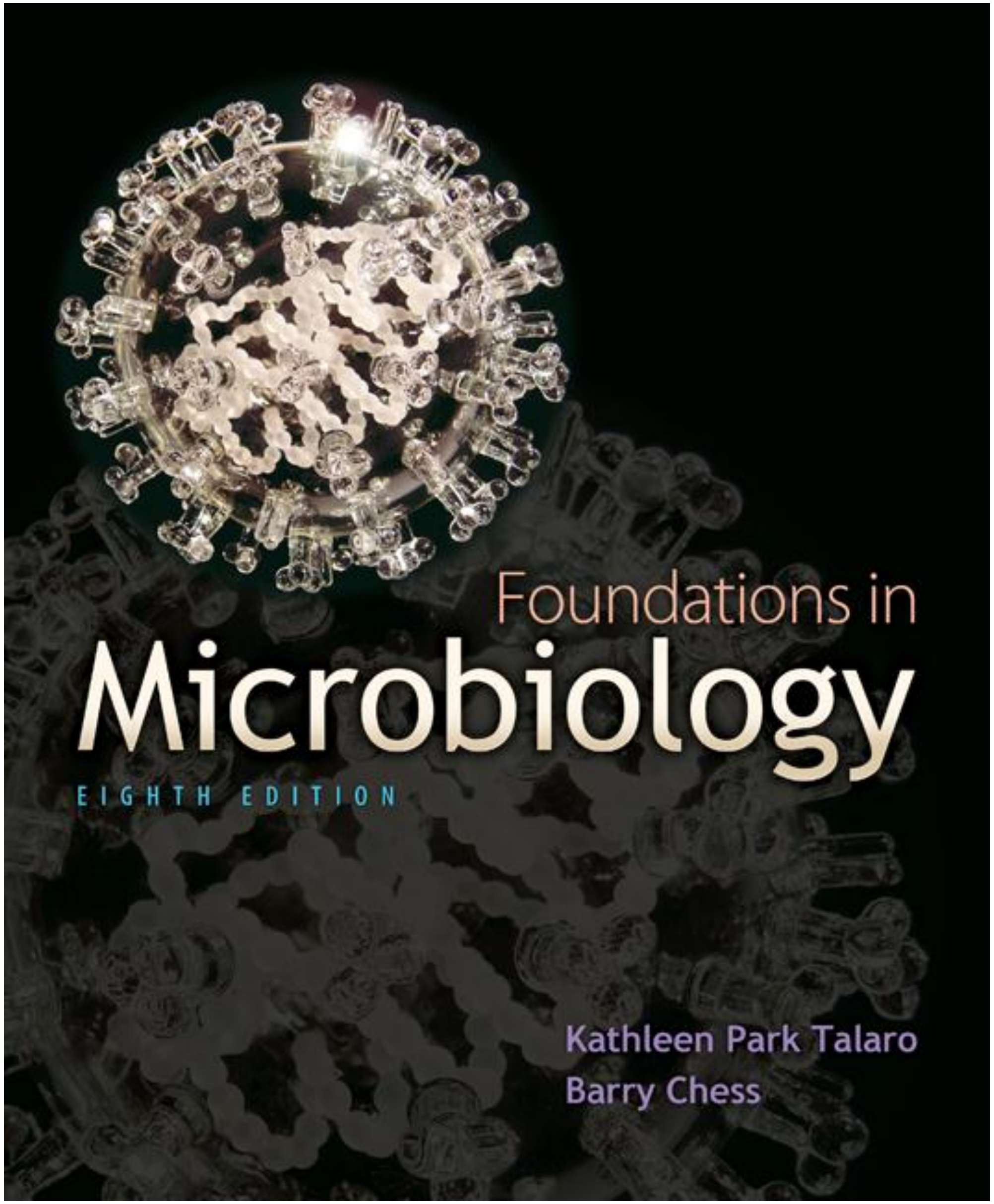
Chapter 11

Physical and Chemical Agents for Microbial Control

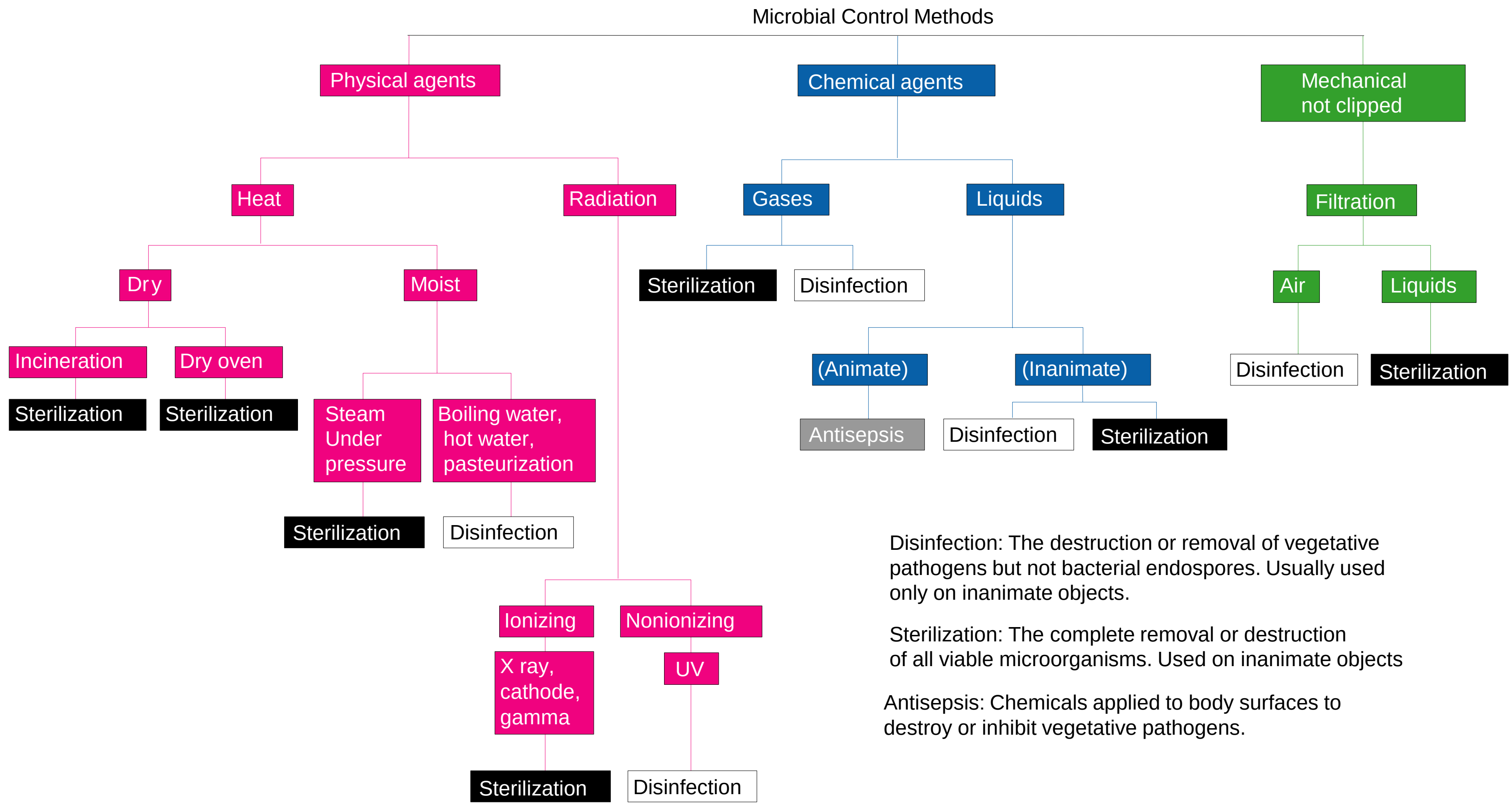


11.1 Controlling Microorganisms

- Physical, chemical, and mechanical methods to destroy or reduce undesirable microbes in a given area (decontamination)
- Primary targets are microorganisms capable of causing infection or spoilage:
  - Vegetative bacterial cells and endospores
  - Fungal hyphae and spores, yeast
  - Protozoan trophozoites and cysts
  - Worms
  - Viruses
  - Prions

General Considerations in Microbial Control

Figure 11.1 An overview of microbial control methods



Disinfection: The destruction or removal of vegetative pathogens but not bacterial endospores. Usually used only on inanimate objects.

Sterilization: The complete removal or destruction of all viable microorganisms. Used on inanimate objects

Antisepsis: Chemicals applied to body surfaces to destroy or inhibit vegetative pathogens.

Relative Resistance of Microbial Forms

- **Highest resistance**
  - Prions, bacterial endospores
- **Moderate resistance**
  - *Pseudomonas sp.*
  - *Mycobacterium tuberculosis*
  - *Staphylococcus aureus*
  - Protozoan cysts
- **Least resistance**
  - Most bacterial vegetative cells
  - Fungal spores and hyphae, yeast
  - Enveloped viruses
  - Protozoan trophozoites

Terminology and Methods of Microbial Control

- **Sterilization** – a process that destroys all viable microbes, including viruses and endospores
- **Disinfection** – a process to destroy vegetative pathogens, not endospores; inanimate objects
- **Antiseptic** – disinfectants applied directly to exposed body surfaces
- **Sanitization** – any cleansing technique that mechanically removes microbes
- **Degermation** – reduces the number of microbes through mechanical means

What is Microbial Death?

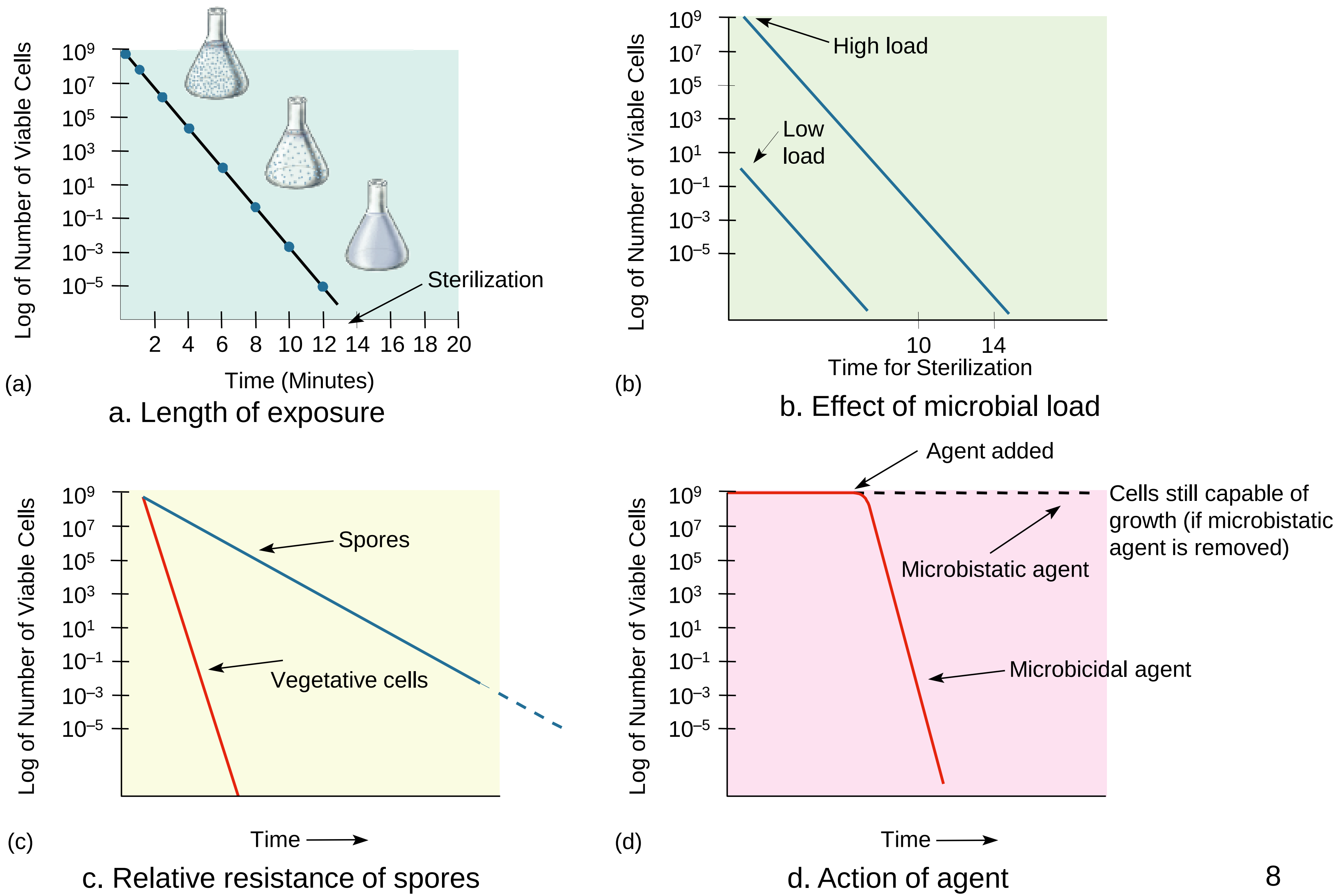
- Hard to detect, microbes often reveal no conspicuous vital signs to begin with

- So, determine by permanent loss of reproductive capability, even under optimum growth conditions – dead cells do not grow!

Factors That Affect Death Rate

- Number of microbes
- Nature of microbes in the population
- Temperature and pH of environment
- Concentration or dosage of agent
- Mode of action of the agent
- Presence of solvents, organic matter, or inhibitors

Figure 11.2 Factors that influence kill rate of microbes



Some Practical Concerns in Selecting Antimicrobial Agents

Selection of method of control depends on circumstances:

- Does the application require sterilization?
- Is the item to be reused?
- Can the item withstand heat, pressure, radiation, or chemicals?
- Is the method suitable?
- Will the agent penetrate to the necessary extent?
- Is the method cost- and labor-efficient and is it safe?

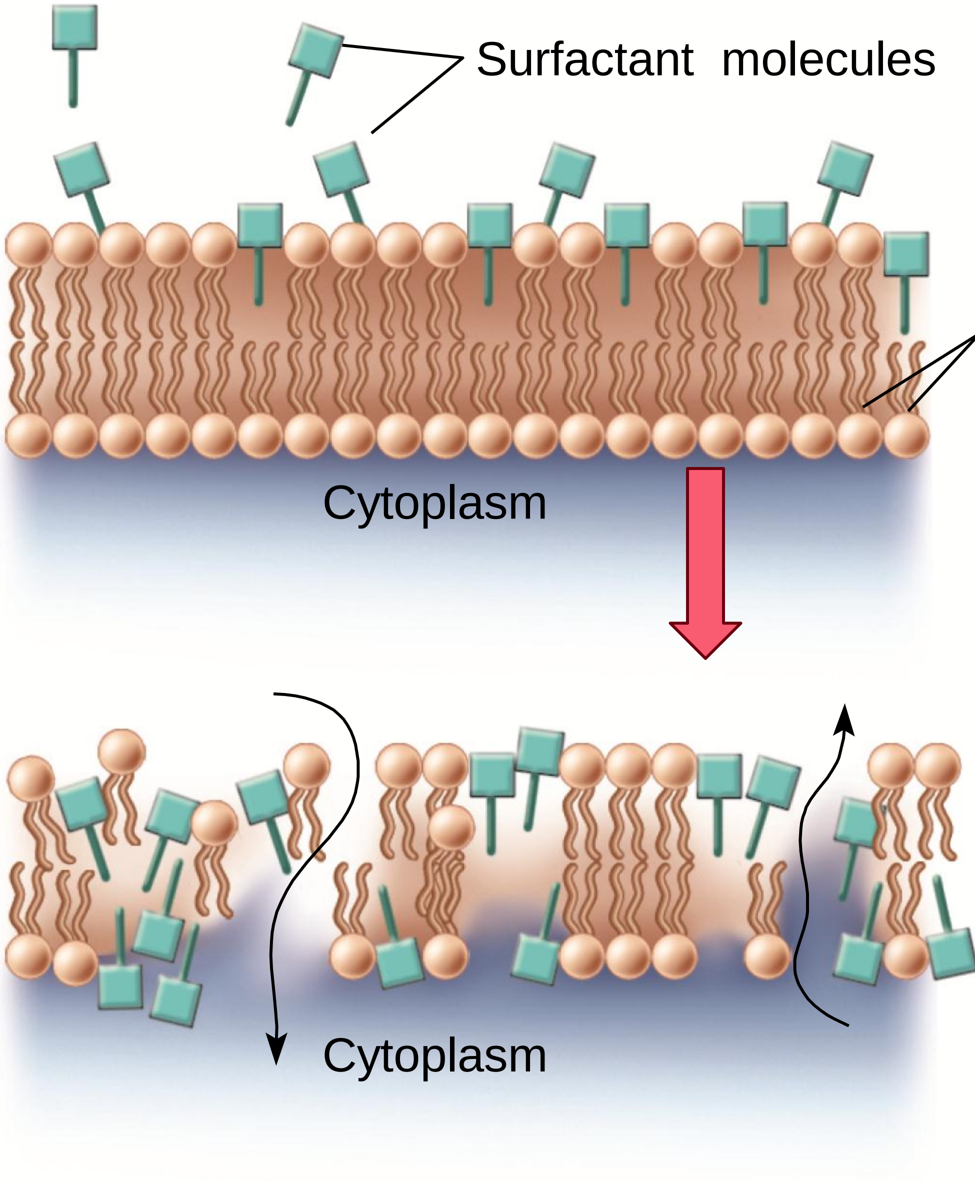
How Antimicrobial Agents Work: Their Modes of Action

Cellular targets of physical and chemical agents:

1. **The cell wall** – cell wall becomes fragile and cell lyses; some antimicrobial drugs, detergents, and alcohol

2. **The cell membrane** – loses integrity; detergent **surfactants**

Figure 11.3 Mode of action of surfactants on the cell membrane



Surfactants insert into the lipid bilayer and disrupts it

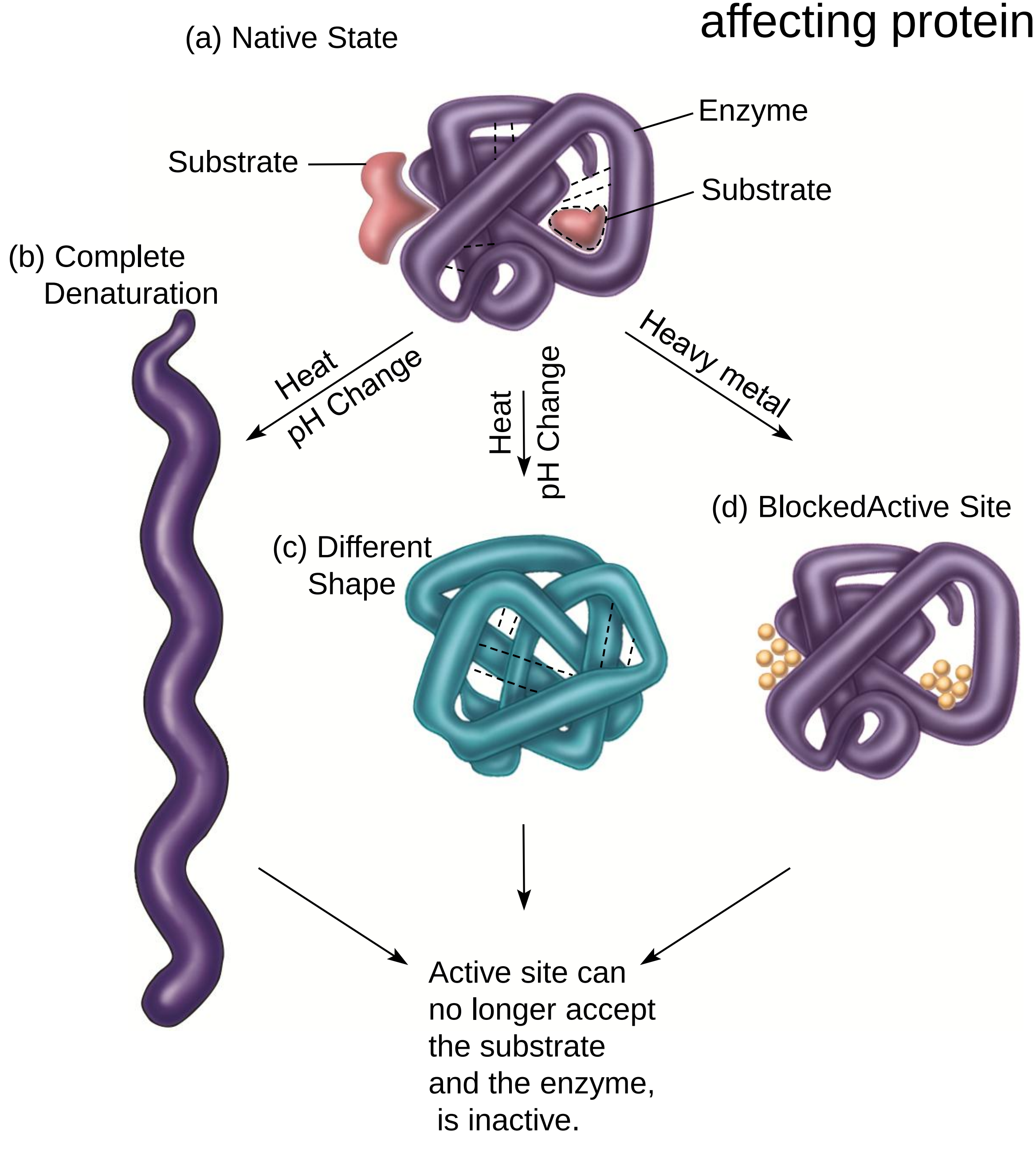
How Antimicrobial Agents Work: Their Modes of Action

Cellular targets of physical and chemical agents:

3. **Protein and nucleic acid synthesis** – prevention of replication, transcription, translation, peptide bond formation, protein synthesis; chloramphenicol, ultraviolet radiation, formaldehyde

4. **Proteins** – disrupt or denature proteins; alcohols, phenols, acids, heat

Figure 11.4 Modes of action affecting protein function



11.2 Physical Methods of Control: Heat

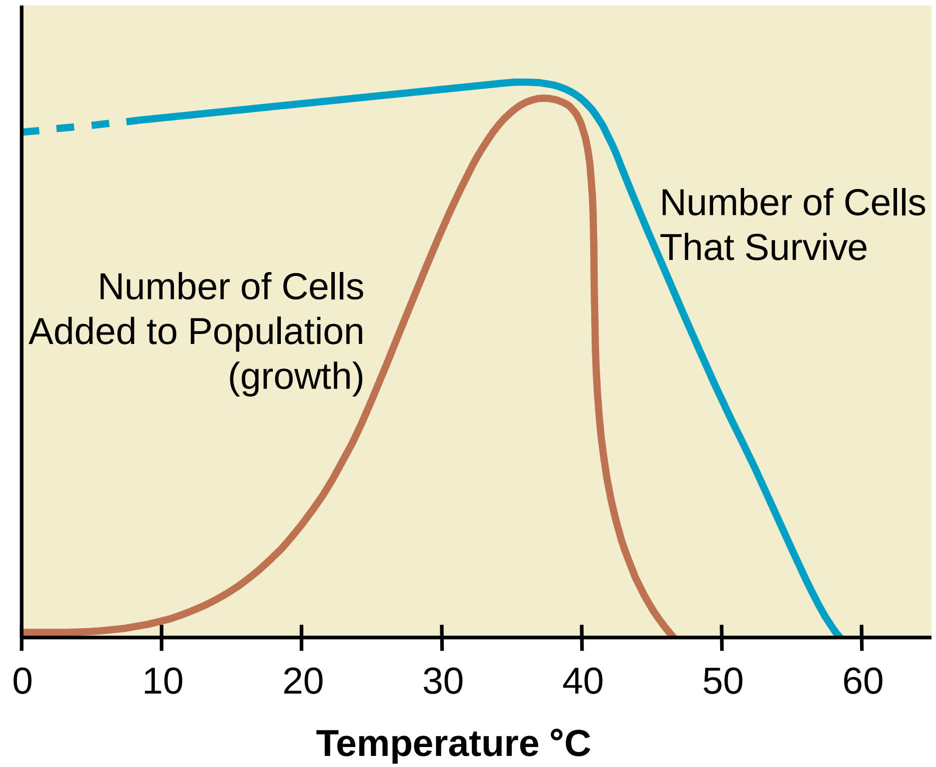
1. Heat – moist and dry
2. Cold temperatures
3. Desiccation
4. Radiation
5. Filtration

Mode of Action and Relative Effectiveness of Heat

- **Moist heat** – lower temperatures and shorter exposure time; coagulation and denaturation of proteins

- **Dry heat** – moderate to high temperatures; dehydration, alters protein structure; incineration

Figure 11.5 Comparative effects of temperature on growth rate and survival of a mesophilic microbe

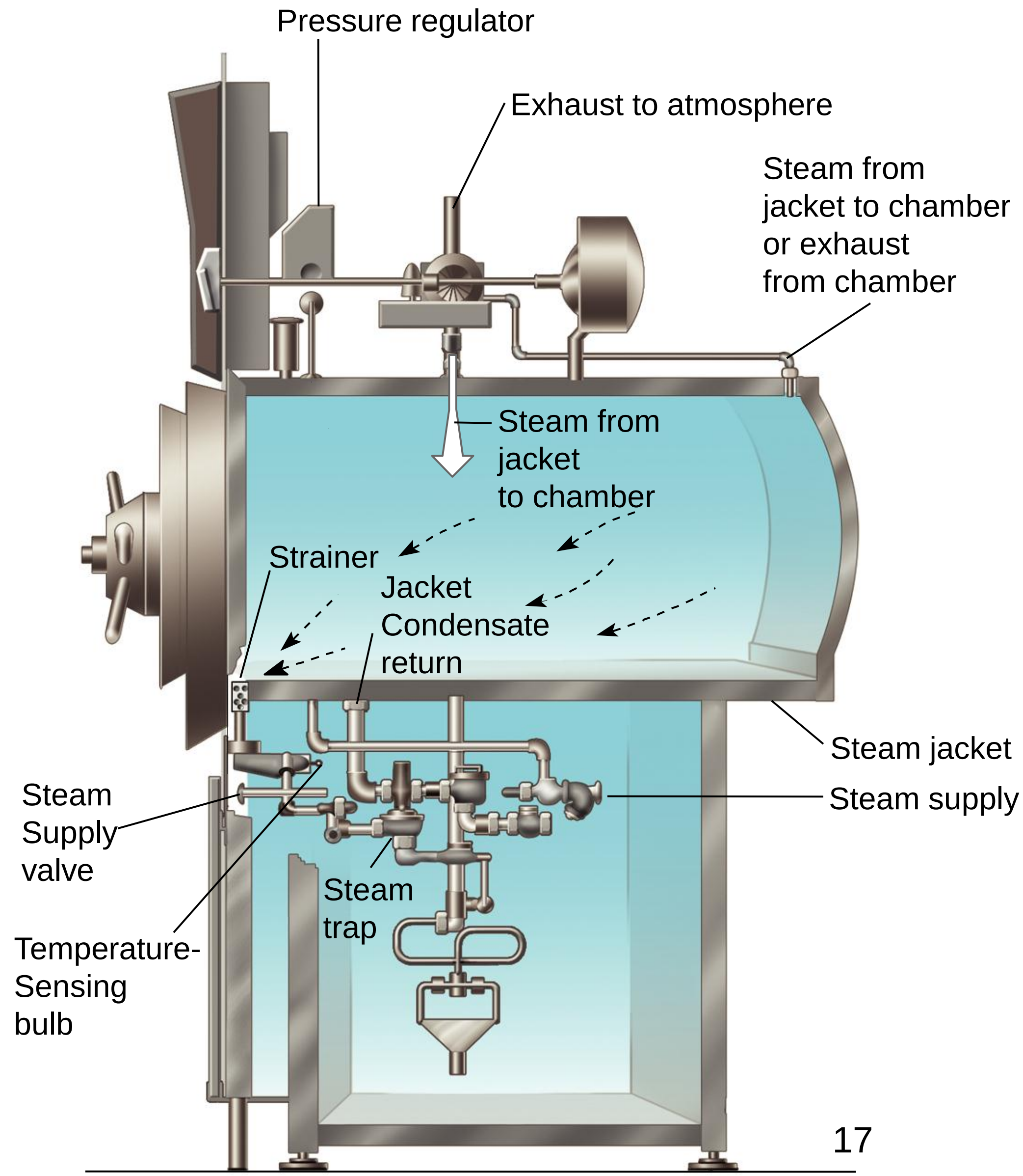


Thermal Death Measurements

- Bacterial endospores most resistant – usually require temperatures above boiling
- **Thermal death time** (TDT) – shortest length of time required to kill all test microbes at a specified temperature
- **Thermal death point** (TDP) – lowest temperature required to kill all microbes in a sample in 10 minutes

Figure 11.6 Moist Heat Methods

- **Steam under pressure** – sterilization
- **Autoclave** 15 psi/121^{o}C/10-40min
- Steam must reach surface of item being sterilized
- Item must not be heat or moisture sensitive
- Mode of action – denaturation of proteins, destruction of membranes and DNA



(b)

Nonpressurized Steam

- **Tyndallization** – intermittent sterilization for substances that cannot withstand autoclaving
- Items exposed to free-flowing steam for 30–60 minutes, incubated for 23–24 hours and then subjected to steam again
- Repeat cycle for 3 days
- Used for some canned foods and laboratory media
- Disinfectant

Boiling Water

- Boiling at 100^{o}C for 30 minutes to destroy non-spore-forming pathogens
- Disinfection – does NOT sterilize!!!

Pasteurization

- **Pasteurization** – heat is applied to kill potential agents of infection and spoilage without destroying the food flavor or value
- 63°C–66°C for 30 minutes (batch method)
- 71.6°C for 15 seconds (flash method)
- Not sterilization – kills non-spore-forming pathogens and lowers overall microbe count; does not kill endospores or many nonpathogenic microbes

Figure A – a small flash pasteurizer used by dairies for calf milk.

Dry Heat: Hot Air and Incineration

Dry heat using higher temperatures than moist heat

- Incineration: flame or electric heating coil
  - Ignites and reduces microbes and other substances
- Dry ovens: (150–180^{o}C) coagulate proteins

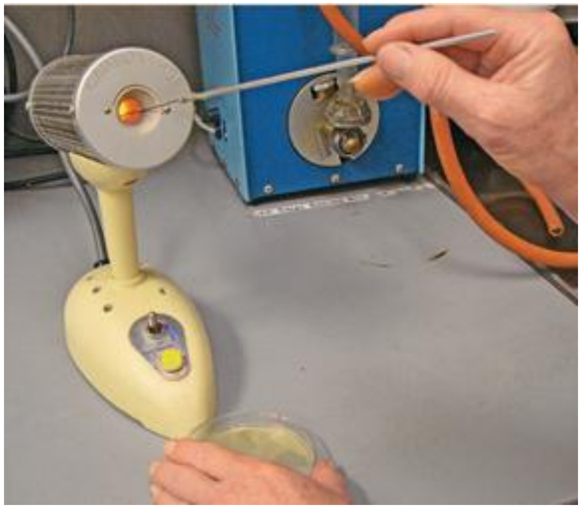


Figure B – Dry heat incineration using an infrared incinerator

The Effects of Cold and Desiccation

- **Microbiostatic**: Cold, slows the growth of microbes
- Refrigeration 0–15^{o}C and freezing <0^{o}C
- Used to preserve food, media, and cultures

The Effects of Cold and Desiccation

- Gradual removal of water from cells, leads to metabolic inhibition
- Not effective microbial control – many cells retain ability to grow when water is reintroduced
- **Lyophilization**: Desiccation, freeze drying; preservation

11.3 Physical Methods of Control: Radiation

- **Ionizing radiation** – deep penetrating power that has sufficient energy to cause electrons to leave their orbit, breaks DNA
  - Gamma rays, X-rays, cathode rays
  - Used to sterilize medical supplies and food products

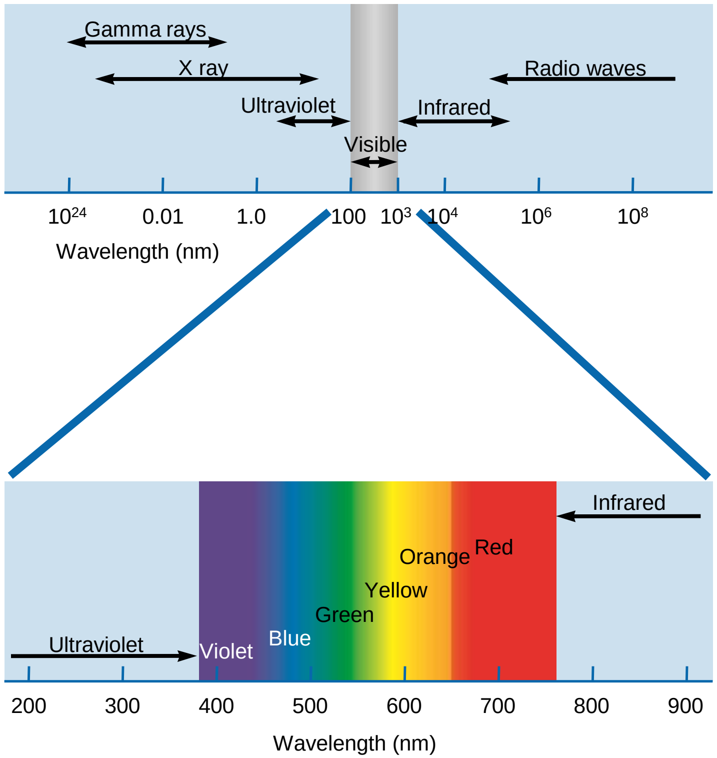


Figure 11.7 The electromagnetic spectrum

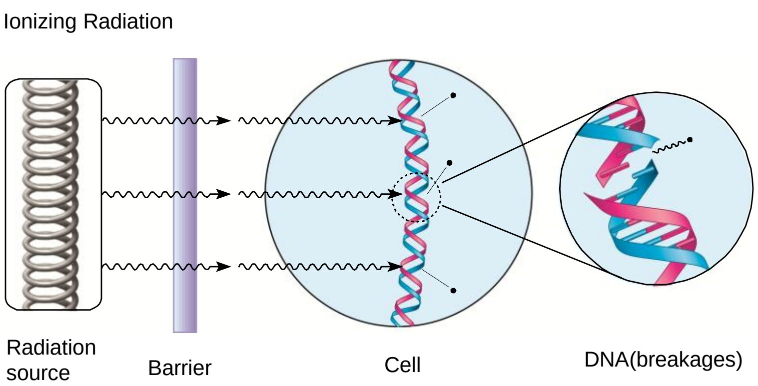


Figure 11.9 Sterilizing with Ionizing Radiation

- Preserving food with ionizing radiation

Exposed to X-rays, 5 days later

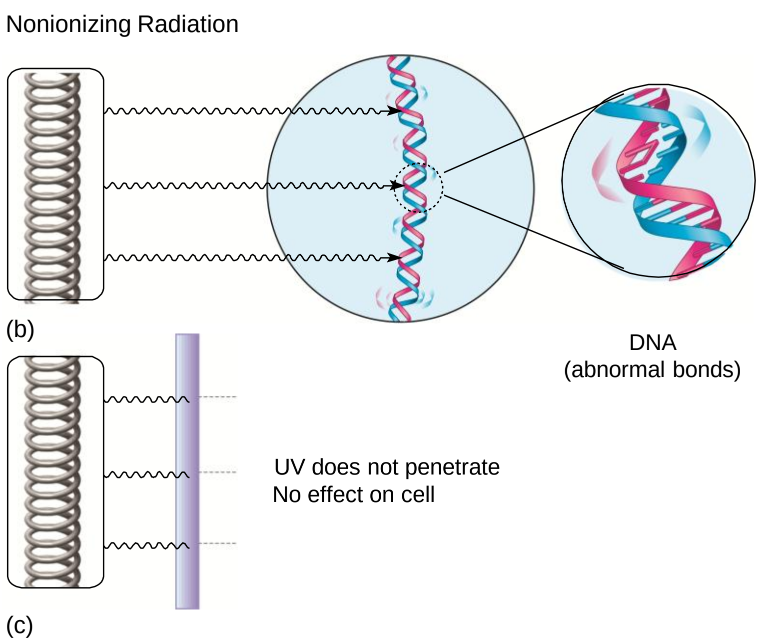
© Kathy Park Talaro

Not exposed to X-rays, 5 days later

© Kathy Park Talaro

Radiation

- **Nonionizing radiation** – little penetrating power so it must be directly exposed
  - UV light creates pyrimidine dimers, which interfere with replication



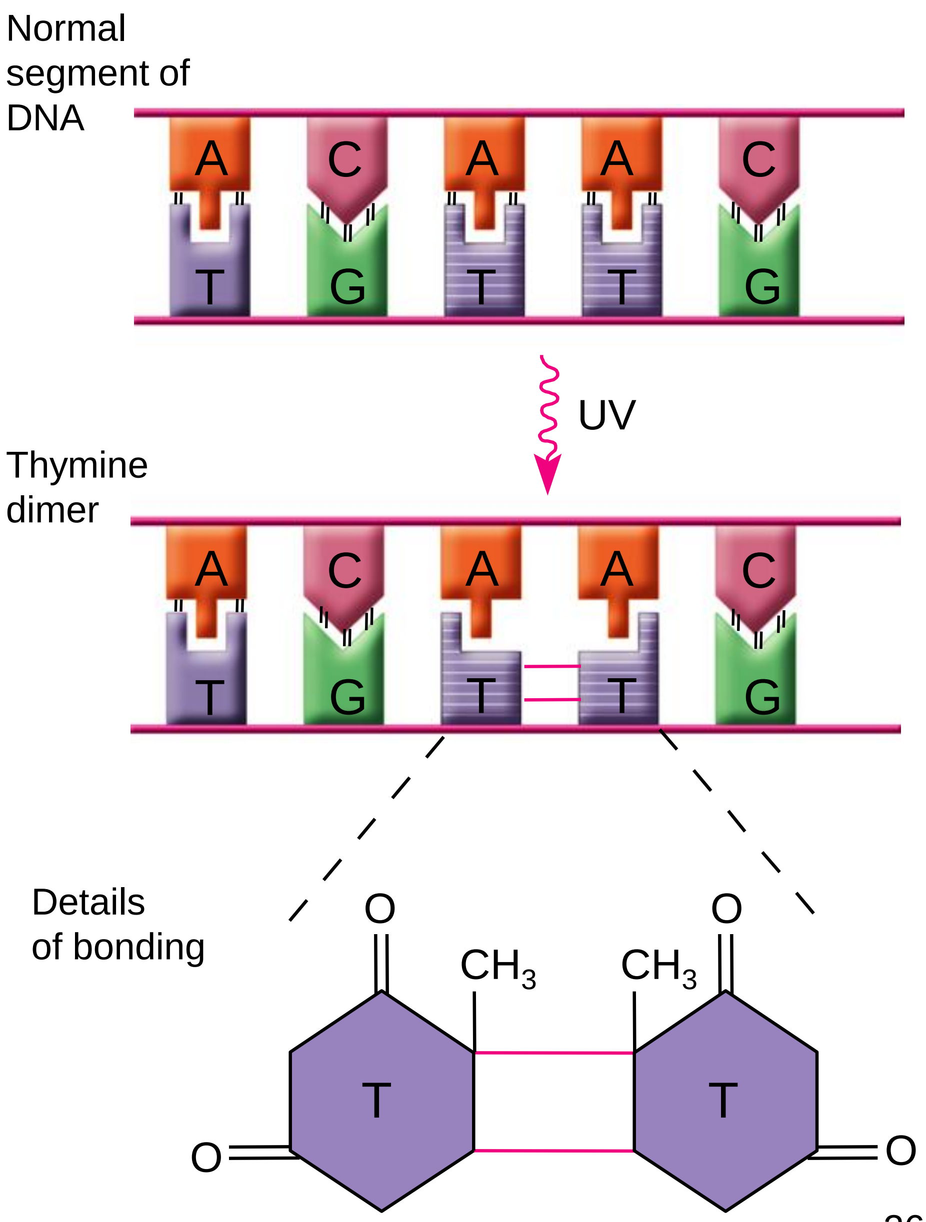


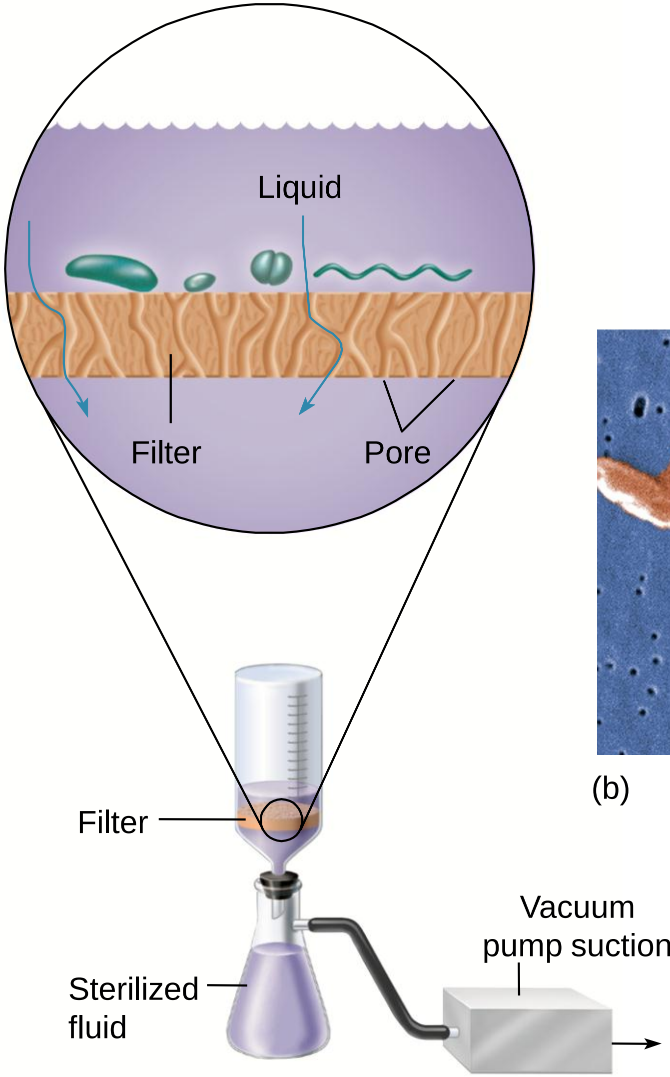
Figure 11.11 An ultraviolet (UV) treatment system for disinfection of water.

- Sterilizing air, water or surfaces

Photo courtesy of ITT Water & Wastewater

11.4 Using Filtration to Remove Microbes

- Physical removal of microbes by passing a gas or liquid through filter
- Used to sterilize heat sensitive liquids and air in hospital isolation units and industrial clean rooms



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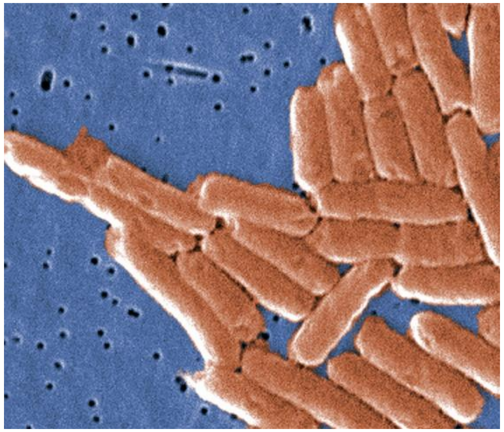


(b)

Figure 11.12 Membrane Filtration

11.5 Chemical Agents in Microbial Control

- Disinfectants, antiseptics, sterilants, degermers, and preservatives
- Some desirable qualities of chemicals:
  - Rapid action in low concentration
  - Solubility in water or alcohol, stable
  - Broad spectrum, low toxicity
  - Penetrating
  - Noncorrosive and nonstaining
  - Affordable and readily available

Choosing a Microbicidal Chemical

- **High-level germicides**: kill endospores; may be sterilants
  - Devices that are not heat sterilizable and intended to be used in sterile environments (body tissue)
- **Intermediate-level**: kill fungal spores (not endospores), tubercle bacillus, and viruses
  - Used to disinfect devices that will come in contact with mucous membranes but are not invasive
- **Low-level**: eliminate only vegetative bacteria, vegetative fungal cells, and some viruses
  - Clean surfaces that touch skin but not mucous membranes

Factors that Affect Germicidal Activities of Chemical Agents

- Nature of the material being treated
- Degree of contamination
- Time of exposure
- Strength and chemical action of the germicide

Categories of Chemical Agents

1. Halogens
2. Phenolics
3. Chlorhexidine
4. Alcohols
5. Hydrogen peroxide
6. Aldehydes
7. Gases
8. Detergents & soaps
9. Heavy metals
10. Dyes
11. Acids and Alkalis

Halogens

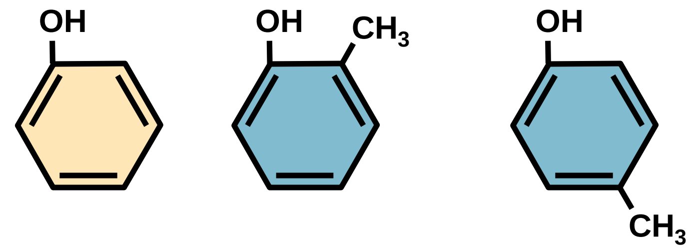
- **Chlorine**: Cl_2, **hypochlorites** (chlorine bleach), chloramines
  - Denaturate proteins by disrupting disulfide bonds
  - Intermediate level
  - Unstable in sunlight, inactivated by organic matter
  - Water, sewage, wastewater, inanimate objects

- **Iodine**: I_2, **iodophors** (betadine)
  - Interferes with disulfide bonds of proteins
  - Intermediate level
  - Milder medical and dental degerming agents, disinfectants, ointments

Phenolics

- Disrupt cell walls and membranes and precipitate proteins
- Low to intermediate level – bactericidal, fungicidal, virucidal, not sporicidal
  - **Lysol**
  - **Triclosan**: antibacterial additive to soaps

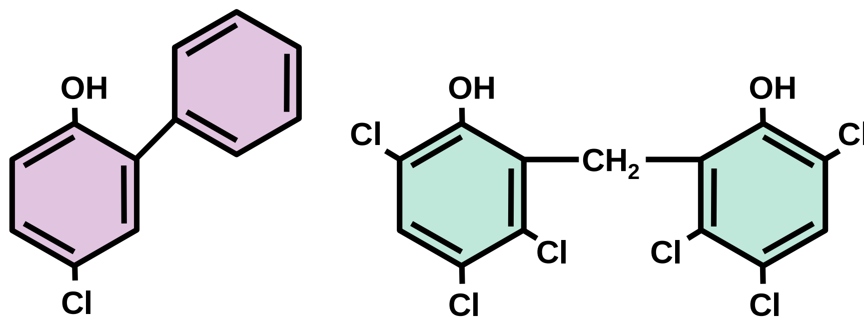
Figure 11.13 Examples of phenolic structures



Phenol (basic aromatic ring structure)

o - cresol

p - cresol



Chlorophene (a chlorinated phenol)

Hexachlorophene (a bisphenol)

Chlorhexidine

- A surfactant and protein denaturant with broad microbicidal properties
- Low to intermediate level
- Hibiclens, Hibitane
- Used as skin degerming agents for preoperative scrubs, skin cleaning, and burns

Alcohols

- Ethyl, isopropyl in solutions of 50-95%
- Act as surfactants dissolving membrane lipids and coagulating proteins of vegetative bacterial cells and fungi
- Intermediate level

Hydrogen Peroxide

- Produce highly reactive hydroxyl-free radicals that damage protein and DNA while also decomposing to O_2 gas – toxic to anaerobes
- Antiseptic at low concentrations; strong solutions are sporicidal

Figure 11.14 Processing cabinet for sterilizing medical equipment with peroxide.

Aldehydes

- Kill by alkylating protein and DNA
  - **Glutaraldehyde** in 2% solution (Cidex) used as sterilant for heat sensitive instruments
    - High level
  - **Formaldehyde**: disinfectant, preservative, toxicity limits use
    - Formalin – 37% aqueous solution
    - Intermediate to high level

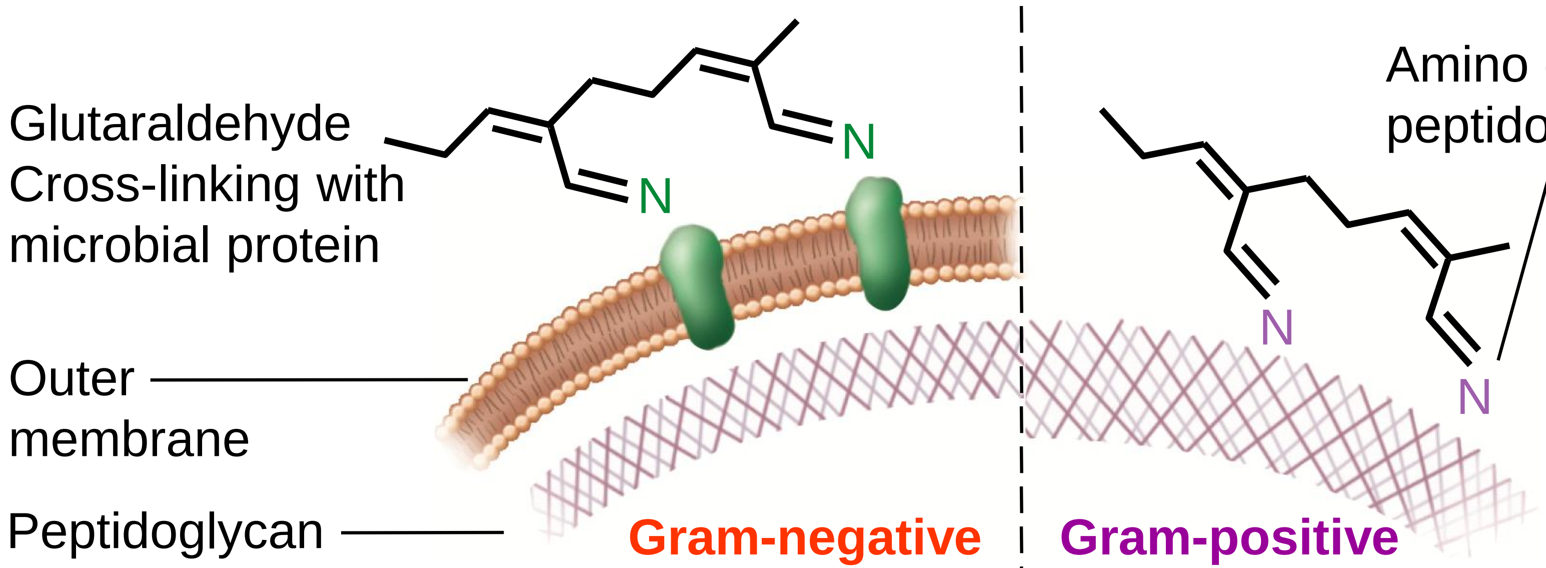


Figure 11.15 Actions of glutaraldehyde on cell walls of gram-negative and gram positive cells.

Gases and Aerosols

- Ethylene oxide, propylene oxide
- Strong alkylating agents
- High level
- Sterilize and disinfect plastics and prepackaged devices, foods

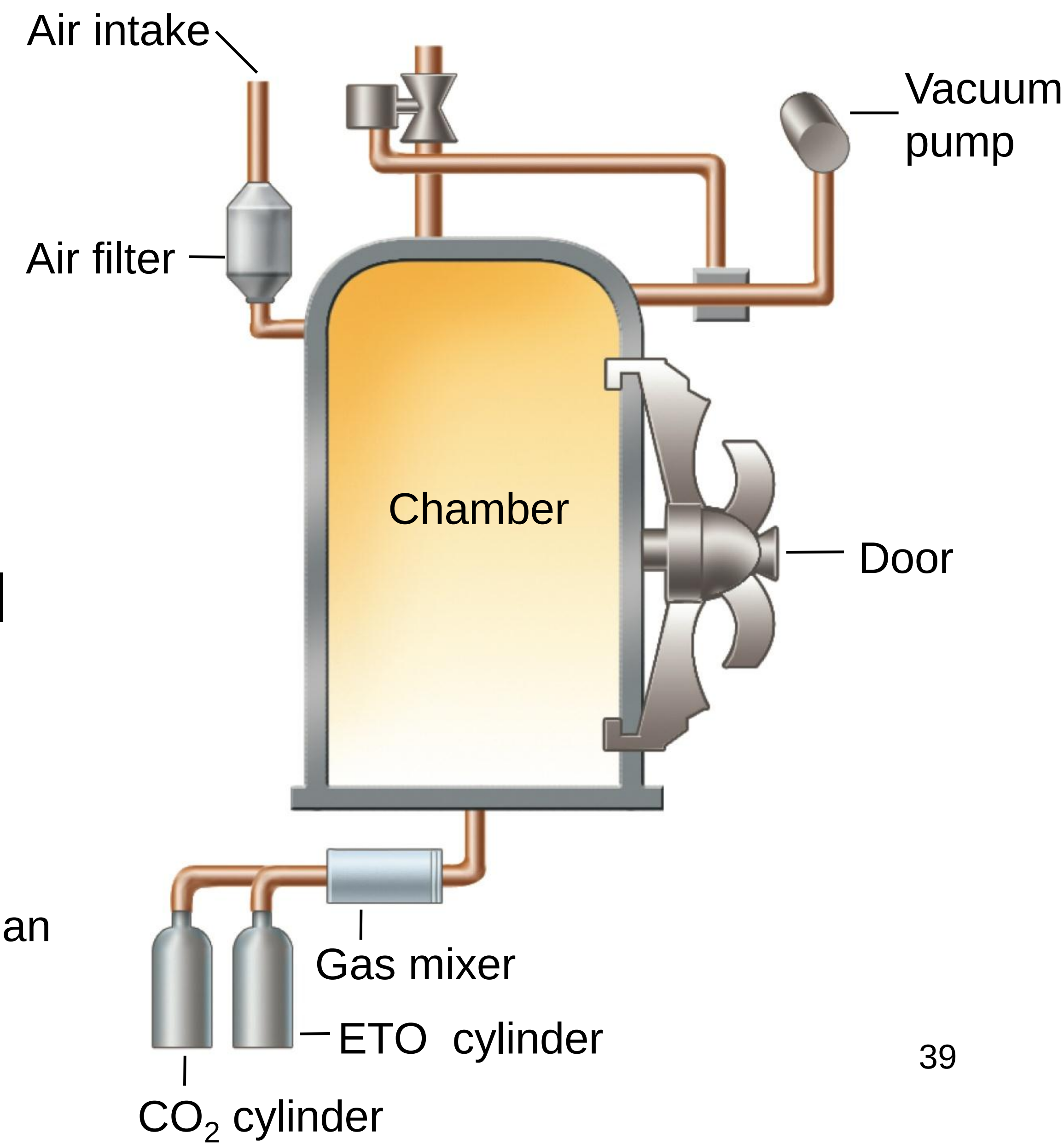
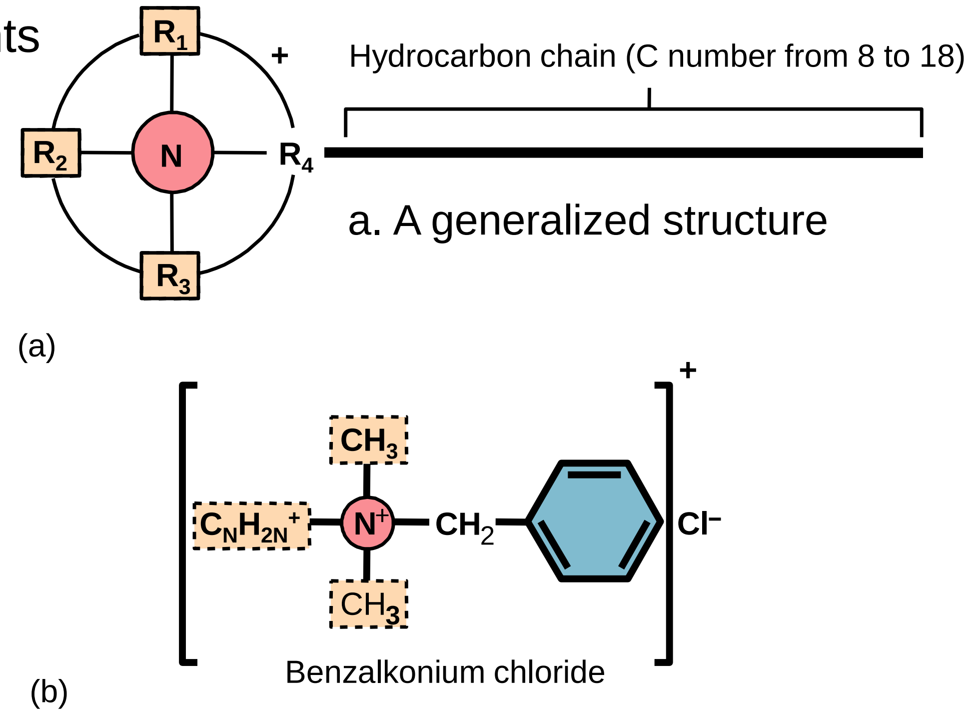


Figure 11.16 Sterilization using an ethylene oxide sterilizer.

Detergents and Soaps

- **Quaternary ammonia compounds (quats)** act as surfactants that alter membrane permeability of some bacteria and fungi
- Very low level

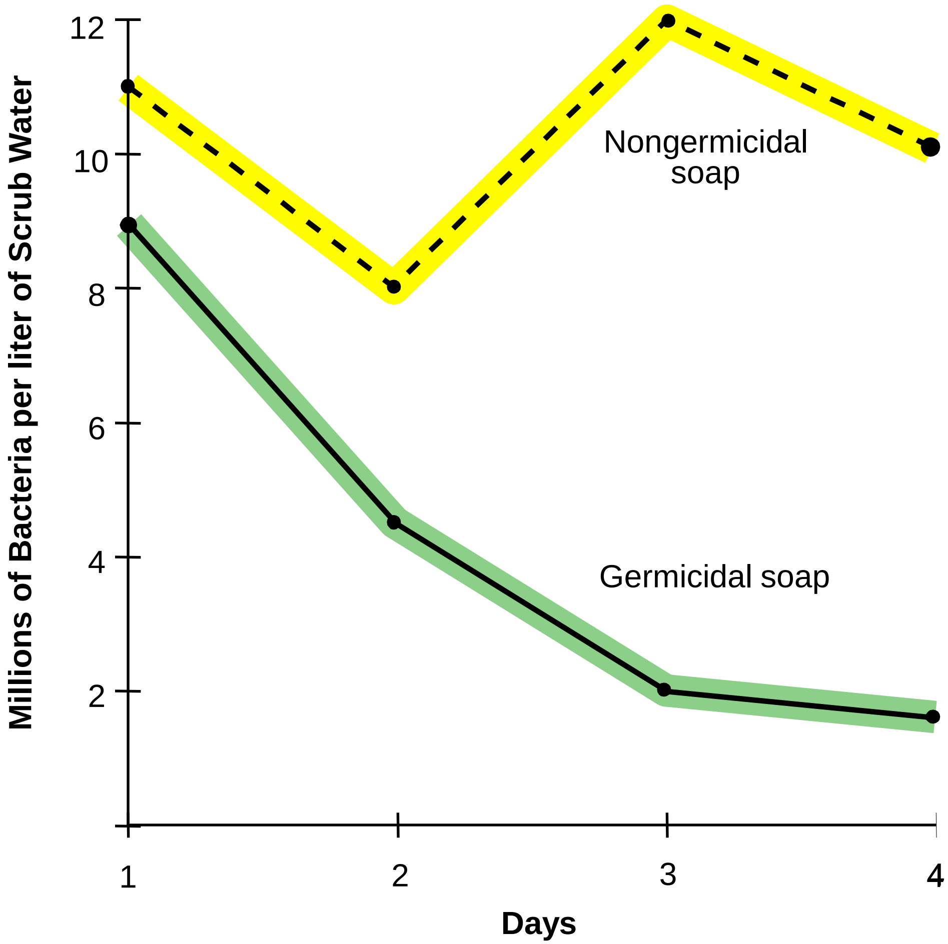
Figure 11.17 The Structure of detergents



Detergents and Soaps

- **Soaps**: mechanically remove soil and grease containing microbes

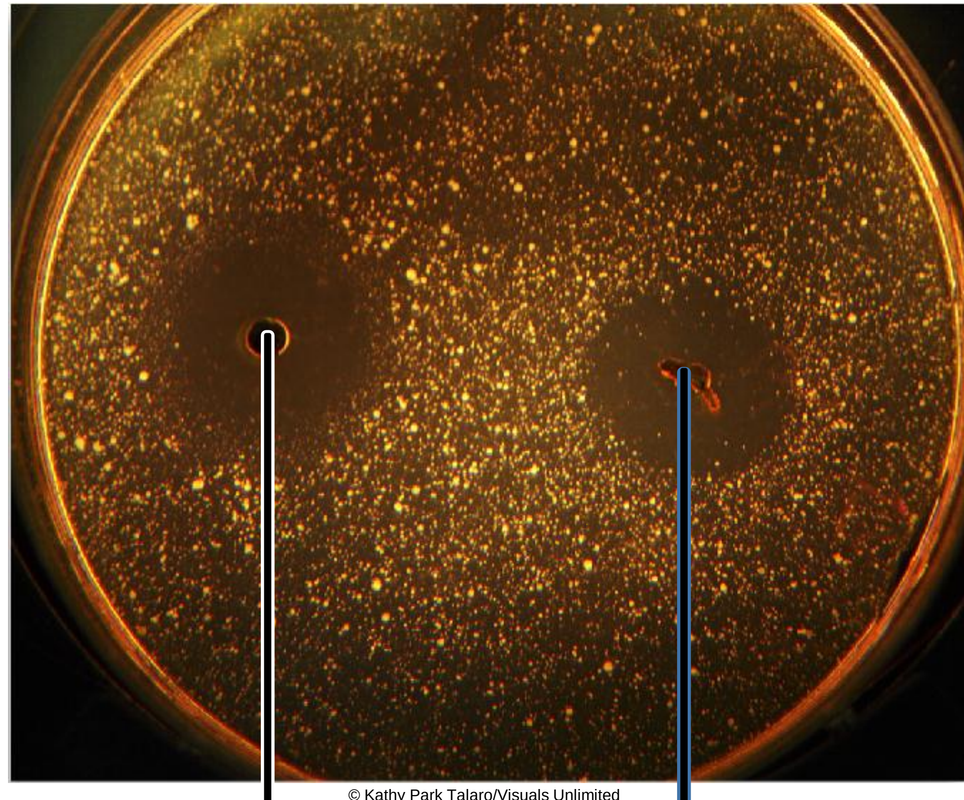
Figure 11.18 Graph showing effects of hand scrubbing



Heavy Metals

Figure 11.19 Demonstration of heavy metals

- Solutions of silver and mercury kill vegetative cells in low concentrations by inactivating proteins
- Oligodynamic action
- Low level
- Merthiolate, silver nitrate, silver



Acids and Alkalis

- Low level of activity
  - Organic acids prevent spore germination and bacterial and fungal growth
  - Acetic acid inhibits bacterial growth
  - Propionic acid retards molds
  - Lactic acid prevents anaerobic bacterial growth
  - Benzoic and sorbic acid inhibit yeast