Chapter 11

Topics

- Controlling Microorganisms
- Physical Control
- Chemical control

Controlling Microorganisms

- **Microbial agents**
- **Sanitation**
- **Effectiveness**
- **Mode of action**

Microbial agents

- -static agents
- -cital agents
- Resistance
- Terms
- Effectiveness
- Mode of action

-static agents

- **Temporarily preventing the growth of microbes**
  - **Bacteriostatic**
  - **Fungistatic**

-cidal

- **Killing or destroying a microorganism**
  - **Germicide**
  - **Bactericide**

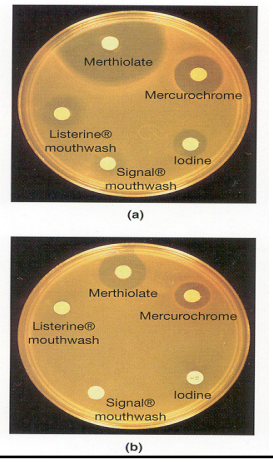
A chemical substance is bacteriostatic if it inhibits the growth of organisms.

A chemical substance is bacteriocidal if it kills the organisms

In like manner viro, sporo and fungi can be substituted for bacterio in the terms bacteriocidal and bacteriostatic.

Resistance

- **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**



S. aureus

E. coli

Terms

- **Sterilization**
- **Disinfection**
- **Antisepsis**

Disinfectants are typically applied to **inanimate objects** to reduce the number of pathogenic organisms.

Antiseptics are generally applied to **living organisms** to destroy microorganisms or limit their growth.

Sterilization is the complete killing of all microorganisms in a material or on an object. A substance is either sterile or not sterile - there is no intermediate.

Effectiveness

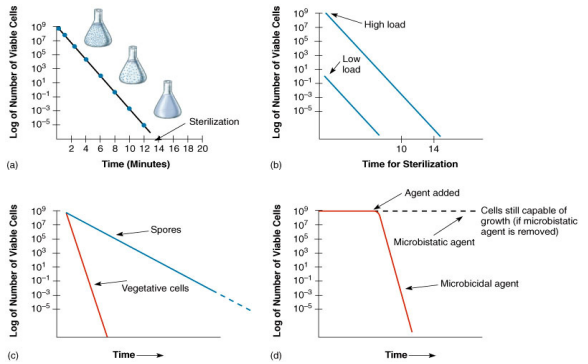
- **Number of microorganisms**
- **Target population (bacteria, fungi, spores, viruses)**
- **Temperature and pH**
- **Concentration of agent**
- **Mode of action**
- **Interfering agents (solvents, debris, saliva, blood, feces)**

Disinfection or Sterilization

Figure 11.2 shows the ways in which disinfection or sterilization are affect by time and 'load' of microorganisms

A concept - the number of organisms present at the beginning affects the time needed to achieve disinfection.

Factors that influence the effectiveness of antimicrobial agents



Modes of action of agents that kill microorganisms

- **Cell wall**
- **Cell membrane**
- **Nucleic acid synthesis**
- **Protein synthesis**
- **Protein function**

THESE WILL BE DISCUSSED IN DETAIL IN THE NEXT CHAPTER AS THEY RELATE TO CHEMOTHERAPEUTIC AGENTS. WE'LL NOTE THE SIMILARITIES AS WE DISCUSS ANTIMICROBIALS

Physical Control

- **Heat**
- **Radiation**
- **Filtration**

Useful for substances not damaged by moist heat - 171°C for one hr.

Autoclave - 121°C for 20 min - time is dependent on configuration of the materials to be sterilized - a one liter flask of broth is easy - a 30 gal bag stuffed with insulating materials may require 1hr or more.

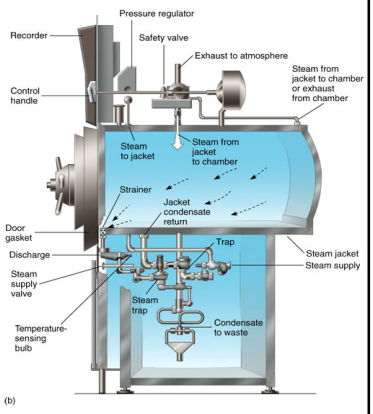
Mode of action

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

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

Cartoon of an AUTOCLAVE



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

Effects of cold and desiccation

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

Refrigeration, freezing excellent to preserve food and other materials - retards microbial growth, but does not effectively kill microorganisms. Microorganisms can be stored in this way.

Drying - many microorganisms are sensitive to drying - but, many are not. For example, *Treponema pallidum* dies if dried. Thus, if toilet seats are dried, there is little chance of spread of syphilis by that route. Freeze drying is used for storing many microorganisms.

Radiation

- Types of radiation
- Modes of action
- Applications

Types of radiation

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

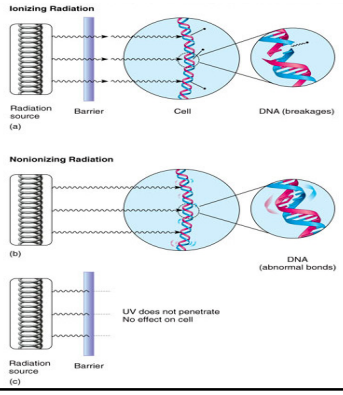
Mode of actions

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

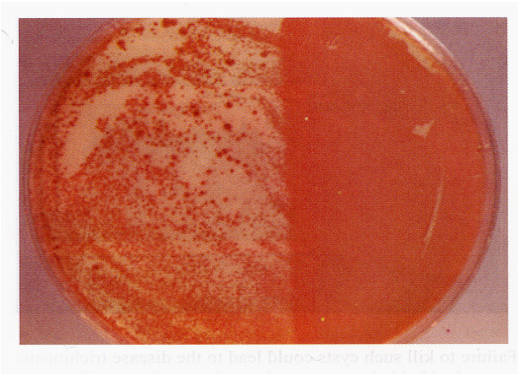
Ionizing radiation is an effective sterilizer. It damages DNA and generates peroxides in the cells. Both effectively sterilize the material. The material does not become radioactive.

Gamma rays and x-rays pass through the material without causing the material to become radioactive. Very effective for the preservation of food.

The effects of ionizing and nonionizing radiation on DNA

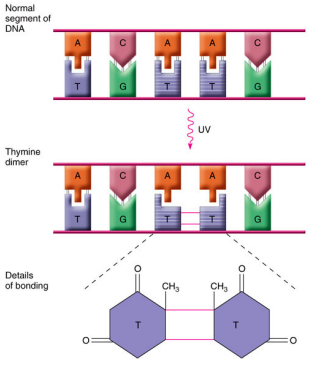


UV radiation of a bacterial lawn on agar



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

Repair Process?



Applications

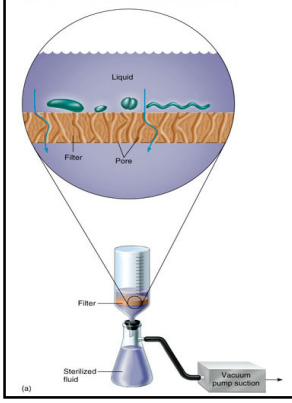
- **Ionizing radiation**
  - **Alternative sterilization method**
  - **Materials sensitive to heat or chemicals**
  - **Some foods (fruits, vegetables, meats)**
- **Nonionizing radiation**
  - **Alternative disinfectant**
  - **Germicidal lamp in hospitals, schools, food preparation areas (inanimate objects, air, water)**

Filtration

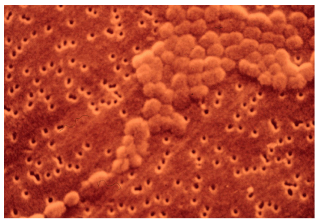
- Removes microbes and spores from liquids and air
- Perforated membrane
  - Pore sizes vary
- Applications
  - Liquids that are sensitive to heat
    - Serum, vaccines, media

Filtration is an effective way to remove microorganisms from a liquid. However, the porosity of the filter is very important. To effectively remove bacteria, a pore size of 0.45 µm is essential, 0.22 µm is preferred.

An example of a filtration system



What was in that water?



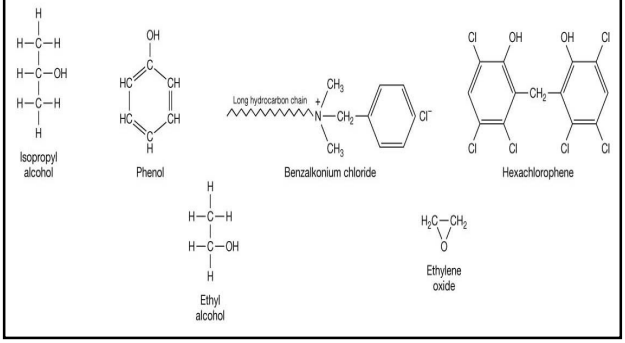
Chemical control

- **Widely used agents**
- **Applications**

Applications

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

Structural formulas of some important disinfectants



Surfactants disrupt the cell membrane - detergents are surfactants

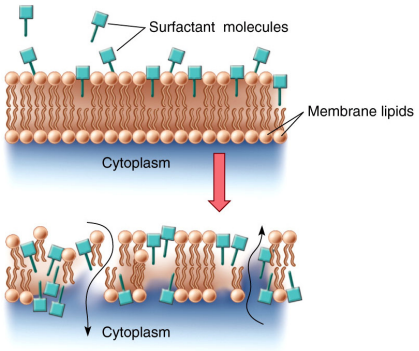
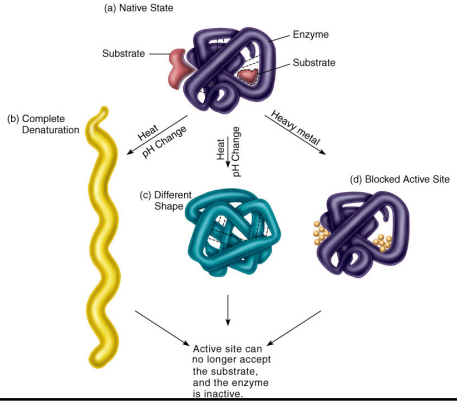
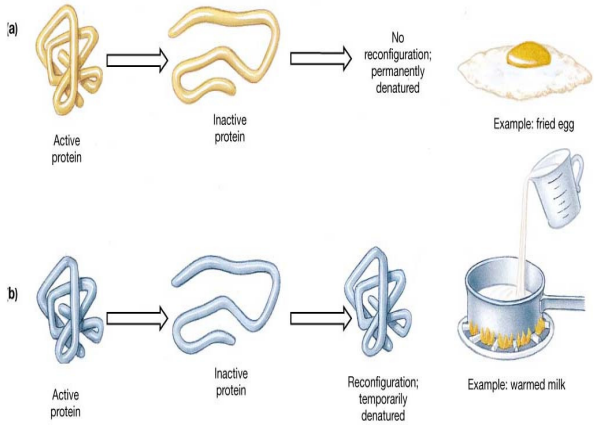


Fig. 11.4 Modes of action affecting protein function





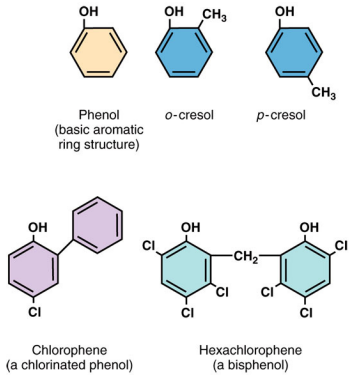
Halogens

- **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



A method of expressing efficacy is the phenol coefficient. The phenol coefficient is determined by testing dilutions of the disinfectant relative to that of phenol.

Alcohols - note that we are NOT talking about weekend parties!!

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

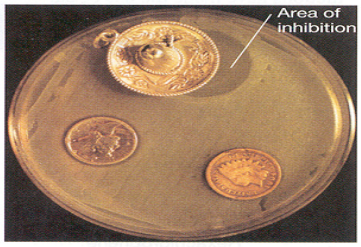
Hydrogen peroxide

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

Heavy metals - note that here we are NOT talking about Iron Maiden or Metallica

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

Heavy metals - silver nitrate solution in infants eyes to prevent infection by *Neisseria* during delivery. Tincture of merthiolate is an organomercury compound used as an antiseptic.



➢ Figure 12.5 **Heavy metals inhibit bacterial growth.** The inhibitory effects of silver ions can be seen as clear zones in which no growth has occurred around the silver charm (which has been pushed aside) and silver dime. The nonsilver coin (a copper penny) has not inhibited growth of the organisms as effectively as the silver objects have.

Demonstration of the effects silver and gold have on microbial growth