Physical and Chemical Agents for Microbial Control

Sterilization, Disinfection

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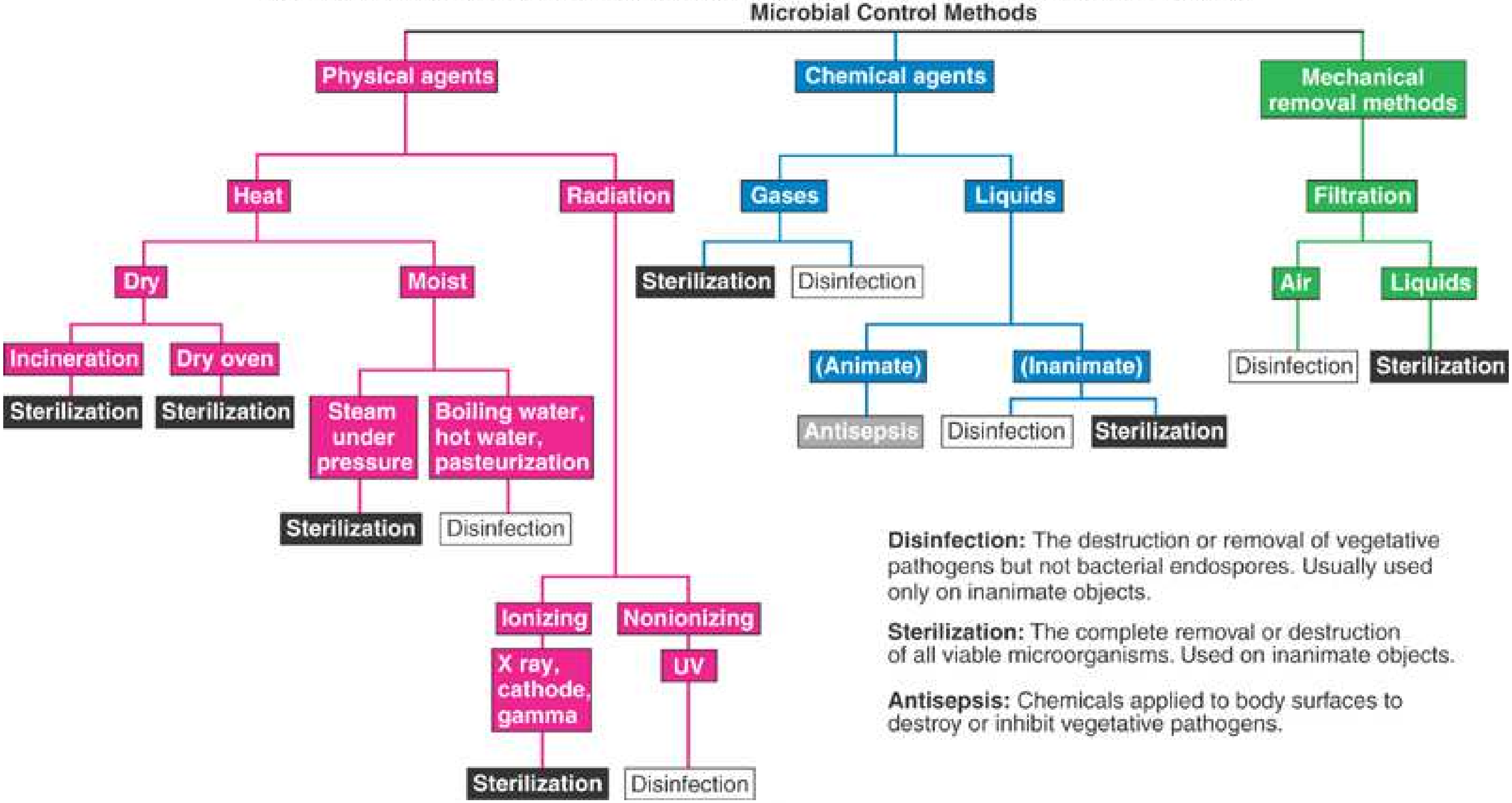
Controlling Microorganisms

- Physical, chemical, and mechanical methods to destroy or reduce undesirable microbes in a given area
- 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



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 Microbes

- Highest resistance
  - bacterial endospores, prions
- 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

Microbial Characteristics

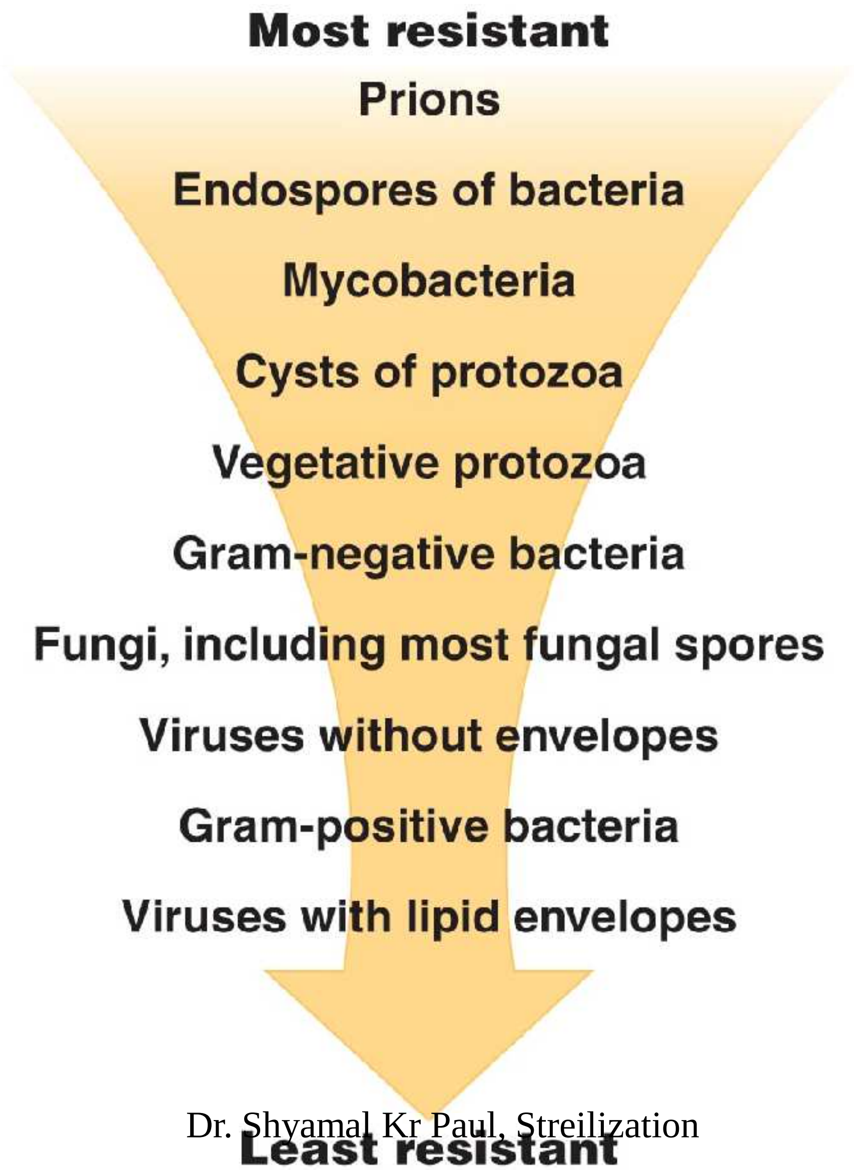


Figure 7.11

Terminology and Methods of Control

- **Sterilization** – a process that destroys all viable microbes, including viruses and endospores; microbicidal
- **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

Microbial death

- Permanent loss of reproductive capability, even under optimum growth conditions

Factors That Affect Death Rate

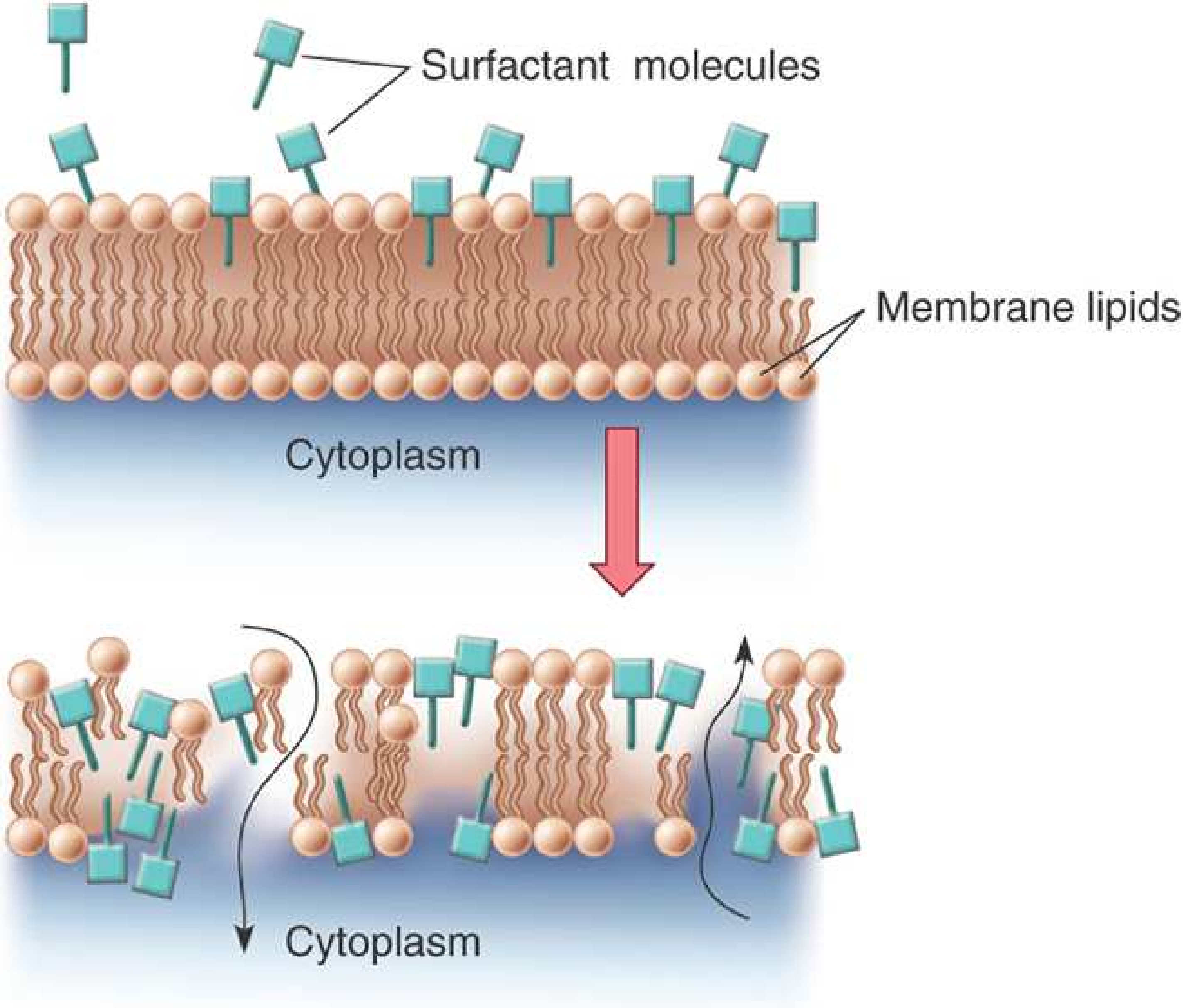
The effectiveness of a particular agent is governed by several factors:

- 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

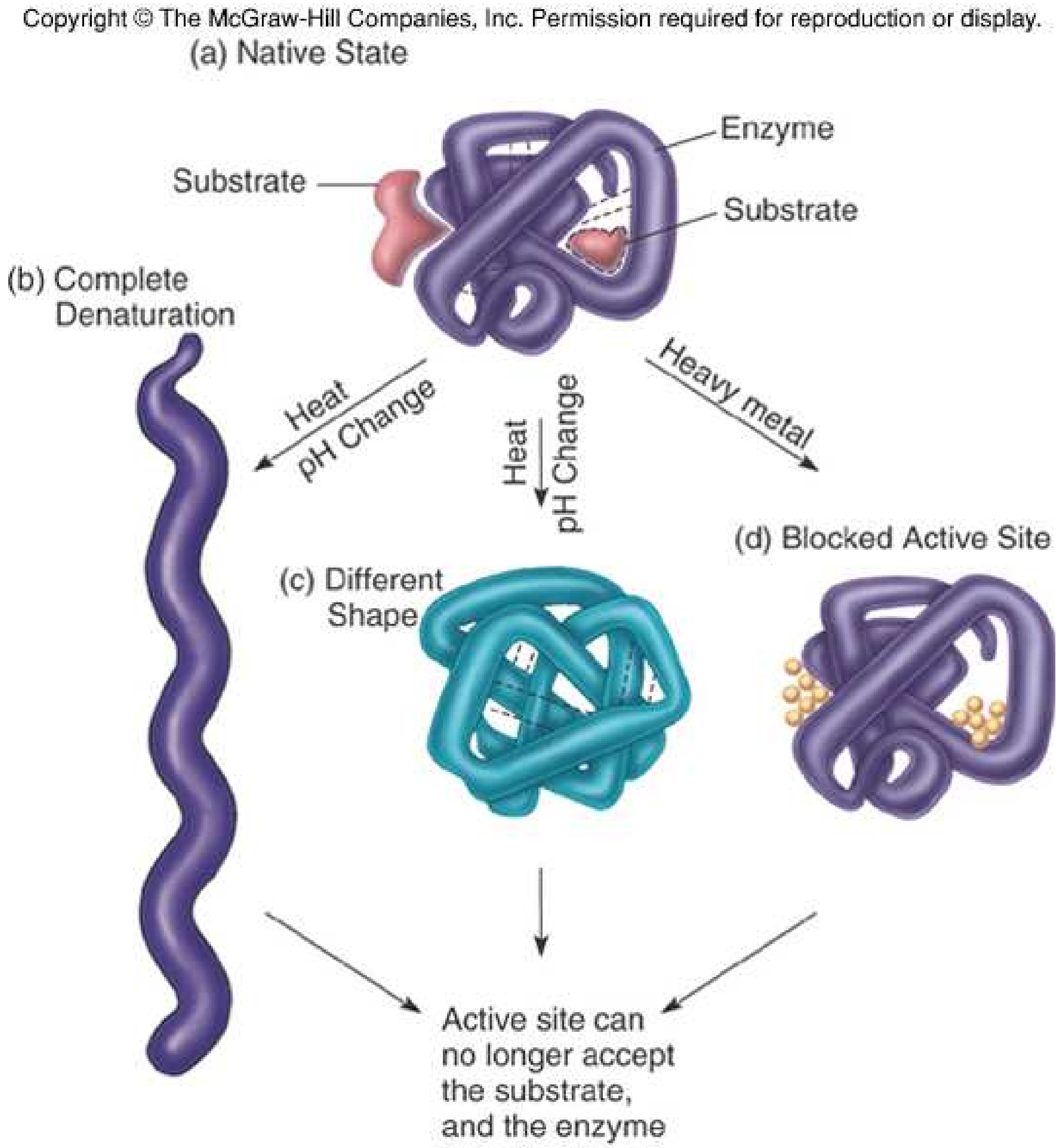
Antimicrobial Agents' Modes of Action

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**
3. Cellular synthetic processes (DNA, RNA) – prevention of replication, transcription; some antimicrobial drugs, radiation, formaldehyde, ethylene oxide
4. Proteins – interfere at ribosomes to prevent translation, disrupt or denature proteins; alcohols, phenols, acids, heat



Surfactant molecules
Membrane lipids
Cytoplasm
Cytoplasm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(a) Native State
Enzyme
Substrate
Substrate
(b) Complete Denaturation
Heat
pH Change
Heat
pH Change
Heavy metal
(c) Different Shape
(d) Blocked Active Site
Active site can no longer accept the substrate, and the enzyme

Methods of Physical Control

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

Heat Resistance and Thermal Death

- Bacterial endospores most resistant – usually require temperatures above boiling

TABLE 11.4

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

Thermal Death Measurements

- **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

Decimal Reduction Time (DRT)

- Minutes to kill 90% of a population at a given temperature

Time (min)	Deaths per Minute	Number of Survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9000	1000
4	900	100
5	90	10
6	9	1

Table 7.2

Physical Antimicrobials

Agent	Mechanisms of Action	Comments
Moist Heat, boiling	Denatures proteins	Kills vegetative bacterial cells and viruses Endospores survive
Moist Heat, Autoclaving	Denatures proteins	121°C at 15 p.s.i. for 30 min kills everything
Moist Heat, Pasteurization	Denatures proteins	Kills pathogens in food products
Dry Heat, Flaming	Incineration of contaminants	Used for inoculating loop
Dry Heat, Hot air oven	Oxidation & Denatures proteins	170°C for 2 hours; Used for glassware & instrument sterilization
Filtration	Separation of bacteria from liquid (HEPA: from air)	Used for heat sensitive liquids
Cold, Lyophilization (also desiccation)	Desiccation and low temperature	Used for food & drug preservation; Does not necessarily kill so used for Long-term storage of bacterial cultures
Cold, Refrigeration	Decreased chemical reaction rate	Bacteriostatic
Osmotic Pressure, Addition of salt or sugar	Plasmolysis of contaminants	Used in food preservation (less effective against fungi)
Radiation, UV	DNA damage (thymine dimers)	Limited penetration
Radiation, X-rays	DNA damage	Used for sterilizing medical supplies
Strong vis. Light		Line-drying laundry

Application of Heat

- Dry heat kills by oxidation (slow, uneven penetration)
- Heat is frequently used to kill microorganisms
- Moist heat kills by protein coagulation (denaturation) so requires lower temperatures or shorter times, but the moisture must penetrate to pathogens to be effective (grease & oil can block)

Moist Heat

- Moist heat kills microbes by denaturing enzymes (coagulation of proteins)
- Boiling (at 100°C, I.e., at sea level) kills many vegetative cells and viruses within 10 minutes
- Autoclaving: steam applied under pressure (121°C for 15 min) is the most effective method of moist heat sterilization—the steam must directly contact the material to be sterilized
- Pasteurization: destroys pathogens (*Mycobacterium tuberculosis*, *Salmonella typhi*, etc.) without altering the flavor of the food—does not sterilize (63°C for 30 seconds)
- Higher temperature short time pasteurization applies higher heat for a much shorter time (72°C for 15 seconds)
- An ultra-high-temperature, very short duration treatment (140°C for 3 sec.) is used to sterilize dairy products

Sterilization Times

- 171º C, 60 minutes, dry heat
- 160º C, 120 minutes, dry heat
- 149º C, 150 minutes, dry heat
- 141º C, 180 minutes, dry heat
- 121º C, 12 hours, dry heat
- 121º C, 15 minutes, moist heat (but don't start the clock until entire item is up to temp—e.g., large volumes fluid)

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

Autoclaving

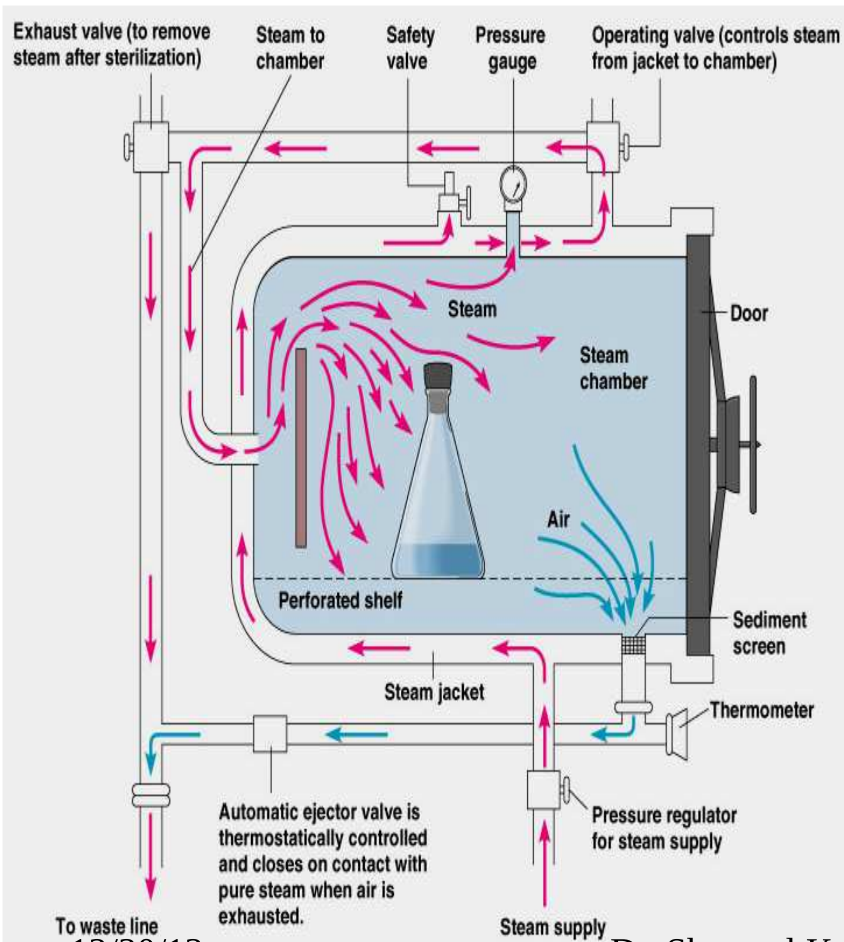
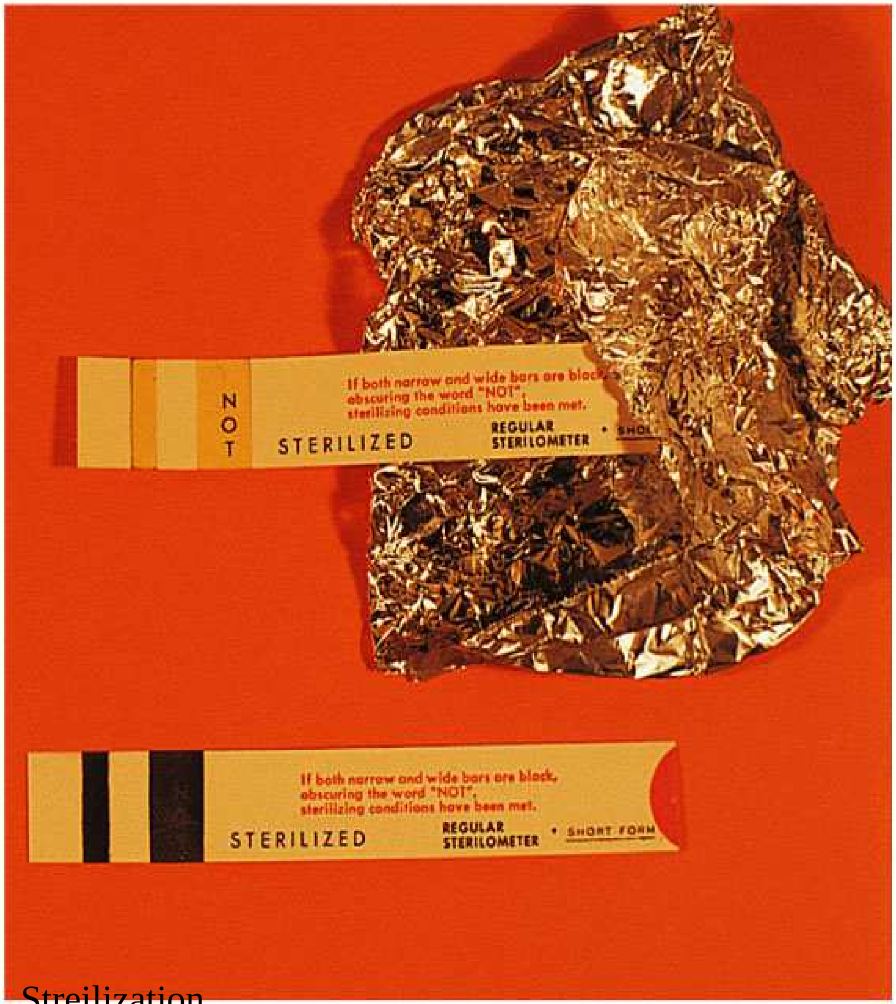


TABLE 7.3 **The Relationship Between the Pressure and Temperature of Steam at Sea Level***

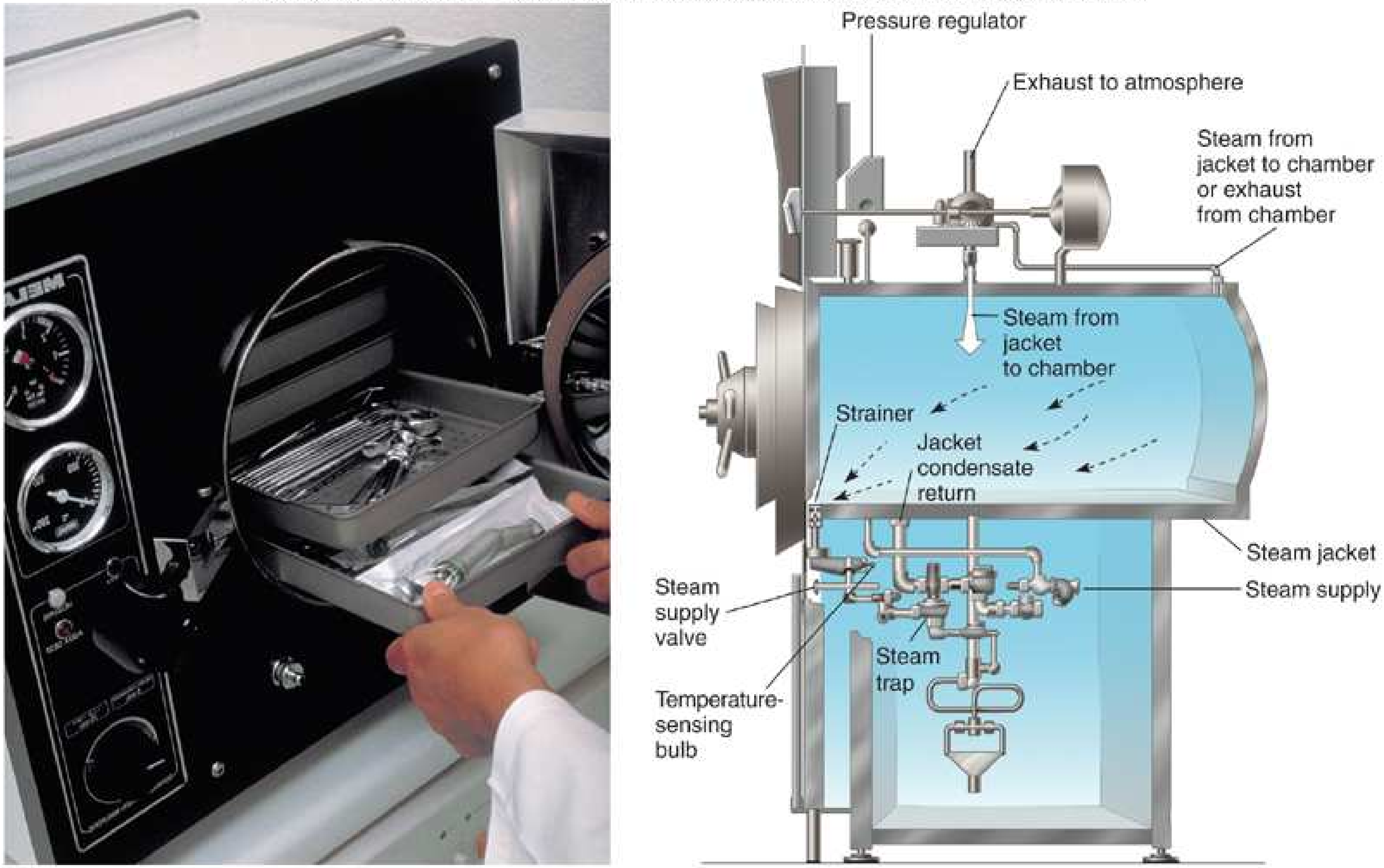
Pressure (psi in excess of atmospheric pressure)	Temperature (°C)
0 psi	100
5 psi	110
10 psi	116
15 psi	121
20 psi	126
30 psi	135

*At higher altitudes the atmospheric pressure is less, which must be taken into account in operation of an autoclave. For example, in order to reach sterilizing temperatures (121°C) in Denver, Colorado, whose altitude is 5280 feet (1600 meters), the pressure shown on the autoclave gauge would need to be higher than the 15 psi shown in the table.

Autoclaving



- Autoclaving
  - Extra time to reach center of solids
  - Paper should be used to wrap instruments
  - Indicators
    - Strips
    - Tape


Pressure regulator
Exhaust to atmosphere
Steam from jacket to chamber or exhaust from chamber
Steam from jacket to chamber
Strainer
Jacket condensate return
Steam jacket
Steam supply
Steam supply valve
Steam trap
Temperature-sensing bulb

(a) (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

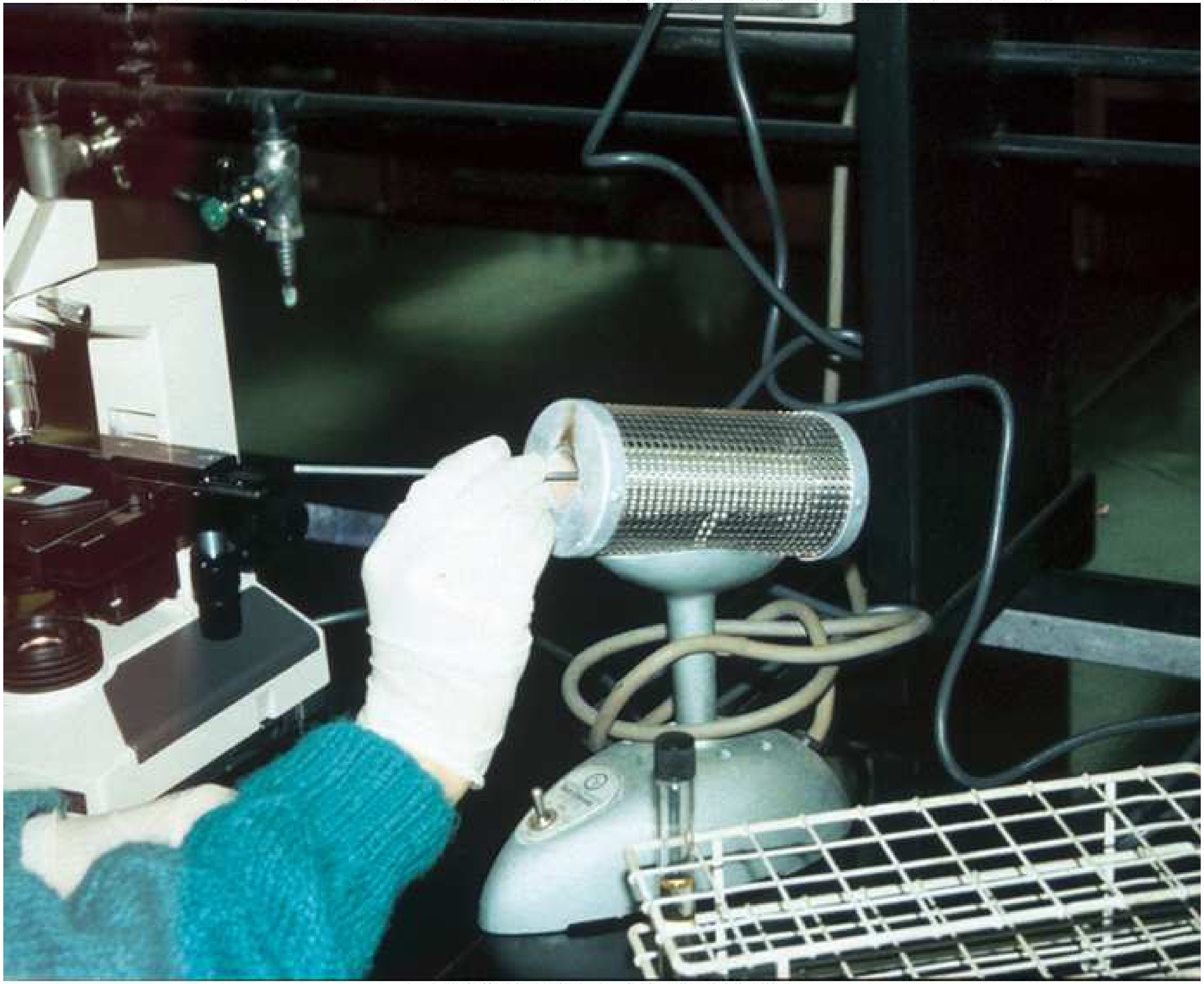
Pasteurization

- **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 (Holding method)
- 71.6°C for 15 seconds (flash method)
  – **Ultra-high-temperature**: 140°C for <1 sec
- Not sterilization - kills non-spore-forming pathogens and lowers overall microbe count; does not kill endospores or many nonpathogenic microbes

Dry Heat

Dry heat using higher temperatures than moist heat

- Incineration – flame or electric heating coil
  - ignites and reduces microbes and other substances
  - Red Heat- 300^{o}C
- Dry ovens – 150-180^{o}C- coagulate proteins



Cold

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

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** – freeze drying; preservation

Radiation

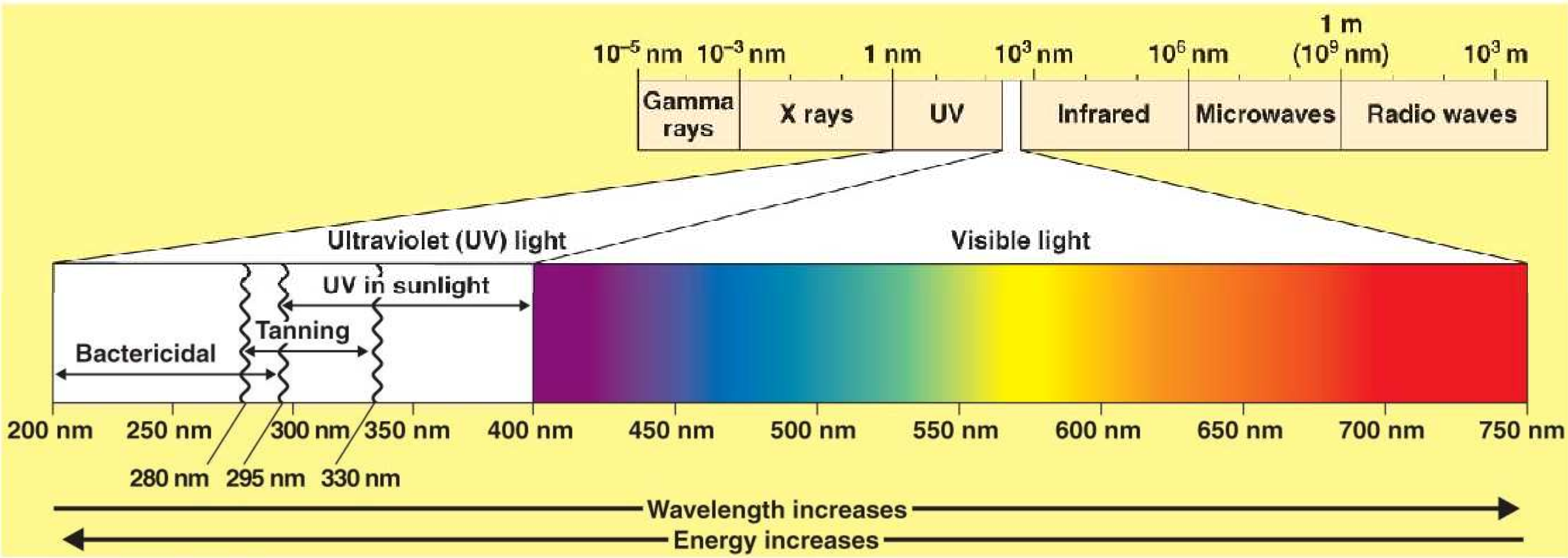


Figure 7.5

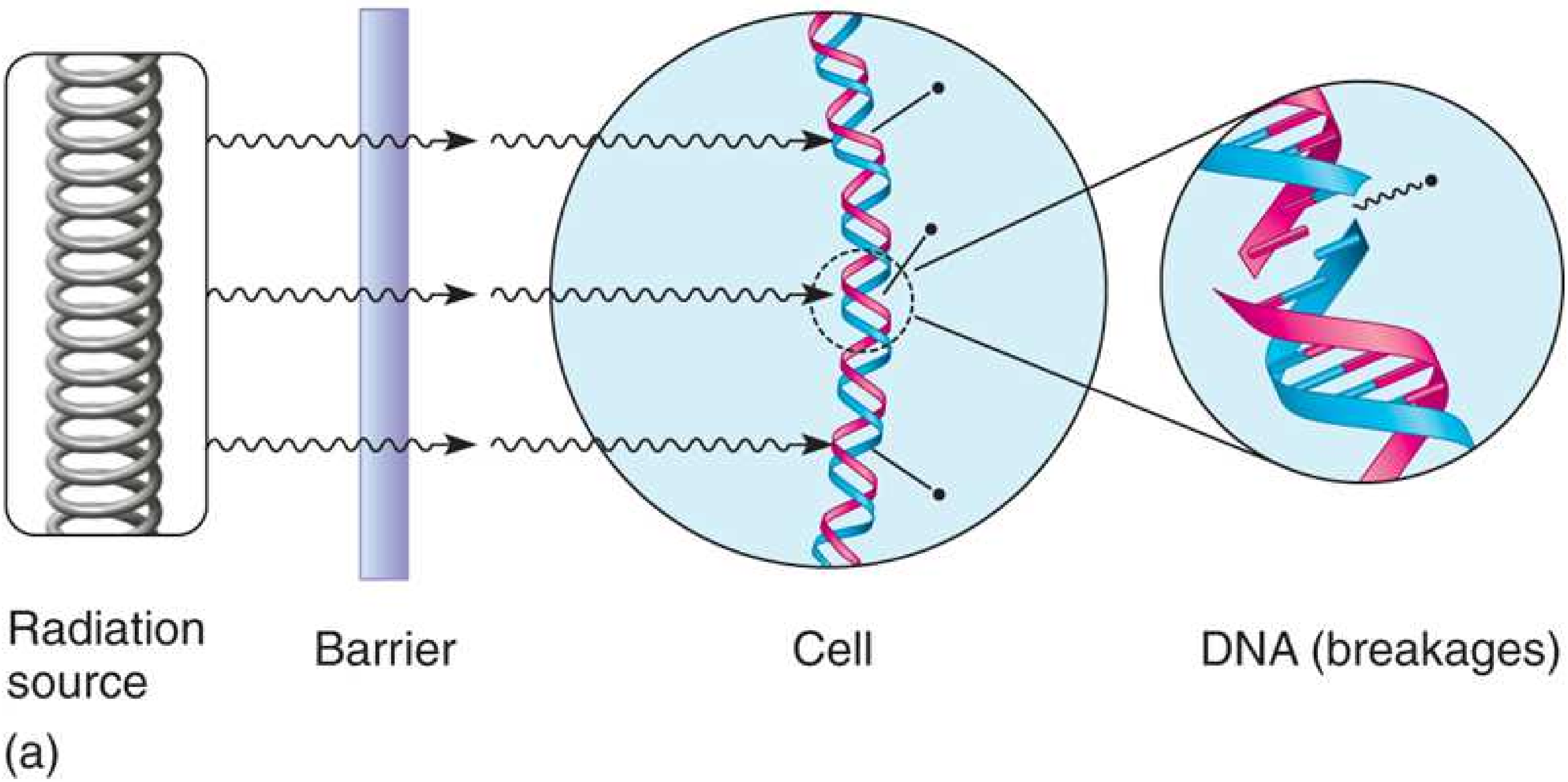
Radiation

- **Ionizing radiation** (X rays, gamma rays, electron beams)
  - Ionizes water to release OH•
  - Damages DNA
- **Nonionizing radiation** (UV, 260 nm)
  - Damages DNA
- **Microwaves** kill by heat; not especially antimicrobial

Radiation

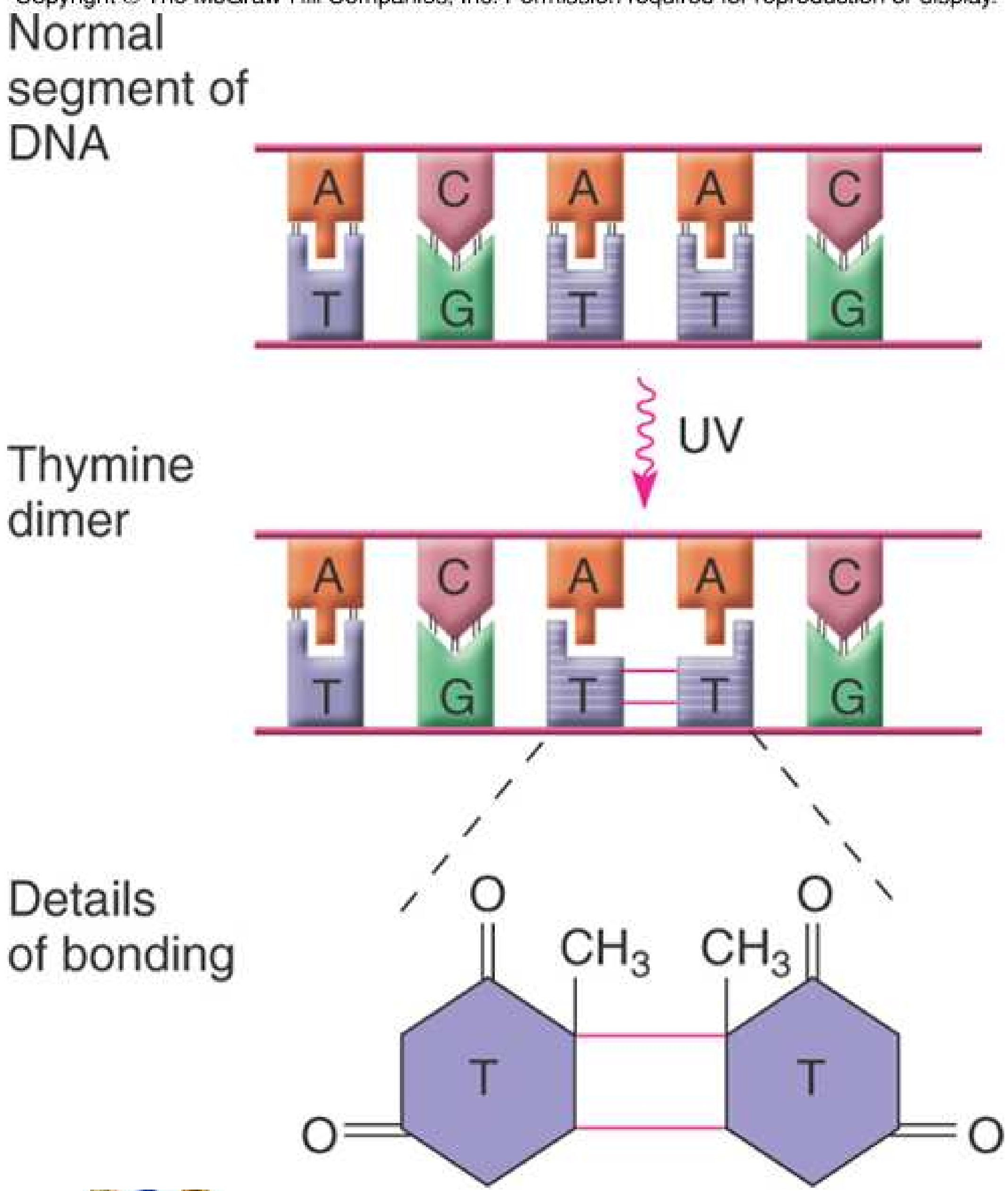
- **Ionizing radiation** – deep penetrating power that has sufficient energy to cause electrons to leave their orbit, breaks DNA,
  - gamma rays, X-rays, cathode rays
  - used to sterilize medical supplies and food products

Ionizing Radiation



Radiation

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


Normal
segment of
DNA
A
C
A
A
C
T
G
T
T
G
UV
Thymine
dimer
A
C
A
A
C
T
G
T
T
G
Details
of bonding
O
O
CH3
CH3
T
T
O
O

Filtration

- 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

Filtration

- **HEPA** removes microbes >0.3 µm
- **Membrane filtration** removes microbes >0.22 µm

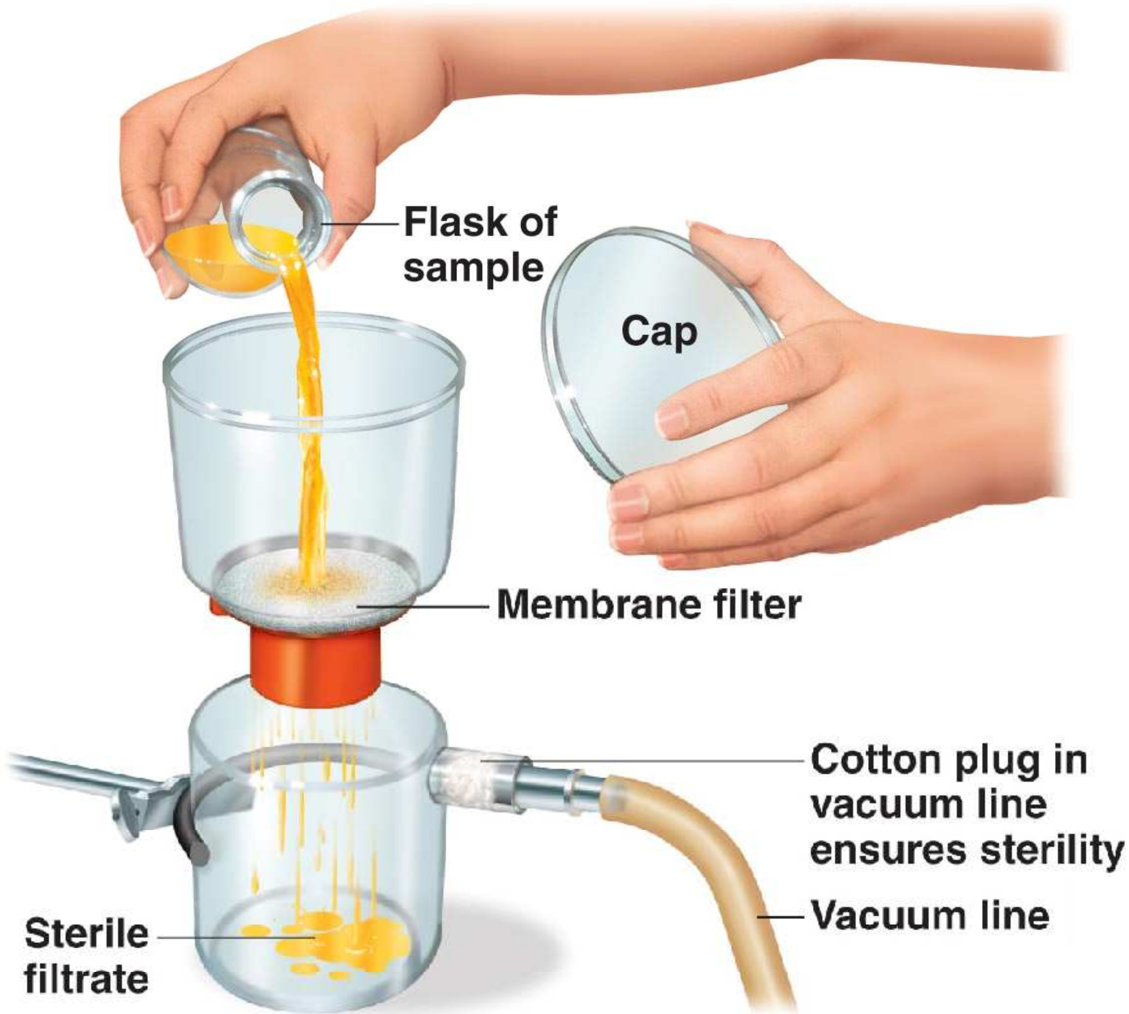
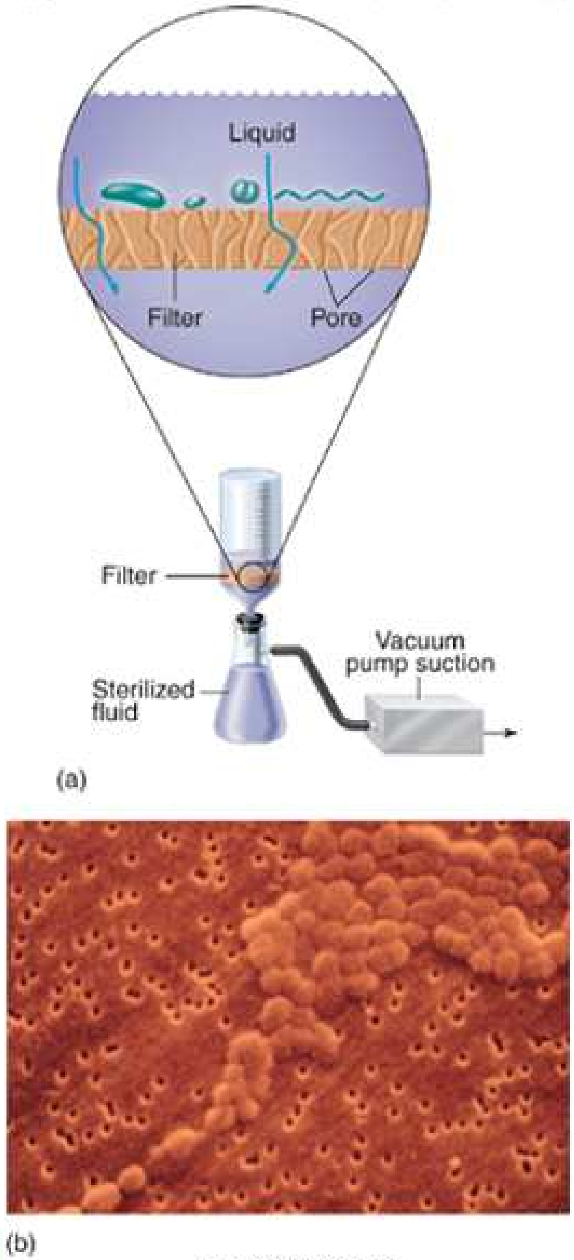


Figure 7.4


Liquid
Filter
Pore
Filter
Vacuum pump suction
Sterilized fluid
(a)
(b)

Chemical Antimicrobials

Agent	Mechanisms of Action	Comments
Surfactants	Membrane Disruption; increased penetration	Soaps; detergents
Quats (cationic detergent)	Denature proteins; Disrupts lipids	Antiseptic - benzalconium chloride, Cepacol; Disinfectant
Organic acids and bases	High/low pH	Mold and Fungi inhibitors; e.g., benzoate of soda
Heavy Metals	Denature protein	Antiseptic & Disinfectant; Silver Nitrate
Halogens	Oxidizing agent Disrupts cell membrane	Antiseptic - Iodine (Betadine) Disinfectant - Chlorine (Chlorox)
Alcohols	Denatures proteins; Disrupts lipids	Antiseptic & Disinfectant Ethanol and isopropyl
Phenolics	Disrupts cell membrane	Disinfectant Irritating odor
Aldehydes	Denature proteins	Gluteraldehyde - disinfectant (Cidex); Formaldehyde - disinfectant
Ethylene Oxide	Denaturing proteins	Used in a closed chamber to sterilize
Oxidizing agents	Denature proteins	Hydrogen peroxide – antiseptic; Hydrogen peroxide – disinfectan; Benzoyl peroxide – antiseptic

Chemical Agents in Microbial Control

- Disinfectants, antiseptics, sterilants, degermers, and preservatives
- 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

Levels of Chemical Decontamination

- 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

What is Disinfection?

Disinfection (in Microbiology) :

1. To kill most of microbial forms except some resistant organisms or bacterium spores

2. Categorizing: High-level ⇔ sterilization
   - Intermediate-level
   - Low level

   → **Not effective for all bacteria or spores**

3. Disinfectant: a substance or method used to kill microbes on surfaces

High-level disinfectants

Used for items involved in invasive procedures but NOT withstand sterilization, e.g. Endoscopes, Surgical instruments

Heat	
Moist heat	75°C to 100°C for 30 min (high)
Liquid	
Glutaraldehyde	2% (high)
Hydrogen peroxide	3% to 25% (high)
Formaldehyde	3% to 8% (high/intermediate)
Chlorine dioxide	Variable (high)
Peracetic acid	Variable (high)
Chlorine compounds	100 to 1000 ppm of free chlorine (high)

Intermediate-level disinfectants

Used for cleaning surface or instruments without bacterial spores and highly resilient organism, eg. Laryngoscopes, Anesthesia breathing circuits…etc

Alcohol (ethyl, isopropyl)	70% to 95% (intermediate)
Phenolic compounds	0.4% to 5.0% (intermediate/ low)
Iodophor compounds	30 to 50 ppm of free iodine/L (intermediate)

Low-level disinfectants

Used to treat non-critical instruments and devices, not penetrating into mucosa surfaces or sterile tissues

Quaternary ammonium compounds	0.4% to 1.6% (low)

Factors that Affect Germicidal Activity of Chemicals

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

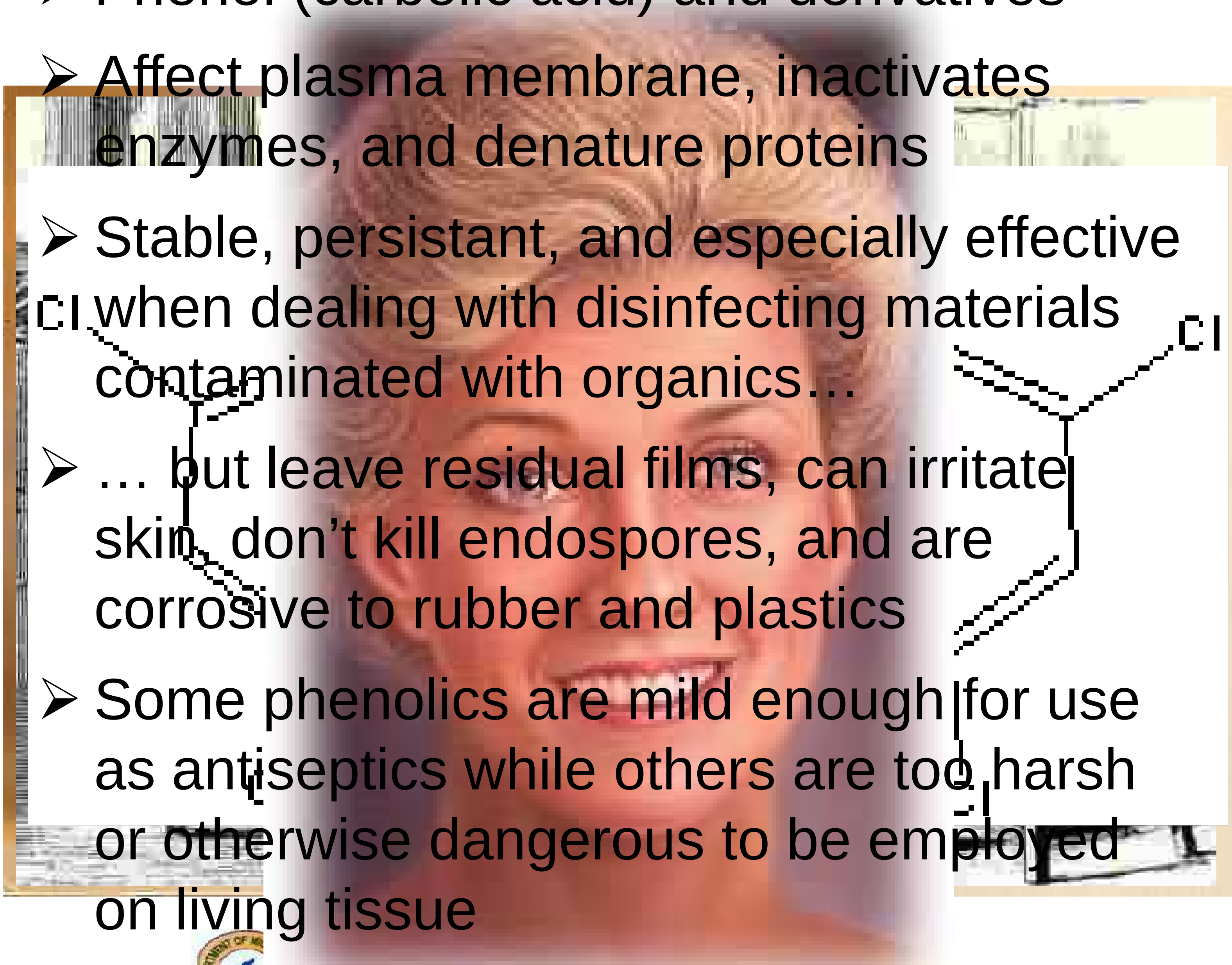
Germicidal Categories

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

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)
  - denature proteins
  - intermediate level
  - milder medical & dental degerming agents, disinfectants, ointments

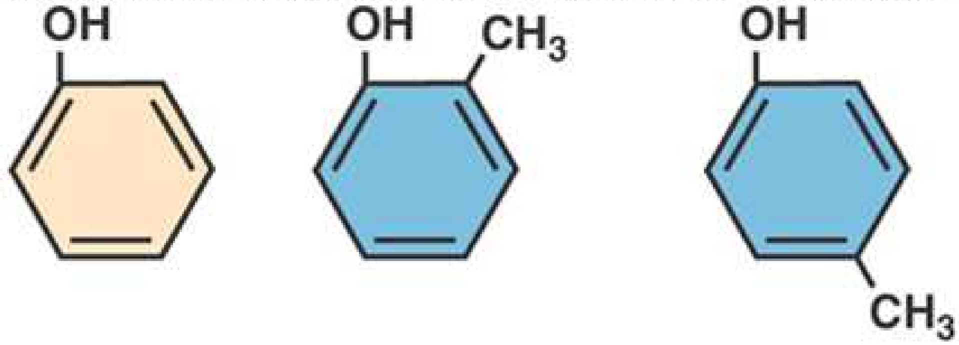
Phenol, Carbolic Acid, & Phenolics



- Phenol (carbolic acid) and derivatives
- Affect plasma membrane, inactivates enzymes, and denature proteins
- Stable, persistant, and especially effective when dealing with disinfecting materials contaminated with organics…
- … but leave residual films, can irritate skin, don't kill endospores, and are corrosive to rubber and plastics
- Some phenolics are mild enough for use as antiseptics while others are too harsh or otherwise dangerous to be employed on living tissue
- Hexachlorophene, Triclosan, Lysol, soap

Phenolics

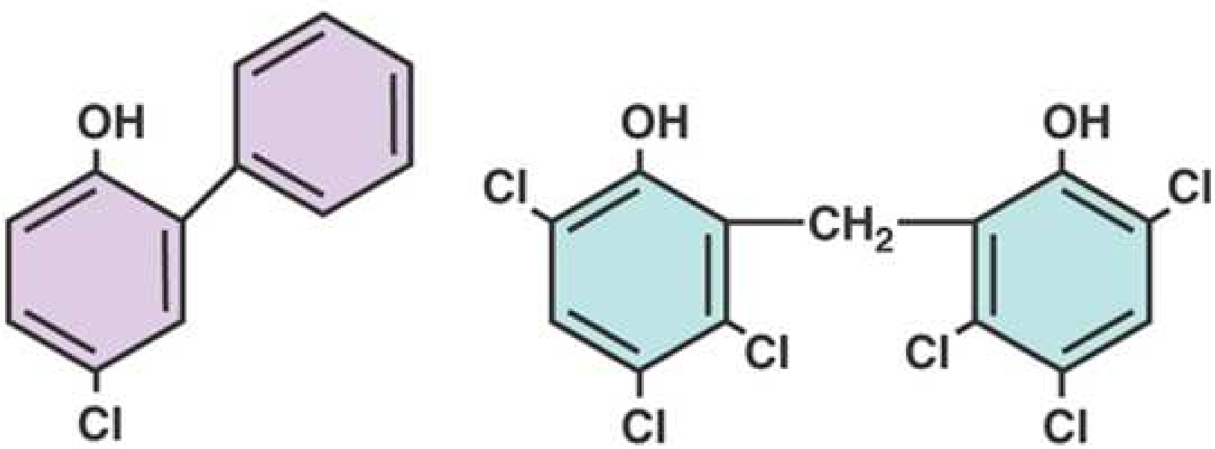
- Disrupt cell walls and membranes and precipitate proteins
- Low to intermediate level - bactericidal, fungicidal, virucidal, not sporicidal
  - lysol
  - triclosan- antibacterial additive to soaps



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

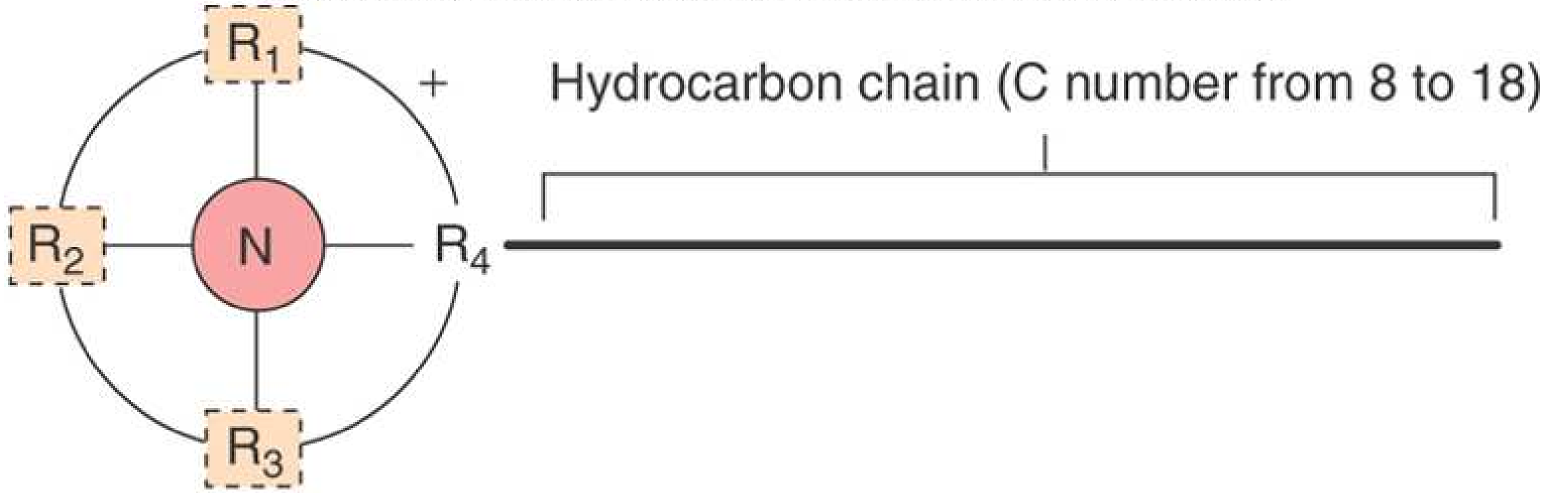
- Ethyl, isopropyl in solutions of 50-95%
- Act as surfactants dissolving membrane lipids and coagulating proteins of vegetative bacterial cells and fungi
- Intermediate level

Hydrogen Peroxide

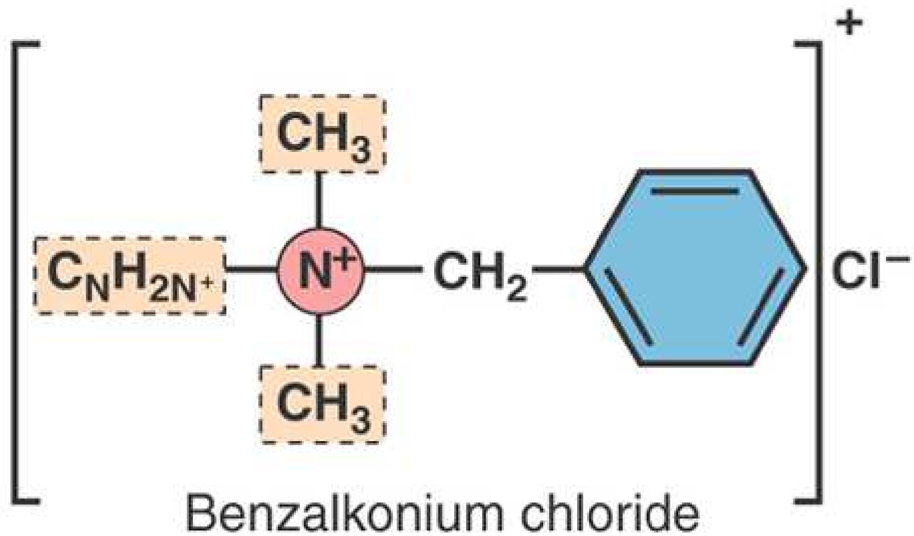
- Weak (3%) to strong (25%)
- Produce highly reactive hydroxyl-free radicals that damage protein and DNA while also decomposing to O_2 gas – toxic to anaerobes
- Antiseptic at low concentrations; strong solutions are sporicidal

Detergents and Soaps

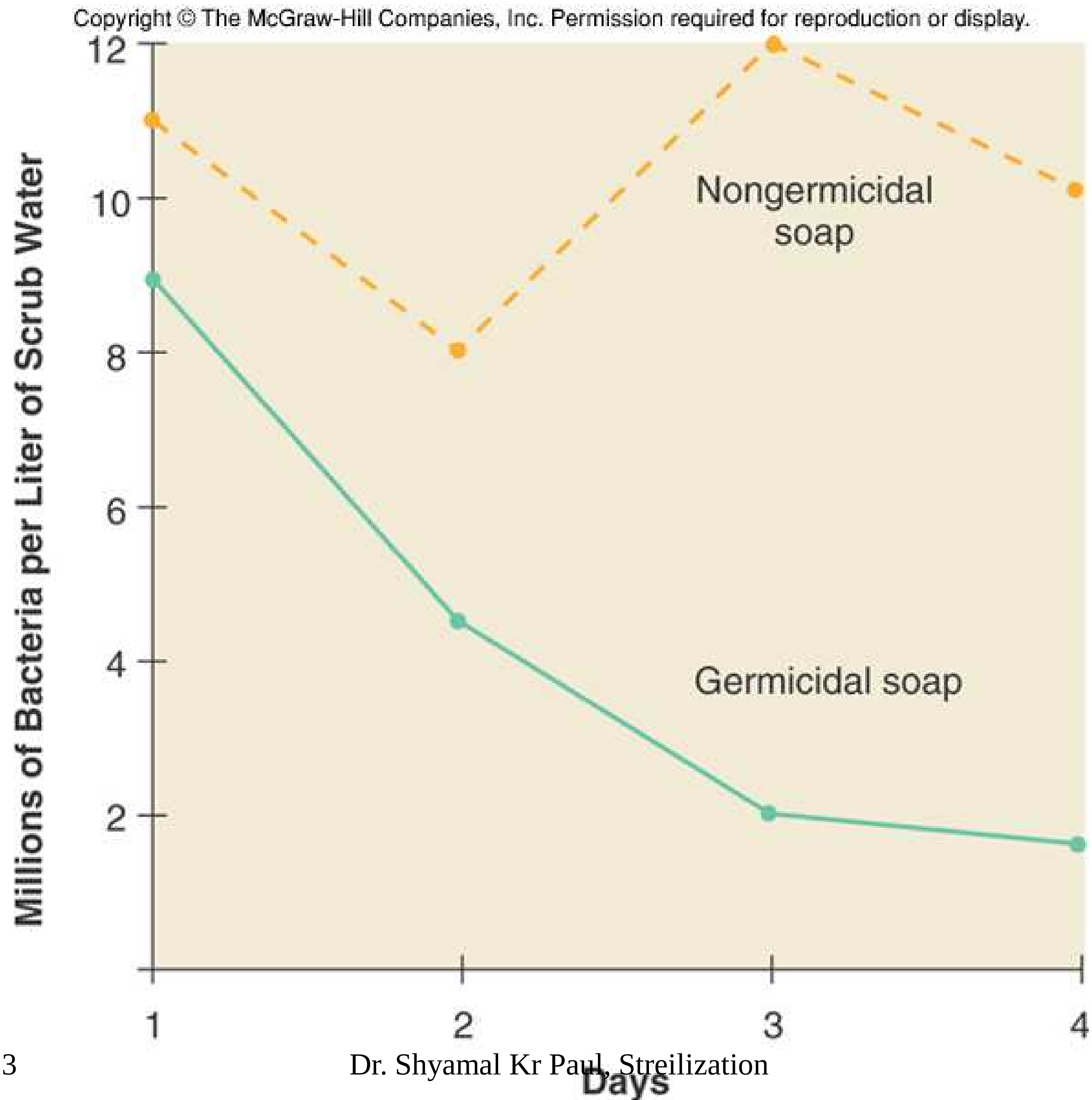
- Quaternary ammonia compounds (quats) act as surfactants that alter membrane permeability of some bacteria and fungi.
- Very low level
- Soaps - mechanically remove soil and grease containing microbes


R1
+
Hydrocarbon chain (C number from 8 to 18)
R2
N
R4
R3

(a)


+
CH3
CNH2N+
N+
CH2
Cl−
CH3
Benzalkonium chloride

(b)



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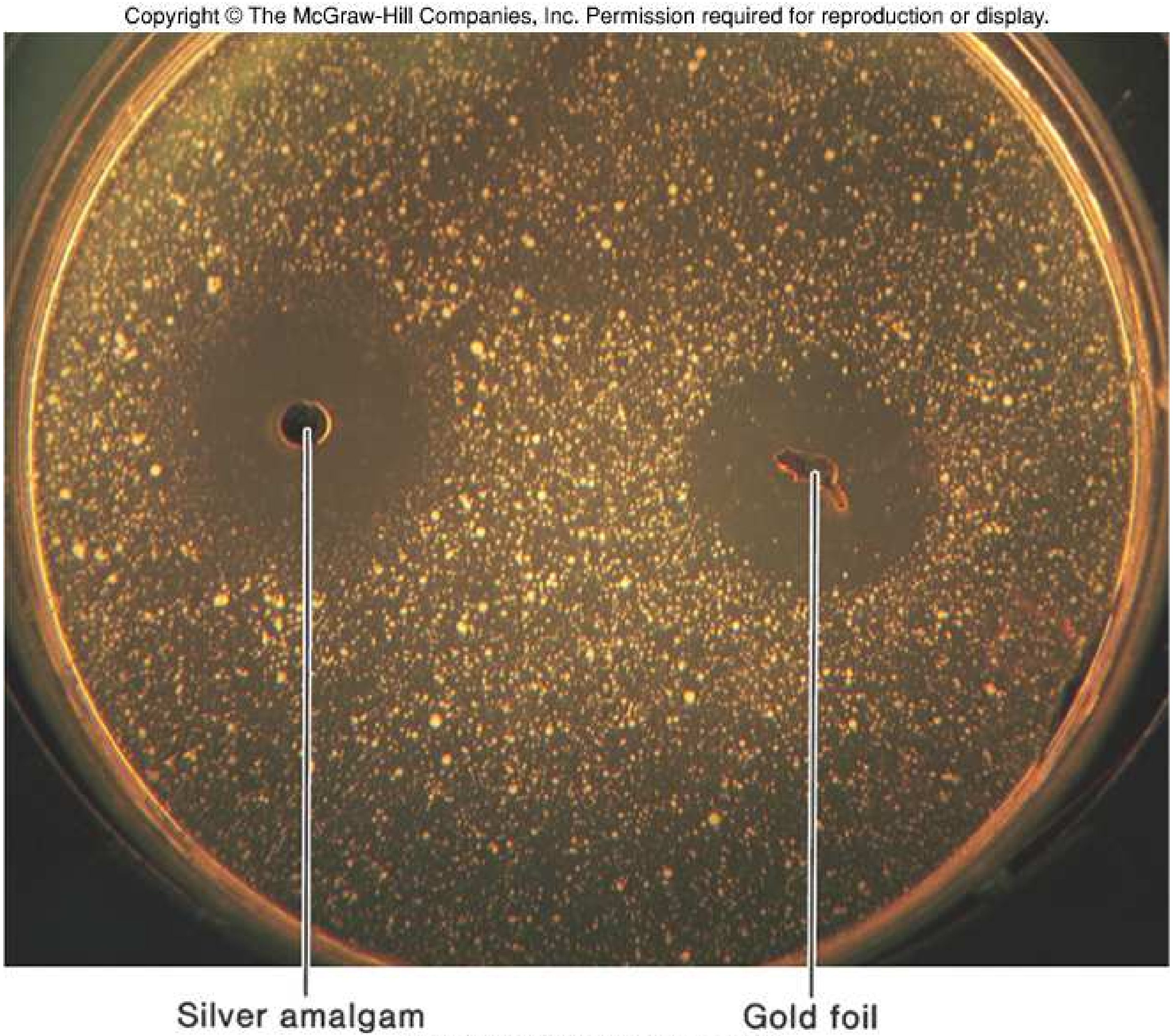
Nongermicidal soap
Germicidal soap
Millions of Bacteria per Liter of Scrub Water
12
10
8
6
4
2
1
2
3
4
Days

Heavy Metals

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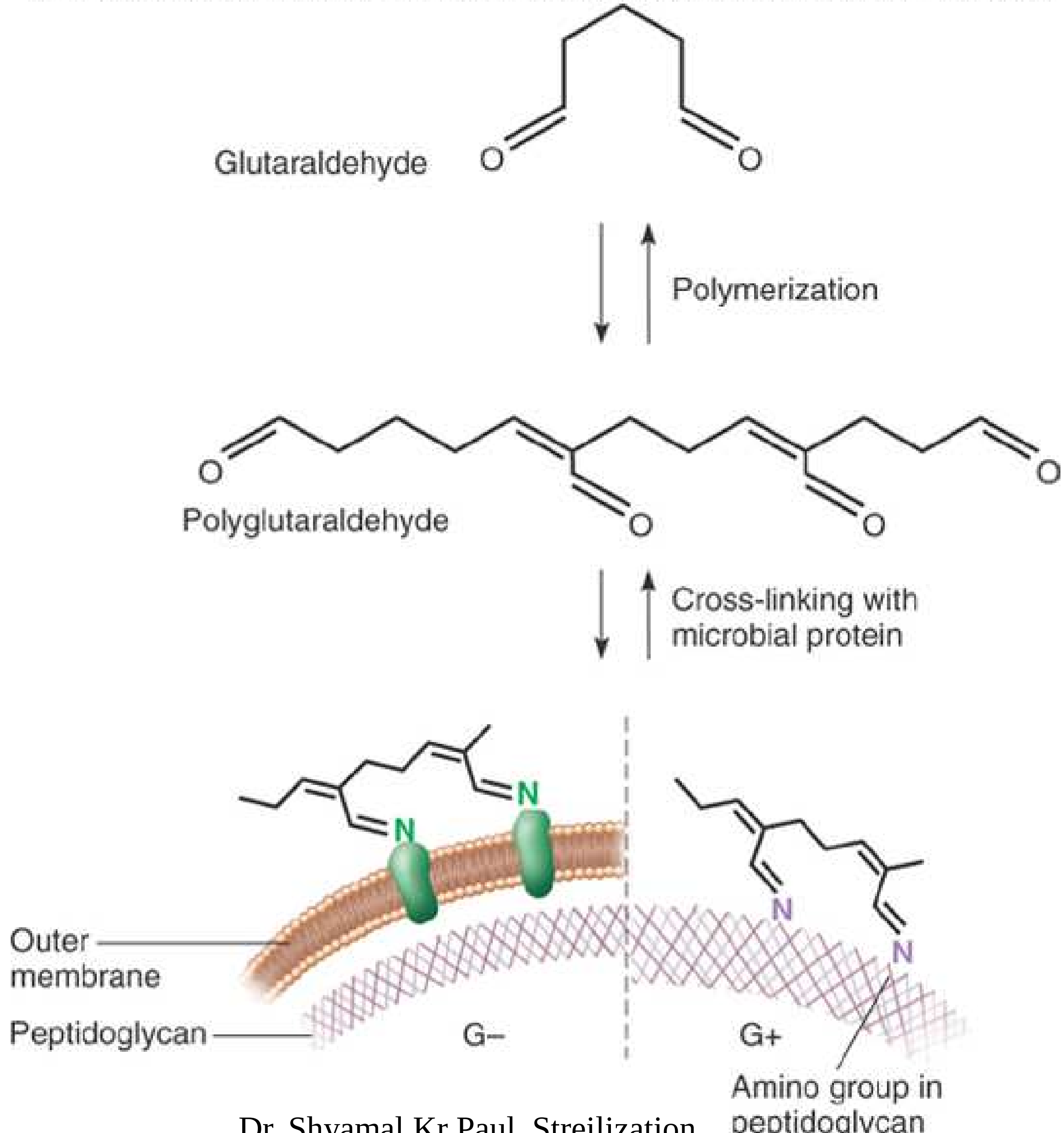
Silver amalgam
Gold foil

Aldehydes

- Glutaraldehyde and formaldehyde 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


Glutaraldehyde
O
O
Polymerization
Polyglutaraldehyde
O
O
O
O
Cross-linking with
microbial protein
N
N
N
N
Outer
membrane
Peptidoglycan
G–
G+
Amino group in
peptidoglycan

Gases and Aerosols

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

TABLE 11.8

Active Ingredients of Various Commercial Antimicrobial Products

Product	Specific Chemical Agent	Antimicrobial Category
Lysol and Chlorox Sanitizing Wipes	Dimethyl benzyl ammonium chloride	Detergent (quat)
Tilex and Lysol Mildew Remover	Sodium hypochlorites	Halogen
Ajax and 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