- Sterilitetsbegreppet
- Avdödningskinetik och faktorer
- Bakteriella endosporer
- Fysikaliska avdödningsmetoder (värme och strålning)
- Mekaniska avdödningsmetoder
- Kemiska avdödningsmetoder (MIC och MLK)

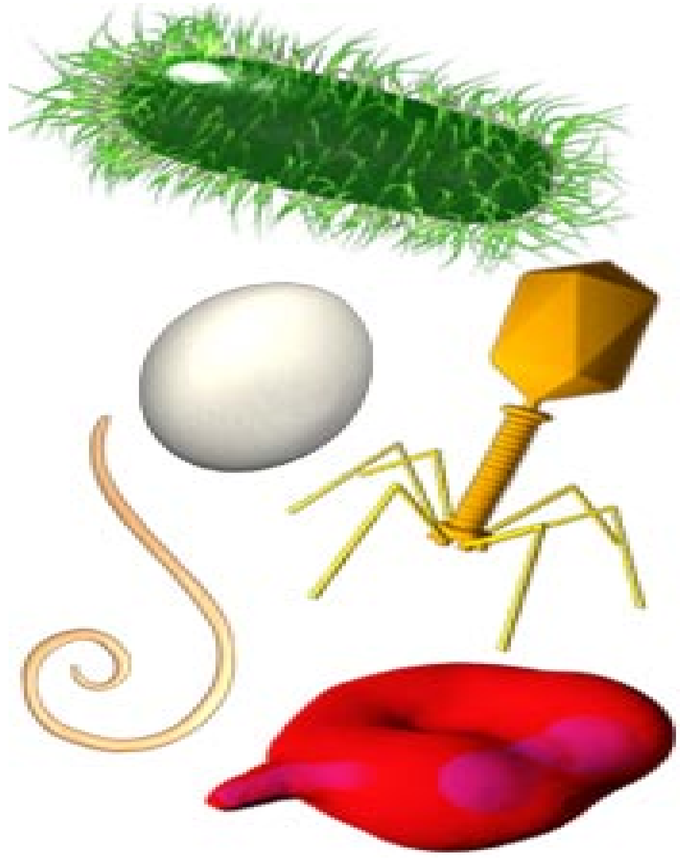
- Joniserande strålning är ett samlingsbegrepp på strålning som har förmågan att slå ut elektroner ur atomer som den kolliderar med, vilket förvandlar atomerna till joner. Joniserande strålning kan antingen vara elektromagnetisk strålning (ultraviolett, röntgen-, och gammastrålning) eller partikelstrålning (energirika elektroner, protoner med mera som har en energi på några elektronvolt).
- Alfastrålning eller α-strålning är en typ av joniserande strålning bestående av alfapartiklar, det vill säga, atomkärnor av helium (två protoner och två neutroner). Alfastrålning avges i samband med radioaktivt sönderfall av typ alfa-sönderfall.
- Betastrålning eller β-strålning är en typ av joniserande strålning bestående av betapartiklar, det vill säga, elektroner och/eller positroner, som uppstår vid radioaktivt betasönderfall. Det är en form av partikelstrålning. Eftersom elektronerna har elektrisk laddning växelverkar de med andra laddade partiklar via elektromagnetisk växelverkan, därför är de lätta att stoppa.
- Gammastrålning eller γ-strålning är fotonstrålning, det vill säga joniserande strålning av fotoner. Gammastrålning är den mest genomträngande formen av strålar som förekommer i samband med radioaktivitet. Gammastrålning finns i den kosmiska strålningen. Den kan stoppas med hjälp av en betongvägg eller bly

Sterilization

Concepts

- *Sterilization* – removal of all living cells from an object, including viruses, spores, viroids and prions

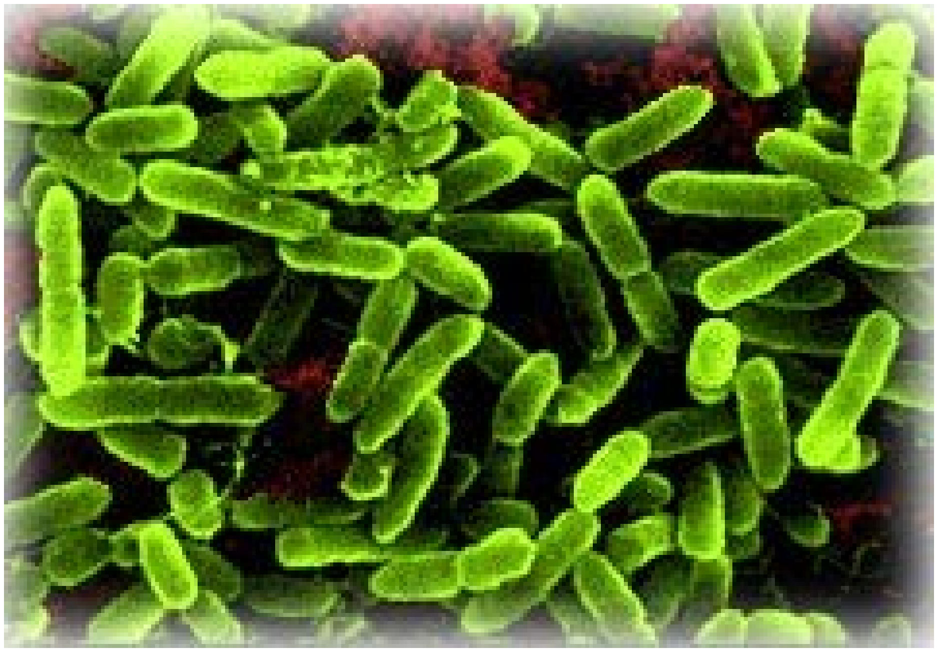
- *Aseptic technique* – techniques with sterile solutions



Sterilization

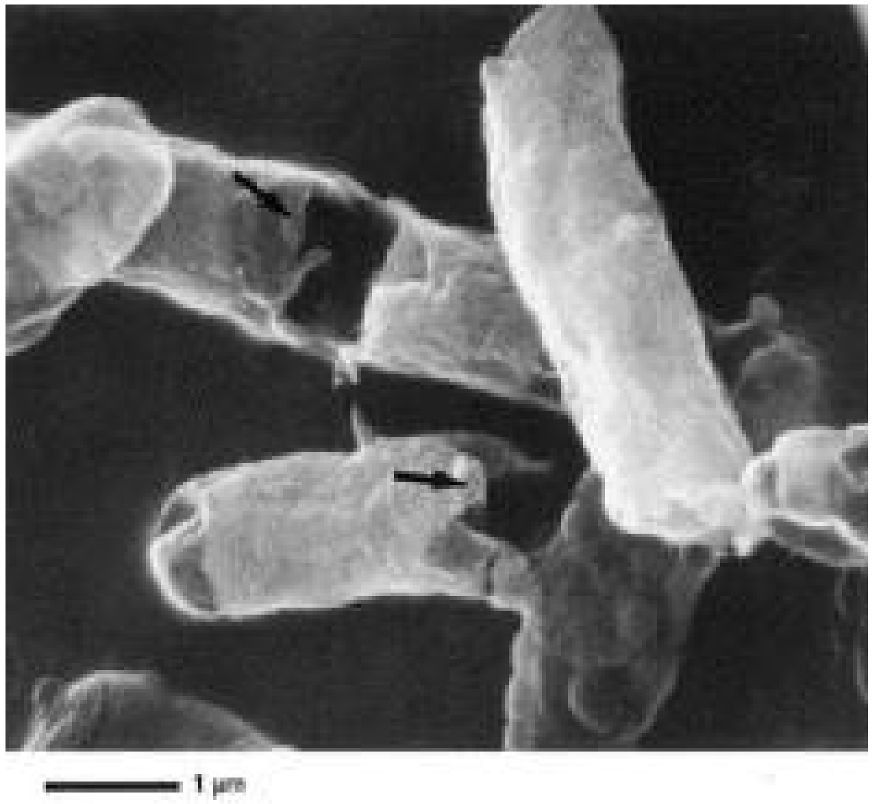
Concepts

- *-static* – bacteriostatic, fugistatic (growth-inhibiting)
- *-cide* – bactericide, algicide, fungicide (killing)



Killing kinetics

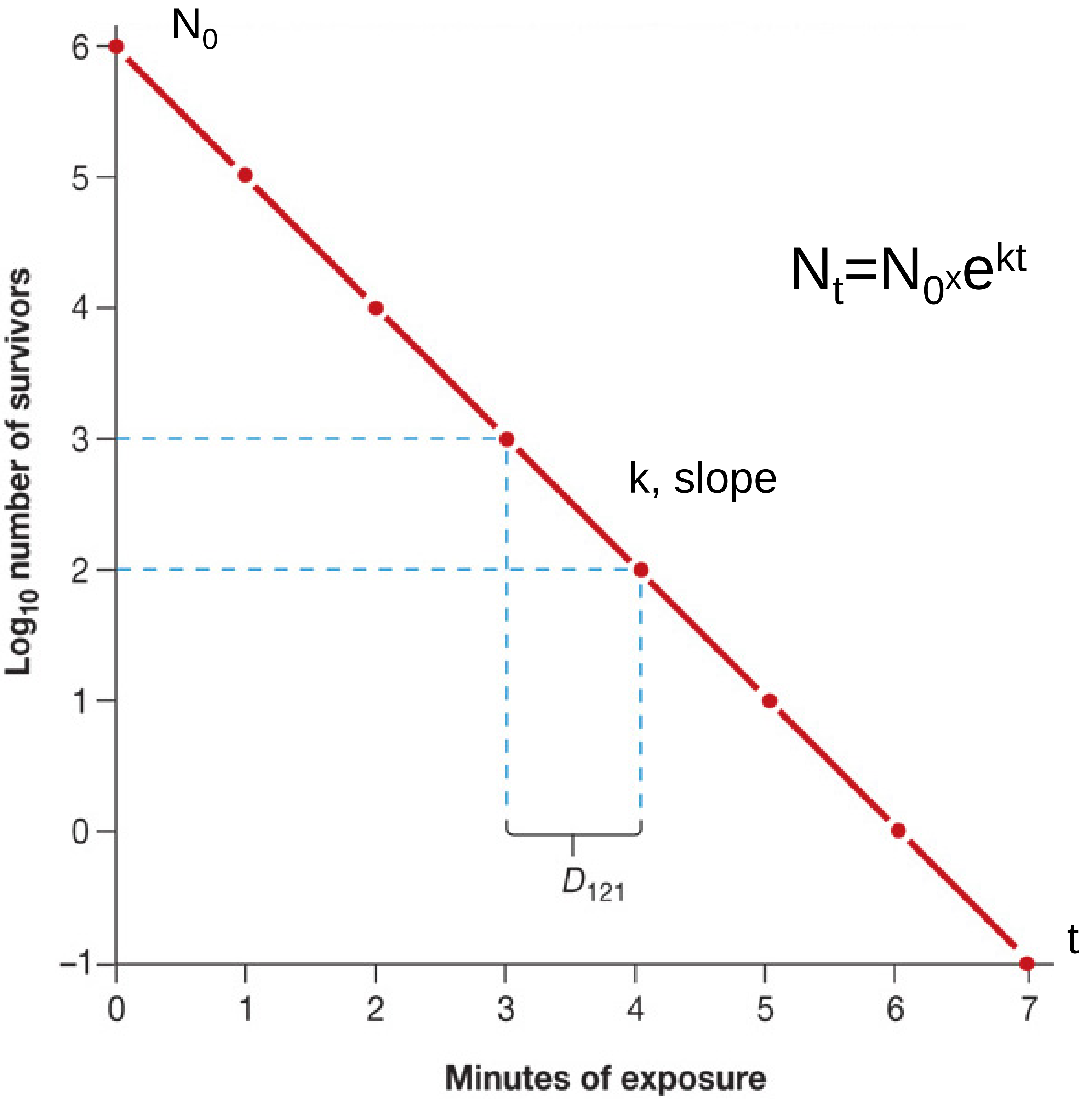
- TDP – thermal death point: the lowest temperature at which all the bacteria in a population will be killed within 10 minutes



- TDT – thermal death time: the length of time before all microorganisms in a population will be killed at a certain temperature

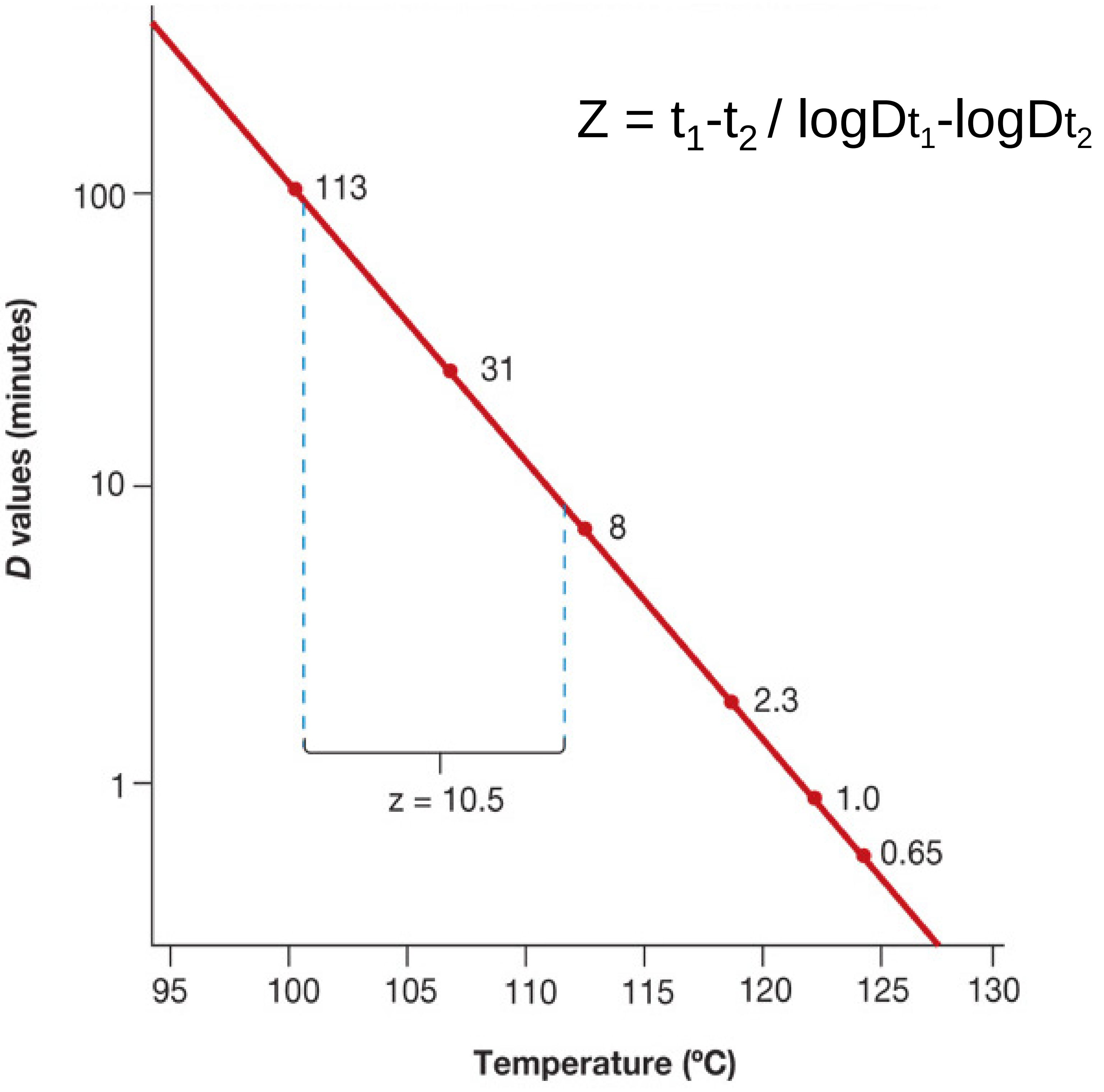
Decimal reduction time (*D* or *D value*)

time required to kill 90% of microorganisms or spores in a sample at a specific temperature



Z value

increase in temperature required to reduce *D* by 1/10



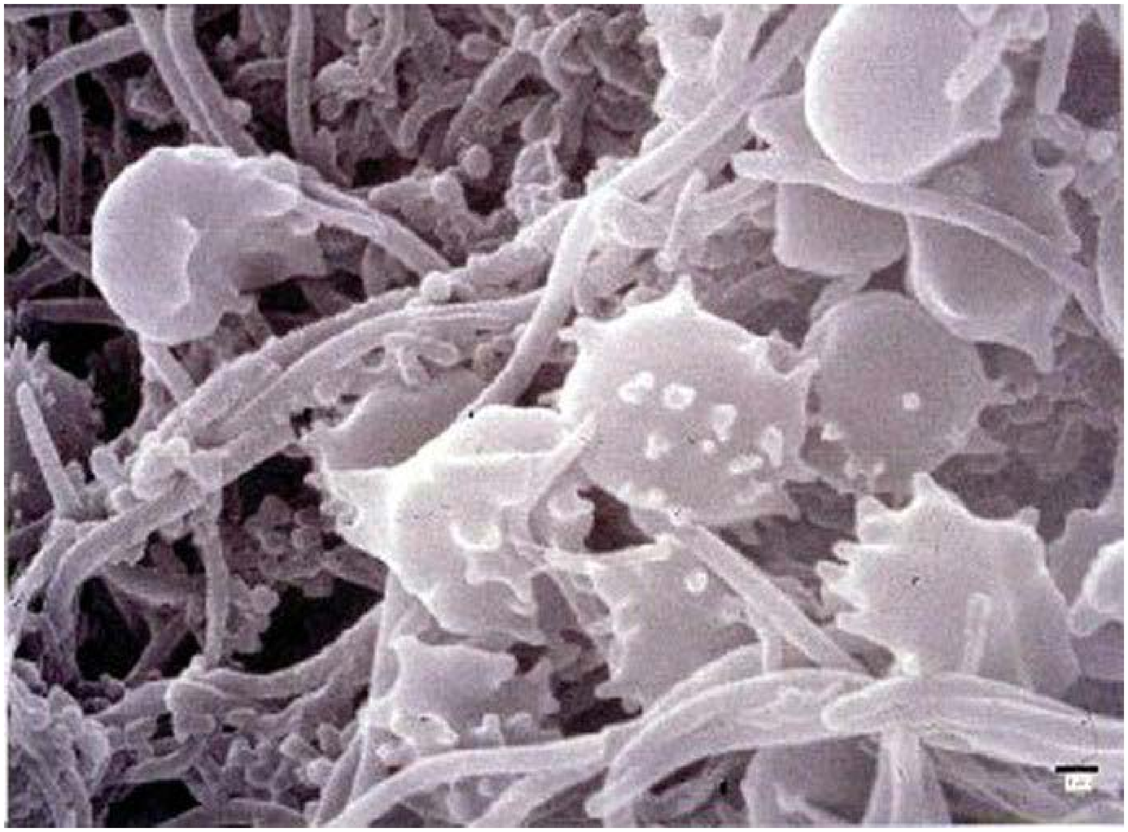
D- and Z-values

Table 7.3 ***D* Values and *z* Values for Some Food-Borne Pathogens**

Organism	Substrate	*D* Value (°C) in Minutes	*z* Value (°C)
Clostridium botulinum	Phosphate buffer	$D_{121} = 0.204$	10
Clostridium perfringens (heat-resistant strain)	Culture media	$D_{90} = 3–5$	6–8
Salmonella spp.	Chicken à la king	$D_{60} = 0.39–0.40$	4.9–5.1
Staphylococcus aureus	Chicken à la king	$D_{60} = 5.17–5.37$	5.2–5.8
	Turkey stuffing	$D_{60} = 15.4$	6.8
	0.5% NaCl	$D_{60} = 2.0–2.5$	5.6

Factors that affect cell death

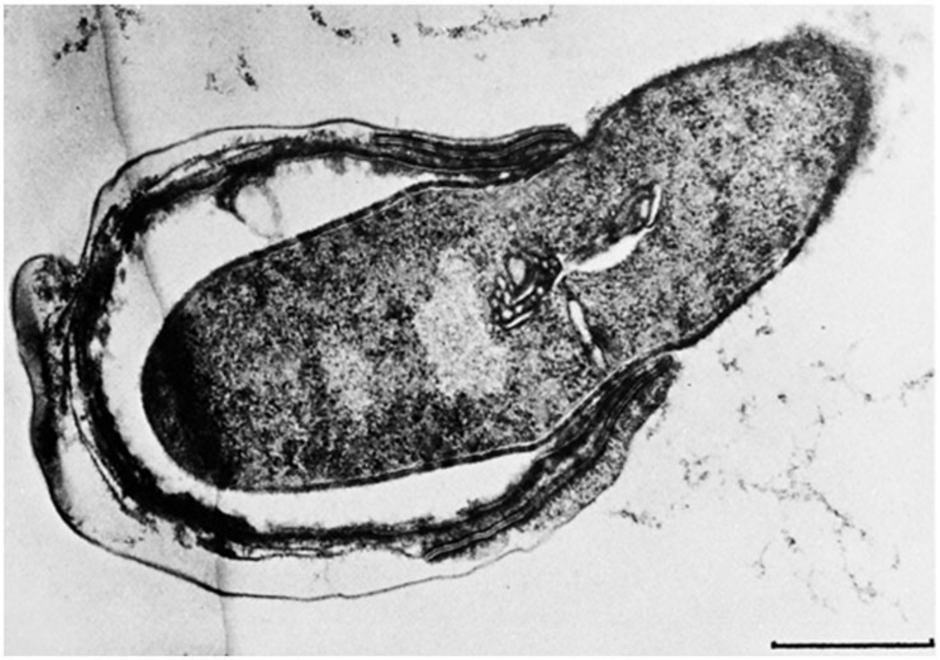
- Time
- Number of cells
- Type of microorganism,
- Method of killing
- Concentration or intensity
- Temperature
- pH
- Organic material
- Biofilm
- Development of resistance



Biofilm, Source: University of Wisconsin-Madison, Department of Bacteriology

The Bacterial Endospore

- formed by some bacteria (*Bacillus* and *Clostridium*)
- normally formed when growth ceases because of lack of nutrients
- Dormant (no metabolism)
- resistant to numerous environmental conditions
  - heat
  - radiation
  - chemicals
  - desiccation



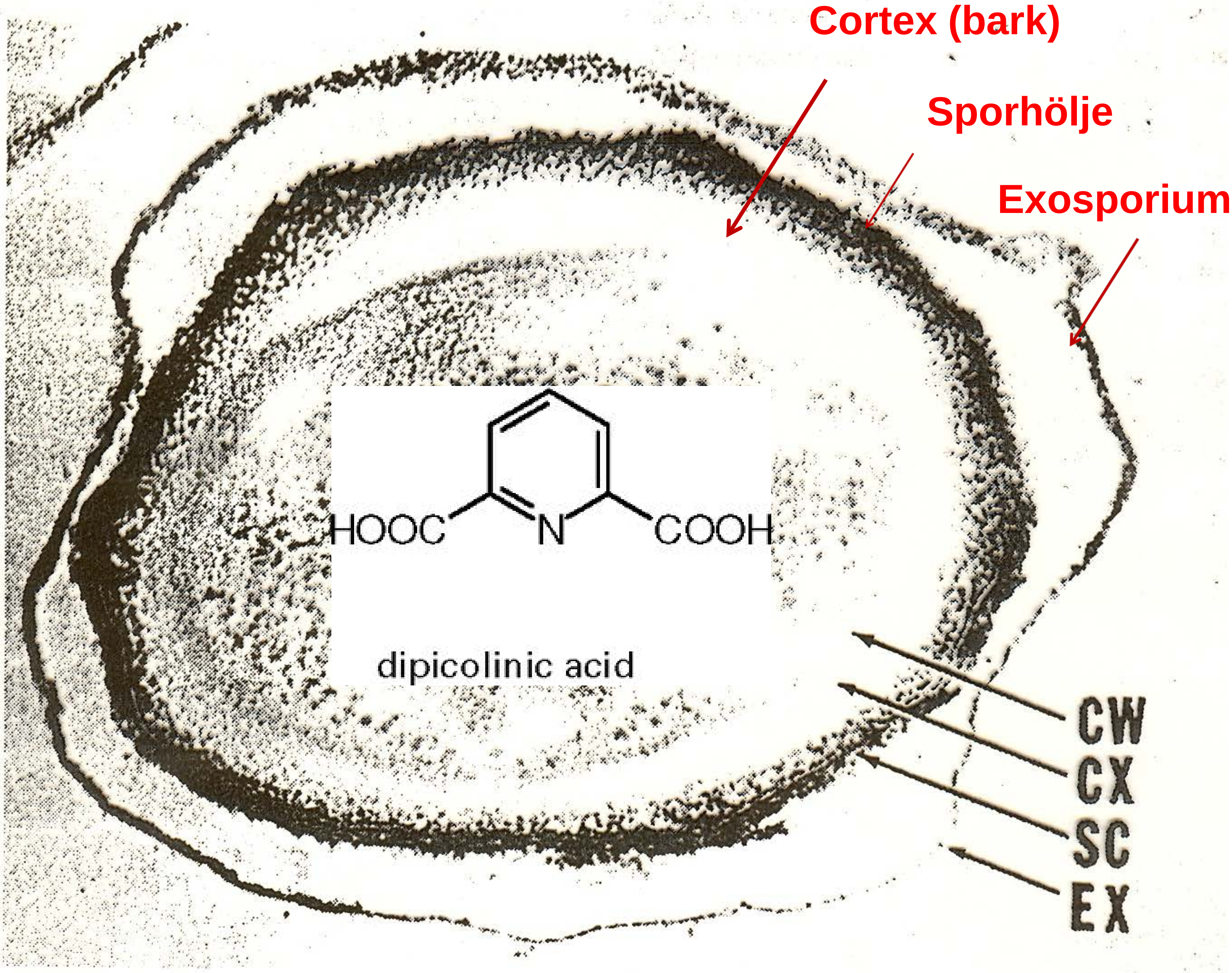
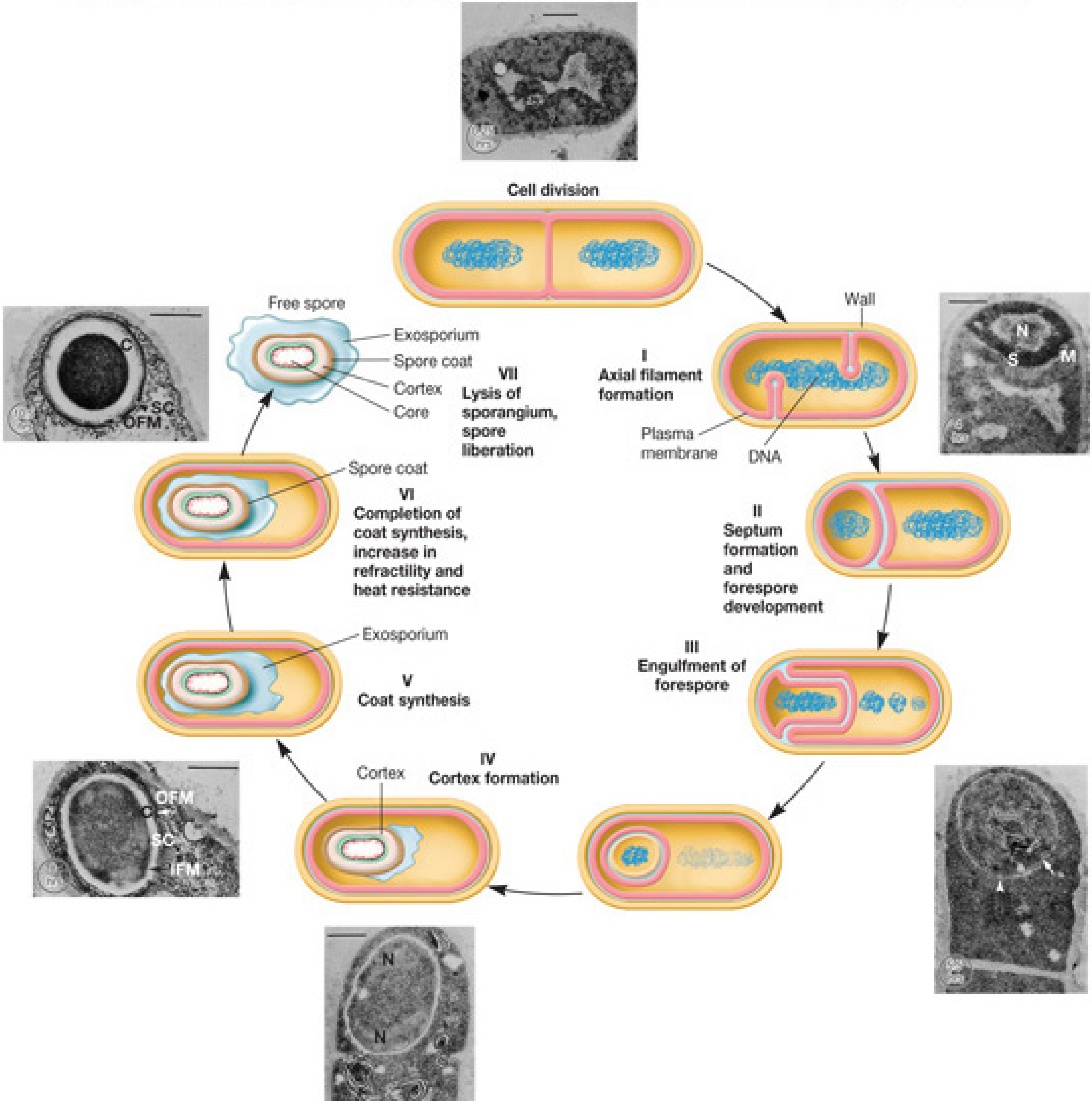
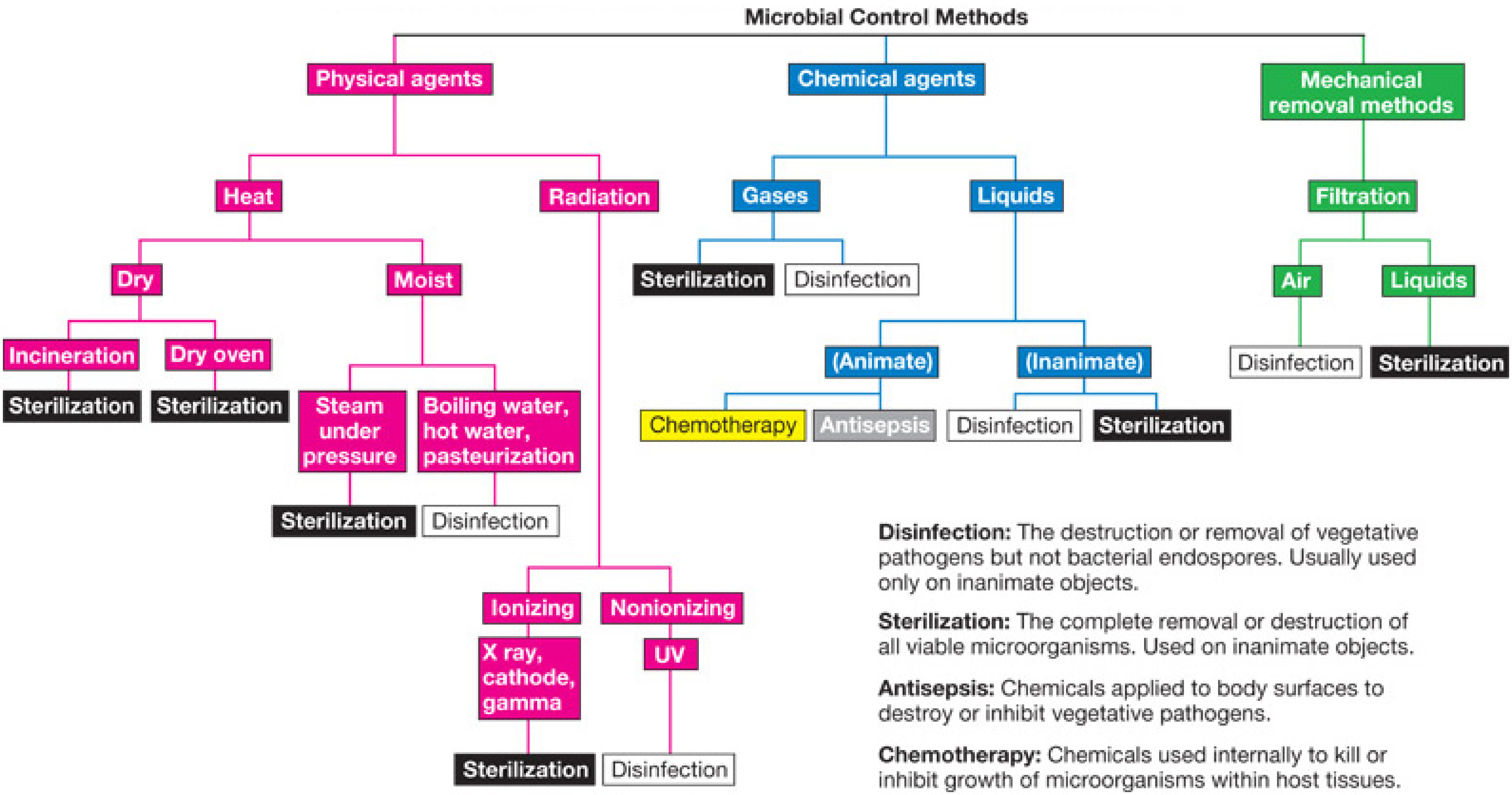


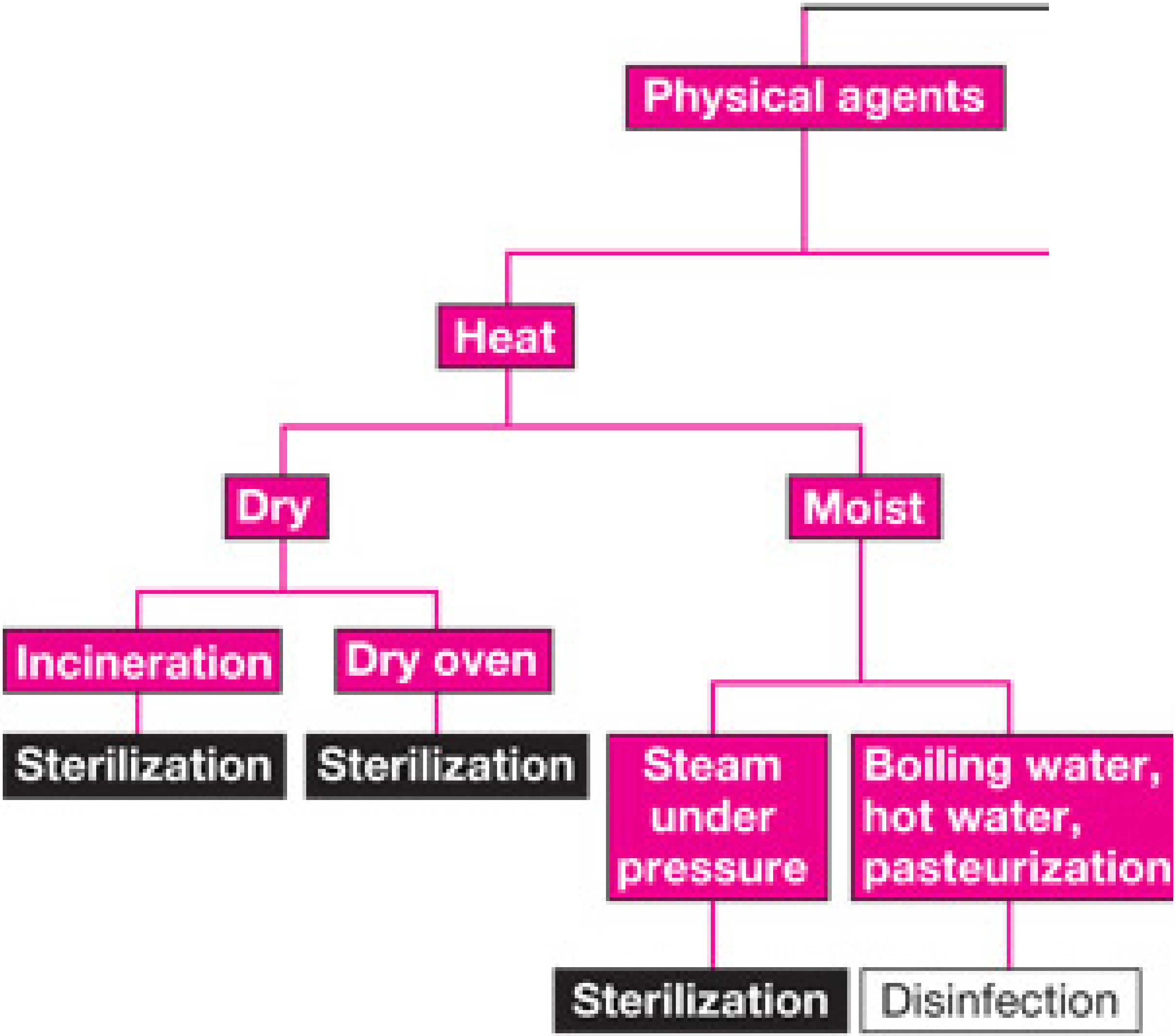
Figure 3.41 Endospore Structure. *Bacillus anthracis* endospore (×151,000). Note the following structures: exosporium, EX; spore coat, SC; cortex, CX; core wall, CW; and the protoplast or core with its nucleoid, N, and ribosomes, CR.



Cell division
Wall
I
Axial filament formation
Plasma membrane
DNA
N
S
M
II
Septum formation and forespore development
III
Engulfment of forespore
IV
Cortex formation
Cortex
N
N
OFM
C
SC
IFM
V
Coat synthesis
Exosporium
VI
Completion of coat synthesis, increase in refractility and heat resistance
Spore coat
VII
Lysis of sporangium, spore liberation
Free spore
Exosporium
Spore coat
Cortex
Core
C
SC
OFM

Control of Microorganisms by Physical and Chemical Agents




Physical agents
Heat
Dry
Moist
Incineration
Dry oven
Sterilization
Sterilization
Steam under pressure
Boiling water, hot water, pasteurization
Sterilization
Disinfection

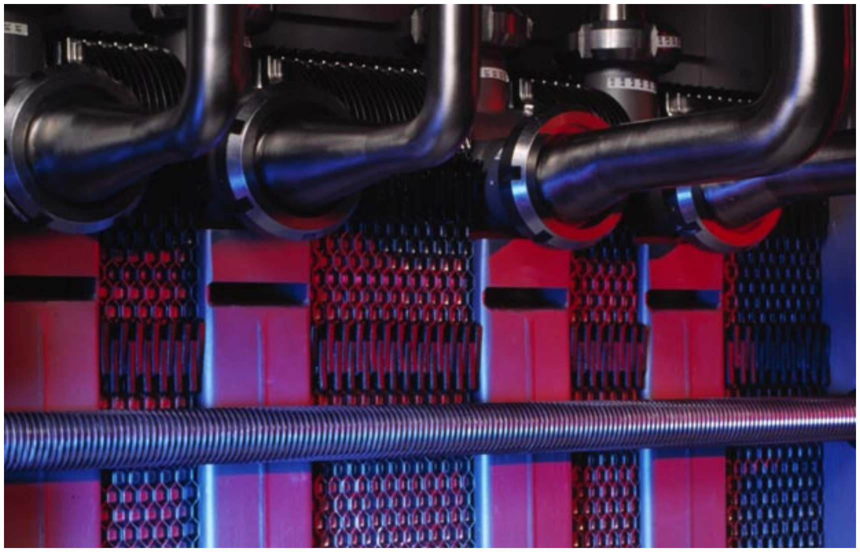
Pasteurization

- LTH, low temperature holding, 63°C, 30 min
- HTST, high temperature short time, 72°C, 15 sec
- UHT, ultra high temperature, 140-150°C, 1-3 sec
- controlled heating at temperatures well below boiling
- used for milk, beer and other beverages
- process does not sterilize but does kill pathogens present and slow spoilage by reducing the total load of organisms present

Louis Pasteur,1822-1895

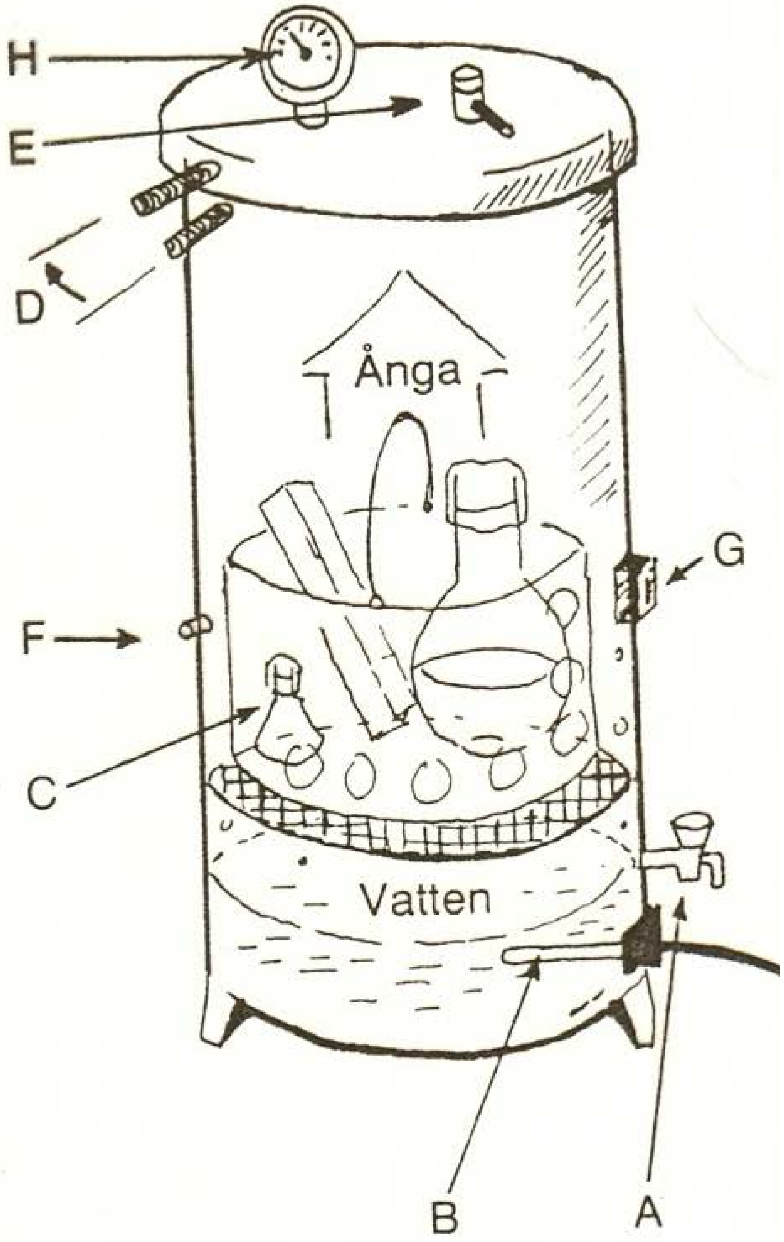
UHT Sterilisation

UHT, or Ultra High Temperature treatment takes place in optimised heat exchangers before packaging. This process minimises heat penetration problems and allows very short heating and cooling times, at the same time minimising unwanted changes in the taste and nutritional properties of the product.

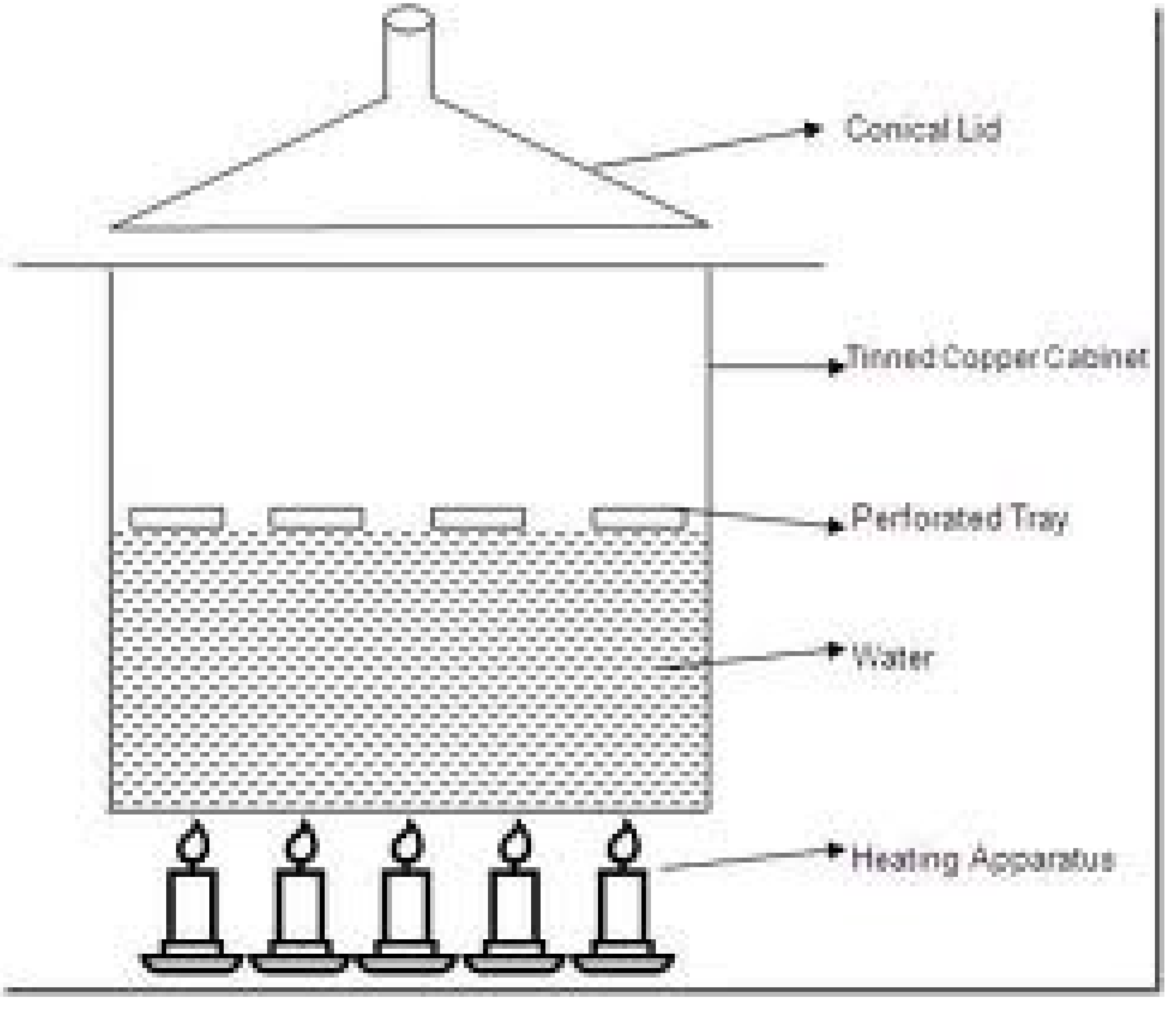


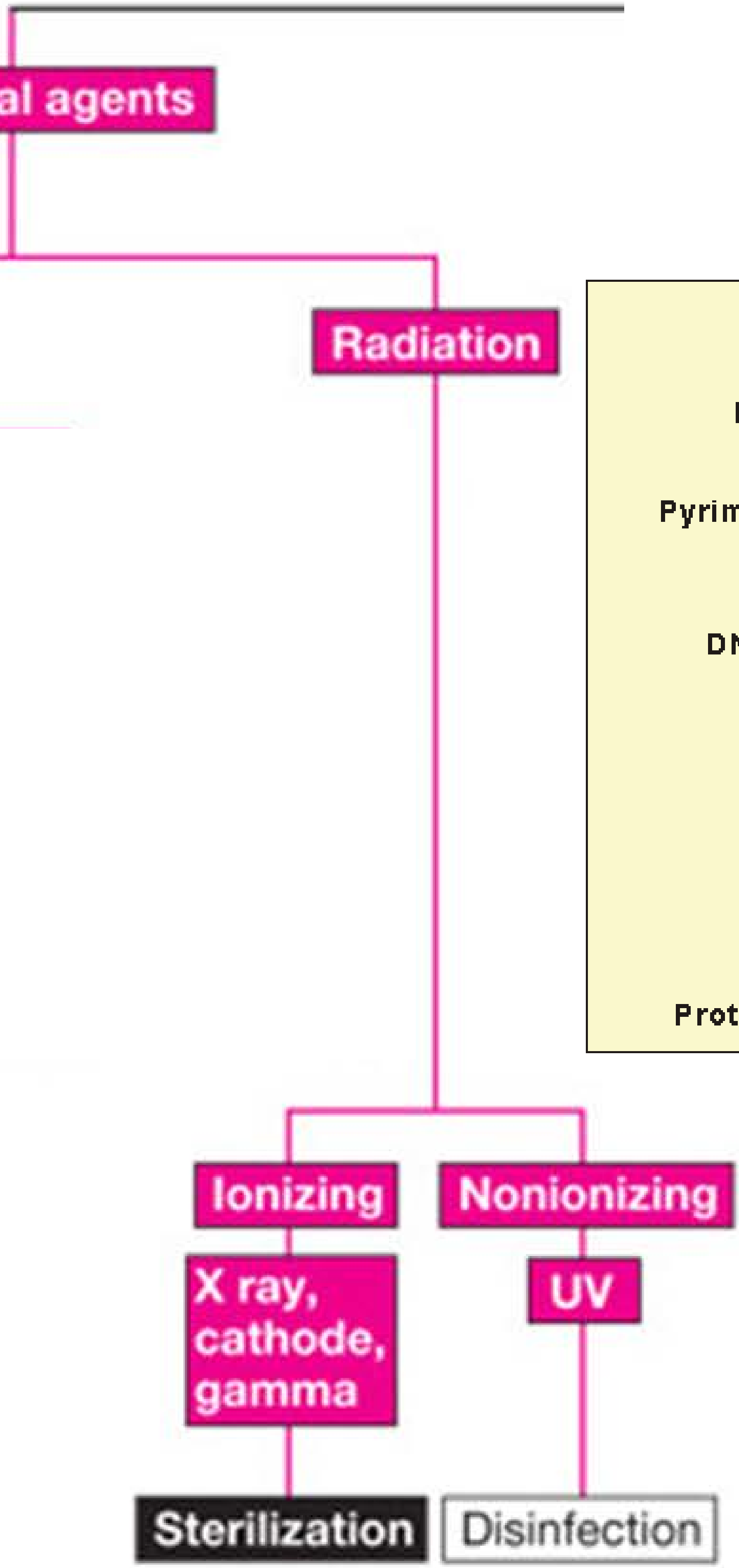
The Autoclave or Steam Sterilizer

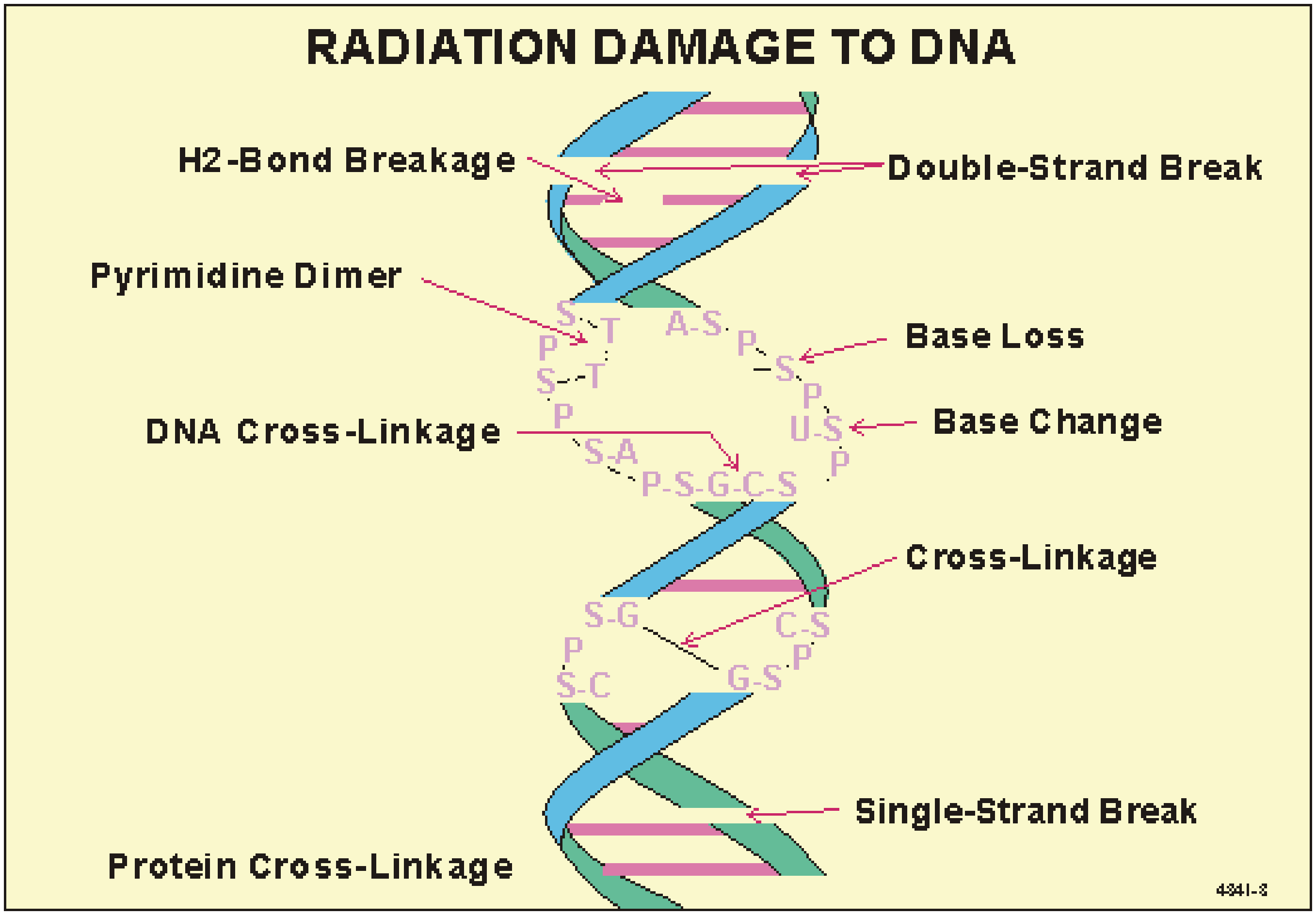
- must be carried out above 100ºC which requires saturated steam under pressure
- carried out using an autoclave
- effective against all types of microorganisms including spores
- degrades nucleic acids, denatures proteins, and disrupts membranes



Tyndallization




al agents
Radiation
Ionizing
Nonionizing
X ray, cathode, gamma
UV
Sterilization
Disinfection


RADIATION DAMAGE TO DNA
H2-Bond Breakage
Double-Strand Break
Pyrimidine Dimer
Base Loss
DNA Cross-Linkage
Base Change
Cross-Linkage
Single-Strand Break
Protein Cross-Linkage

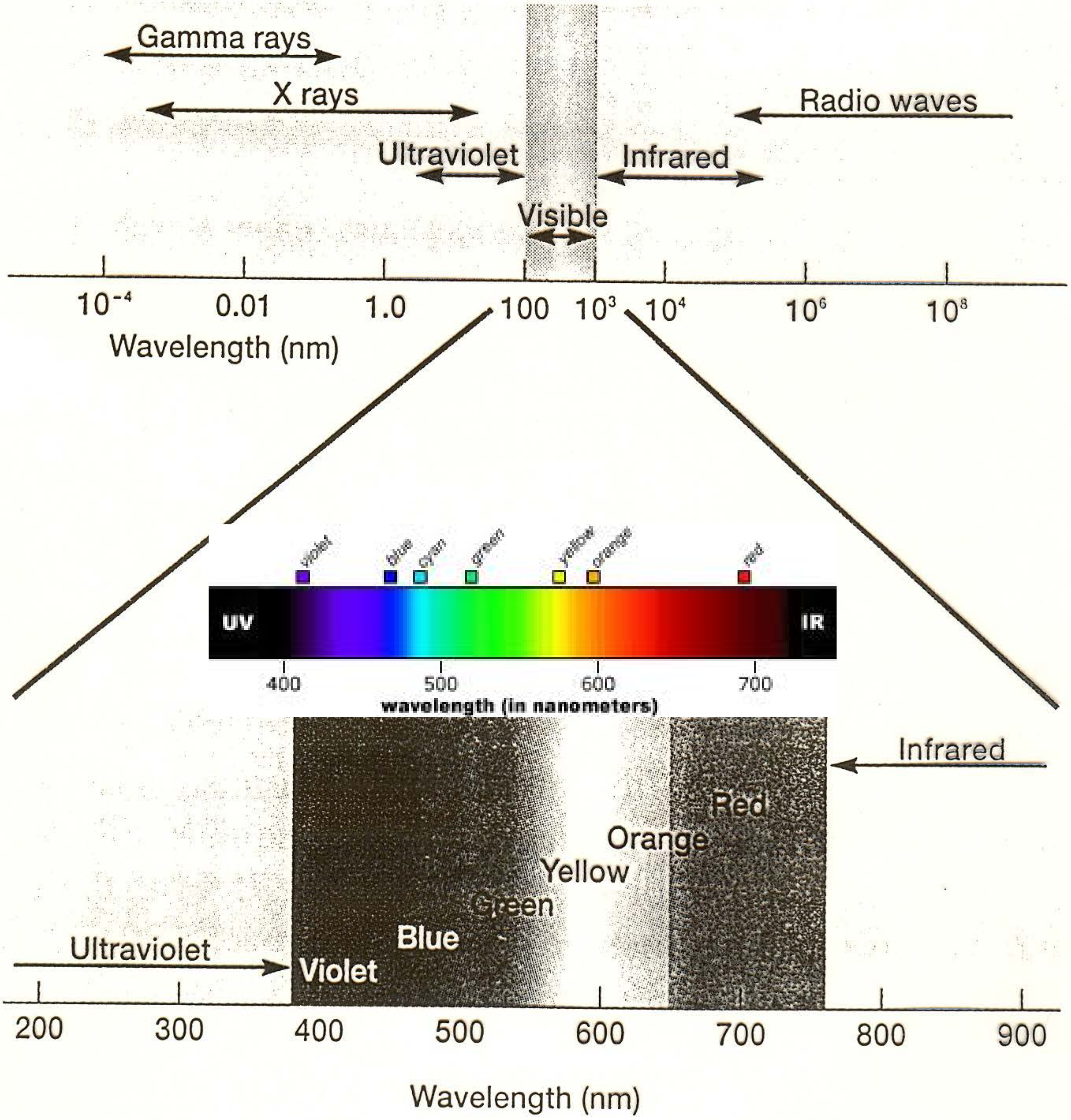
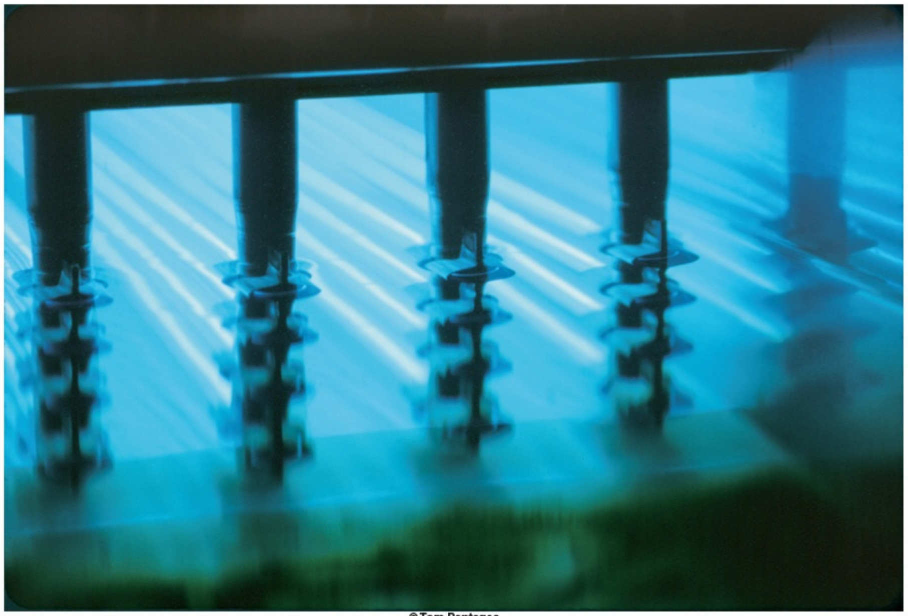


Figure 6.17 The Electromagnetic Spectrum. The visible portion of the spectrum is expanded at the bottom of the figure.

Radiation

Ultraviolet (UV) Radiation

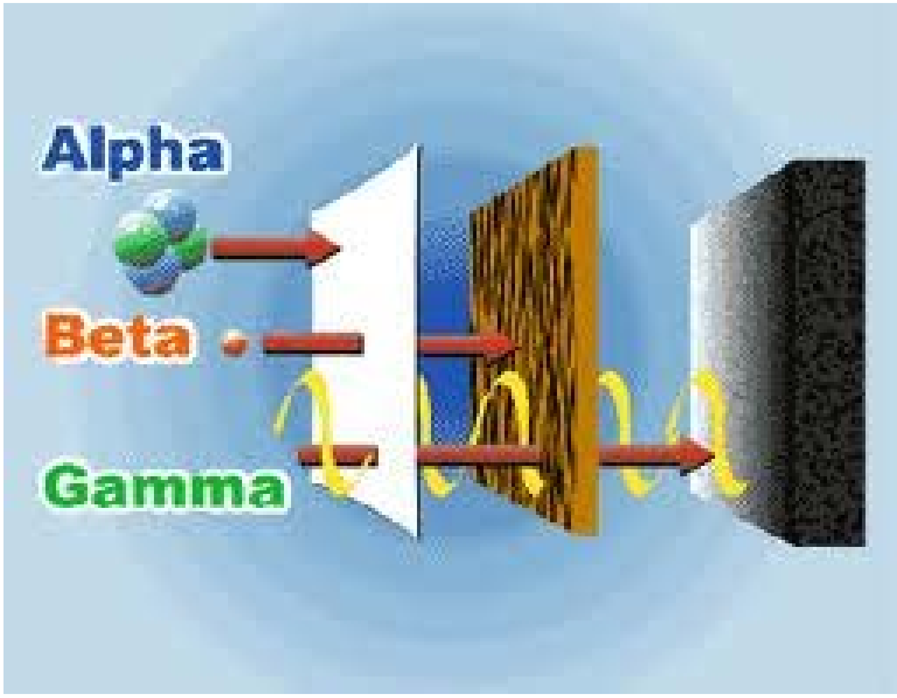
- limited to surface sterilization because it does not penetrate glass, dirt films, water, and other substances
- has been used for water treatment

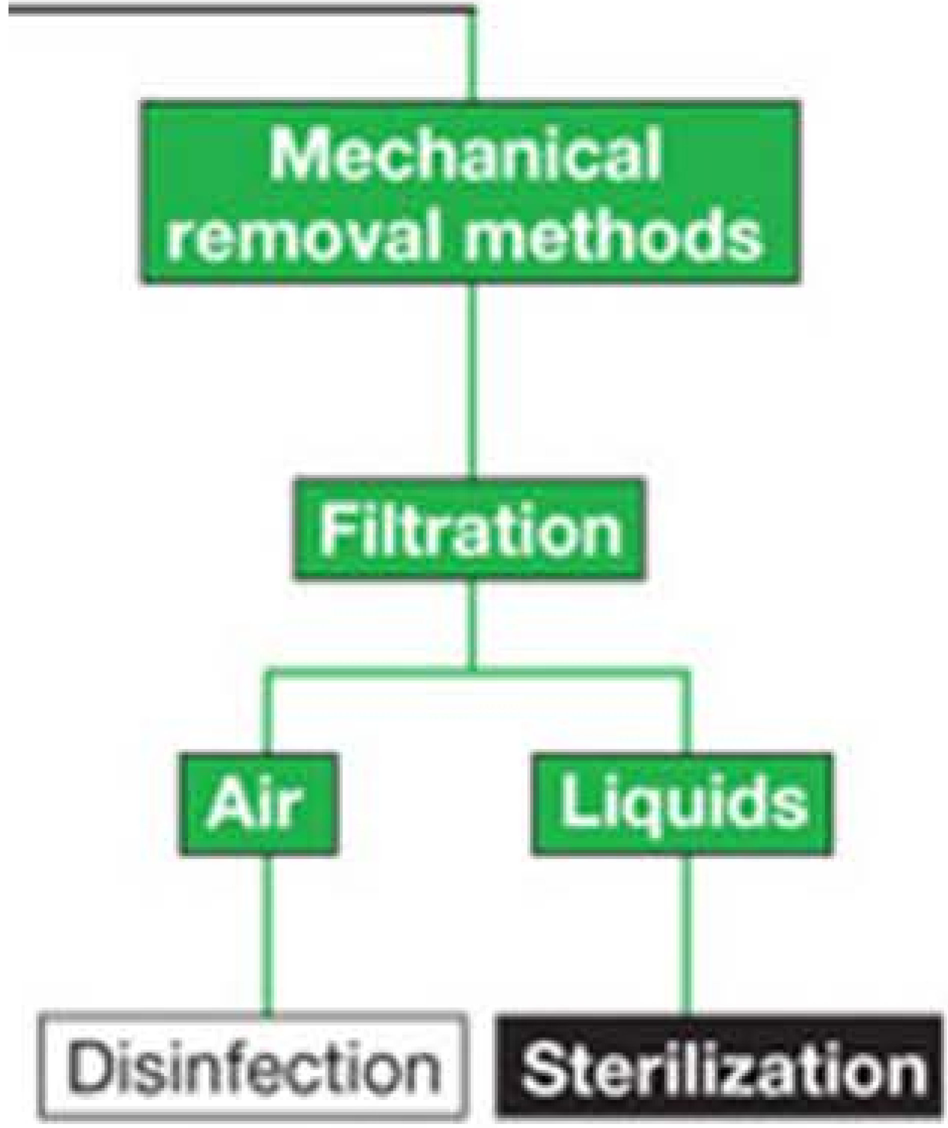


©Tom Pantages

Ionizing Radiation

- penetrates deep into objects
- destroys bacterial endospores; not always effective against viruse
- used for sterilization and pasteurization of antibiotics, hormones, sutures, plastic disposable supplies, and food




Mechanical removal methods
Filtration
Air
Liquids
Disinfection
Sterilization

Filtration

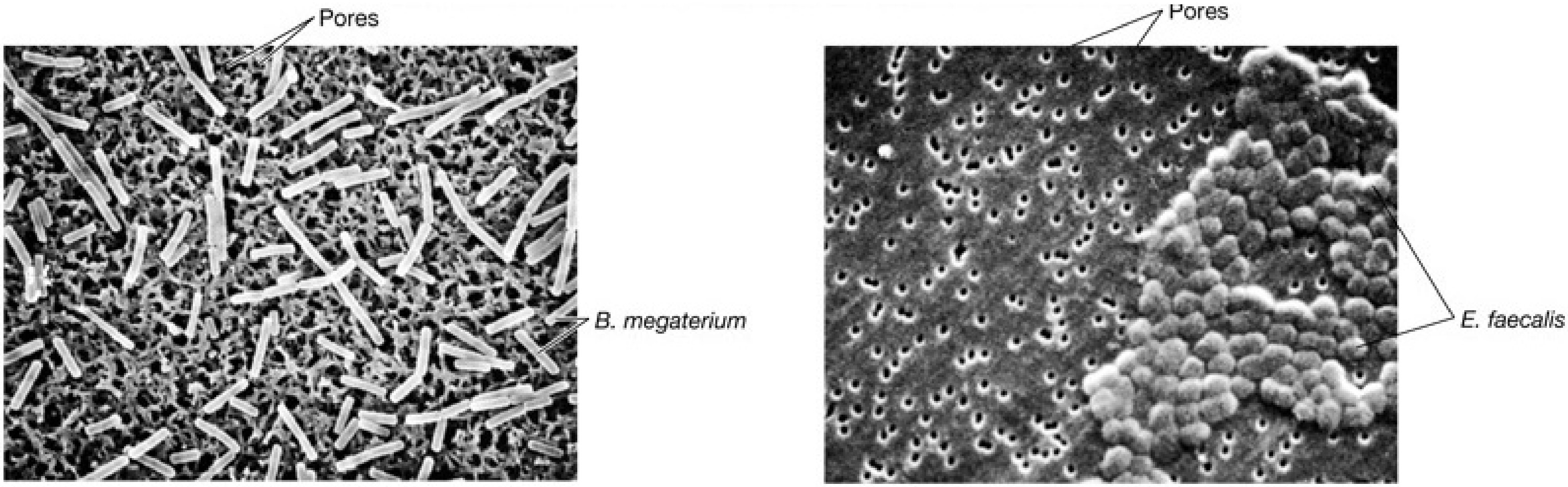
- reduces microbial population or sterilizes solutions of heat-sensitive materials by removing microorganisms
- also used to reduce microbial populations in air

depth filters

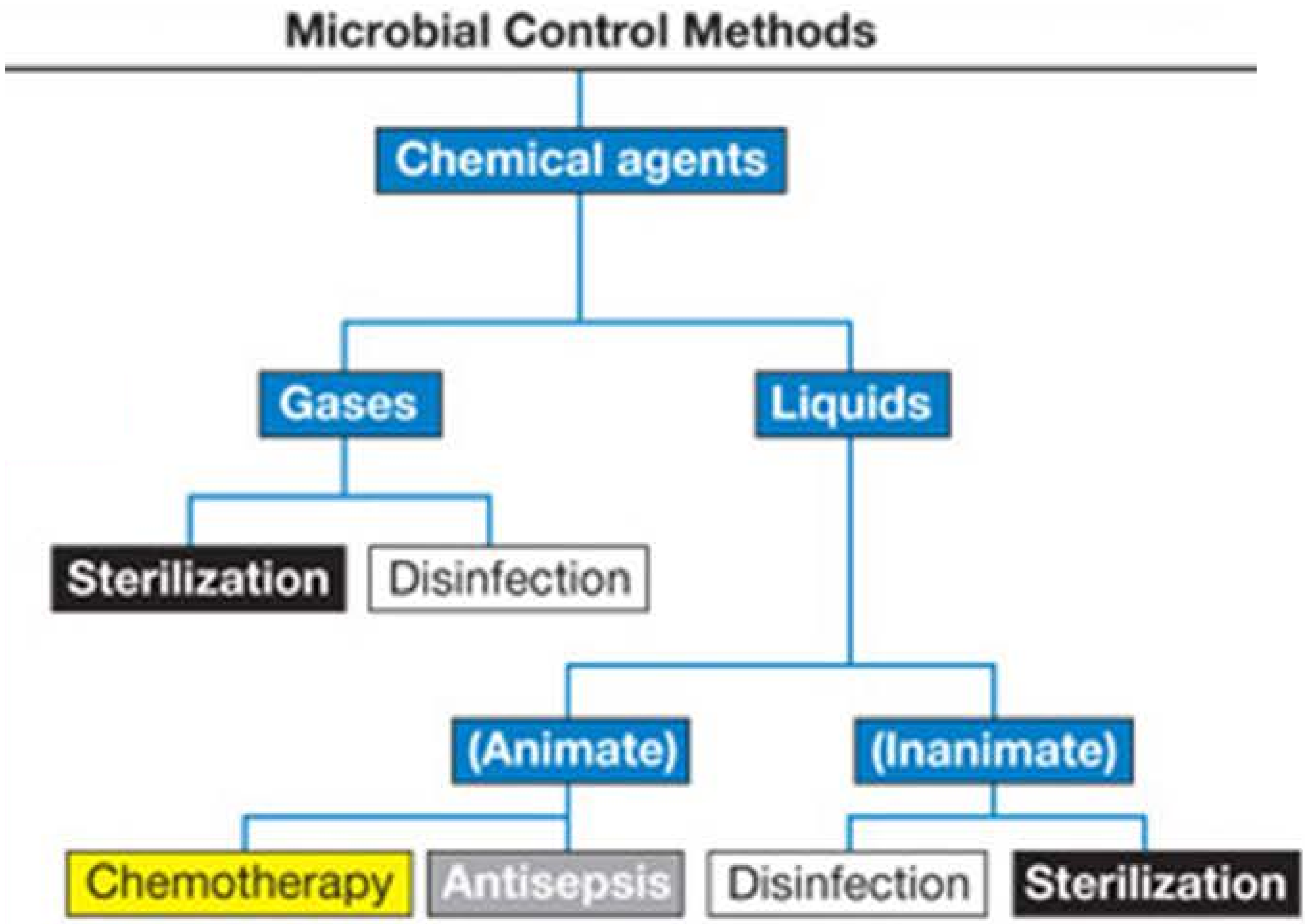
- – thick fibrous or granular filters that remove microorganisms by physical screening, entrapment, and/or adsorption

membrane filters

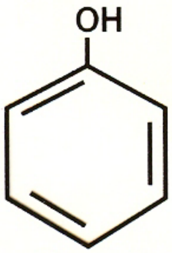
- – porous membranes with defined pore sizes that remove microorganisms primarily by physical screening



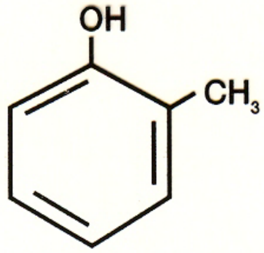
(a) *B. megaterium* (b) *E. faecalis*


Microbial Control Methods
Chemical agents
Gases
Liquids
Sterilization
Disinfection
(Animate)
(Inanimate)
Chemotherapy
Antisepsis
Disinfection
Sterilization

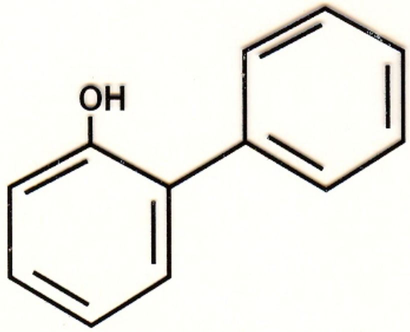
Phenolics



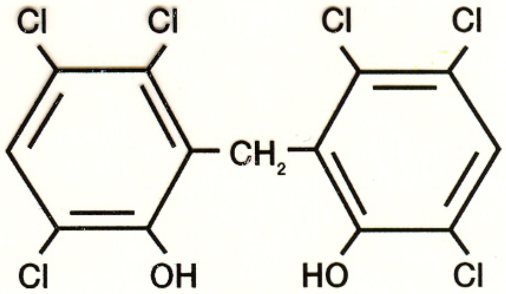
Phenol



Orthocresol



Orthophenylphenol

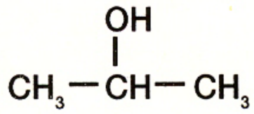


Hexachlorophene

Alcohols

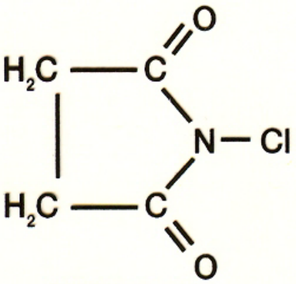
CH_3-CH_2-OH

Ethanol



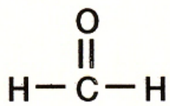
Isopropanol

Halogenated compound

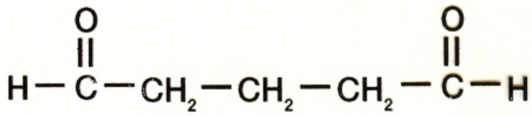


Halazone

Aldehydes

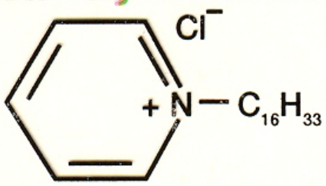


Formaldehyde

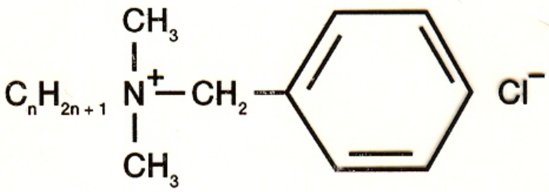


Glutaraldehyde

Quaternary ammonium compounds

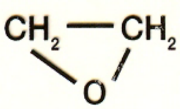


Cetylpyridinium chloride

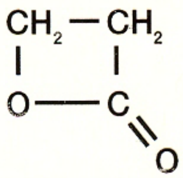


Benzalkonium chloride

Gases



Ethylene oxide



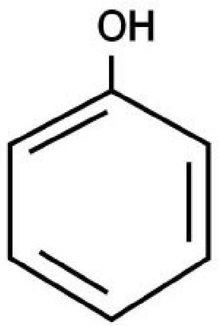
Betapropiolactone

Minimum Inhibitory Concentration (MIC)

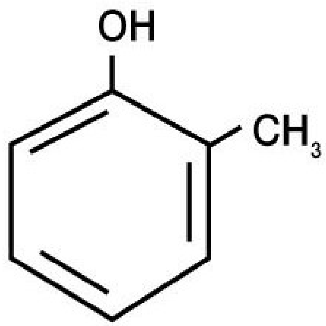
Minimal Lethal Concentration (MLC)

Phenol

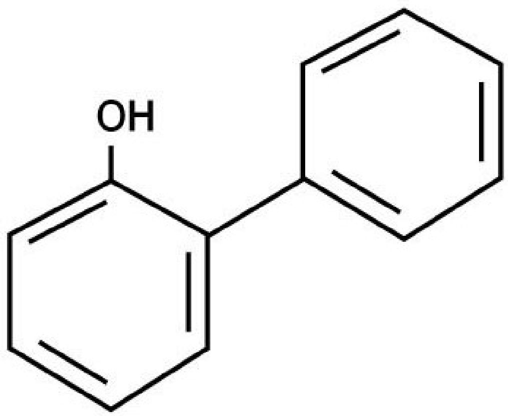
- Causes lysis of cells, destroys the cell membrane and denatures proteins (bactericide: 1-2% solution)
- The effect increases with increasing temperature and decreasing pH
- Modification of phenol with chlorine and methyl groups has resulted in disinfectants with reduced skin irritation and corrosive properties and increased antimicrobial activity.
- The effect is impeded by organic material.
- Hexachlorophene: commercial disinfectant, antiseptic, the antimicrobial effect persists for an extended time, e.g. when used to wash the skin



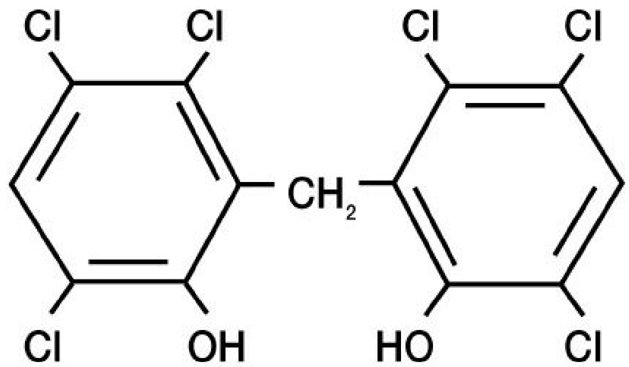
Phenol



Orthocresol



Orthophenylphenol



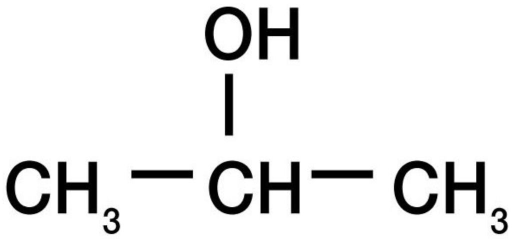
Hexachlorophene

Alcohol

- Bactericidal and fungicidal but NOT sporicidal
- Denatures proteins and destroys membranes (lipids)
- Ethanol: 70% solution most effective
- Phenyl alcohol, eye drops, contact lens solutions
- Benzyl alcohol, injection solutions

- Organic acids: (European code no.)
  Sorbic acid (E200)
  Benzoic acid (E210)
  used in foods

Ethanol

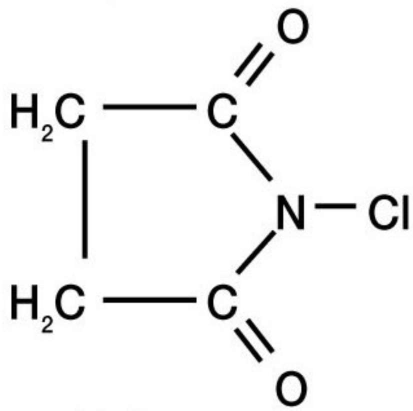


Isopropanol

Halogen compounds

- Strongly oxidizing
- Denatures proteins
- Bactericide, fungicide
- Cl, chlorine gas
- 1 µg/ml is used to disinfect water

- Mechanism:
- $Cl_2 + H_2O \rightarrow HCl + HOCl$

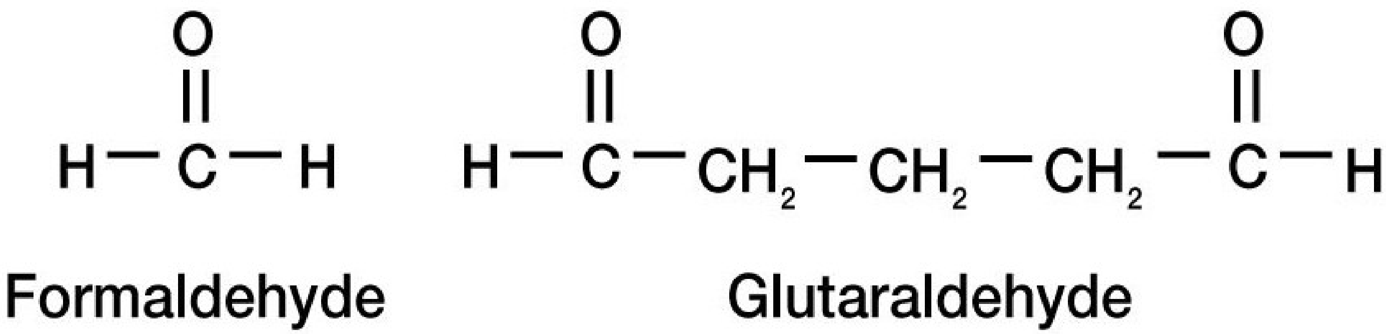


Halazone

- chloramines R - SO_2NCl_2 (chlorophors) give off Cl slowly, e.g. halazone tablets for water purification
- Organic material reduces the effect
- any of five elements: fluorine, chlorine, bromine, iodine, and astatine

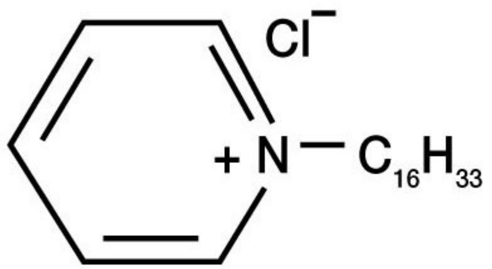
Formaldehyde

- highly reactive molecules, sporicidal
- Mechanism: causes binding of amino groups between proteins, cross-binds DNA/RNA, alkylating
- Very effective, 3-10 mg/l is bactericidal
- Poor penetration, usually used for surface disinfection
- Formalin, 37% formaldehyde in water + 10% methanol (to prevent polymerisation)

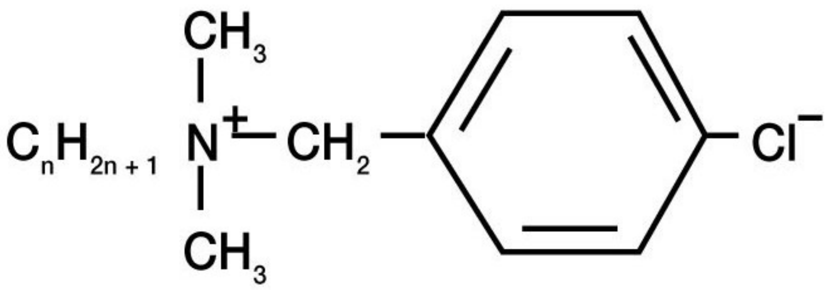


Quaternary ammonium compounds

- detergents that have antimicrobial activity and are effective disinfectants
  - organic molecules with hydrophilic and hydrophobic ends
  - act as wetting agents and emulsifiers
- cationic detergents are effective disinfectants
- Effective against G^+ bacteria
- Alkali solution improves the effect
- Soap (anionic detergents) reduces the effect



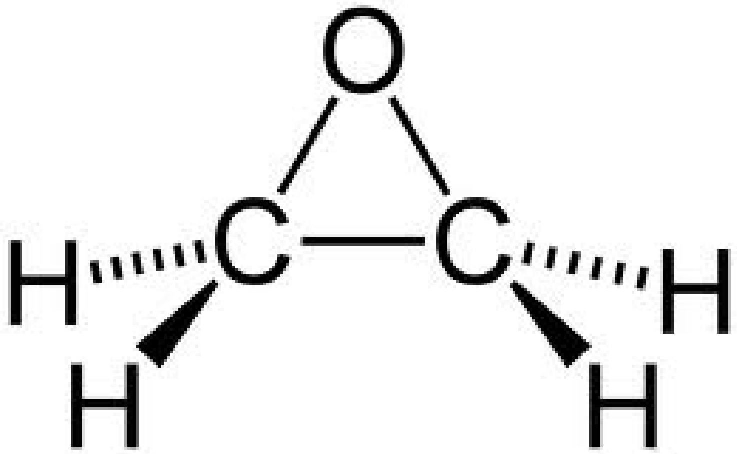
Cetylpyridinium chloride



Benzalkonium chloride

Ethylene oxide

- Used to sterilize heat-sensitive materials, microbicidal and sporicidal
- Boiling point 10.7°C, Very good penetration
- Used with CO_2, freon etc. to avoid explosions, > 3% ethylene oxide in air is explosive!
- Mechanism: ethylene oxide alkylates hydroxyl, carbonyl and sulfhydryl amino groups of enzymes etc.

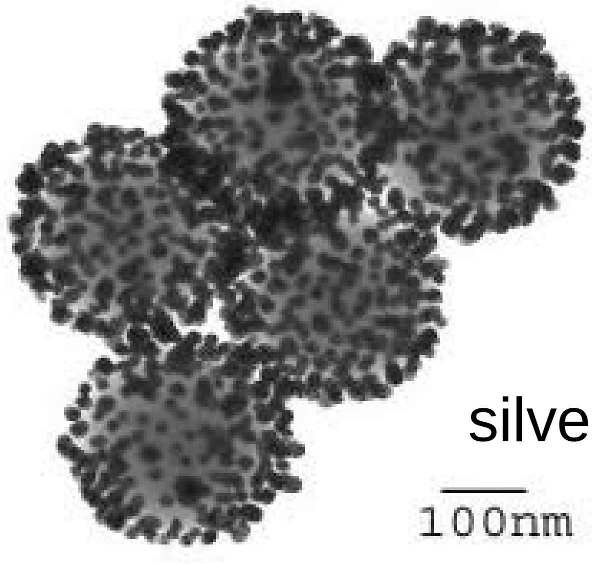


Heavy metals

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

- Silver nitrate ($AgNO_3$) (1% solution) is sometimes used to disinfect the eyes of newborn babies.

- Copper sulphate ($CuSO_4$) fungicide and algicide, agricultural uses



silver nanoparticles