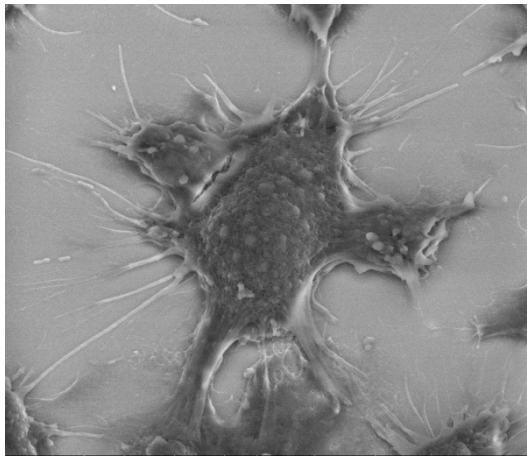
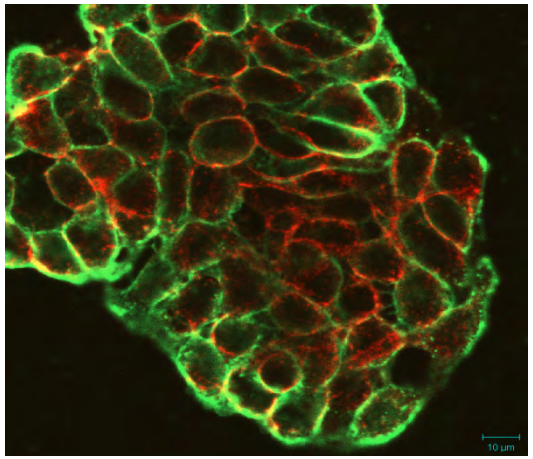
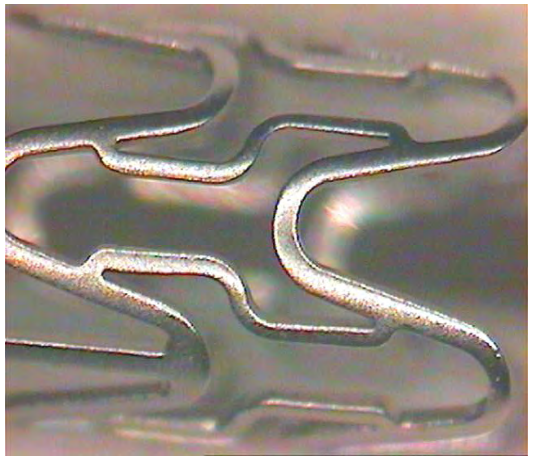
Biological Sciences

B. CHEM. ENGG.







Microbial Growth, Virus, Phage and Control of Microbial Growth

Dr. Ratnesh Jain

Batch
Closed systems microorganisms undergo a predictable pattern of growth characterised by 4 phases

Continuous

Continuous culture methods enable constant cell numbers to be maintained in a constant chemical environment at specified growth rates for prolonged periods of time

Fed-batch

Fed-batch culture is, in the broadest sense, defined as an operational technique in biotechnological processes where one or more nutrients (substrates) are fed (supplied) to the bioreactor during cultivation and in which the product(s) remain in the bioreactor until the end of the run. It is also known as semi-batch culture. In some cases, all the nutrients are fed into the bioreactor.

Synchronous

A synchronous or synchronized culture is a microbiological culture or a cell culture that contains cells that are all in the same growth stage.

Objectives:

- Classify microbes into five groups on the basis of preferred temperature range.
- Explain the importance of osmotic pressure to microbial growth.
- Provide a use for each of the four elements (C, N, S, P) needed in large amounts for microbial growth.
- Explain how microbes are classified on the basis of O_2 needs.
- Identify ways in which aerobes avoid damage by toxic forms of O_2.
- Describe the formation of biofilms and their potential for causing infection.
- Distinguish between chemically defined and complex media.
- Justify the use of each of the following: anaerobic techniques, living host cells, candle jars, selective, differential, and enrichment media.
- Define colony and CFUs and describe how pure cultures can be isolated with streak plates.
- Explain how microbes are preserved by deep-freezing and lyophilization.
- Distinguish between binary fission and budding.
- Define generation time and explain the bacterial growth curve.
- Review some direct and indirect methods of measuring bacterial cell growth.

Microbial Growth

Microbial growth: Increase in cell number, not cell size!

Physical Requirements for Growth: **Temperature**

- Minimum growth temperature
- Optimum growth temperature
- Maximum growth temperature

Five groups based on **optimum growth temperature**

1. **Psychrophiles**
2. **Psychrotrophs**
3. **Mesophiles**
4. **Thermophiles**
5. **Hyperthermophiles**

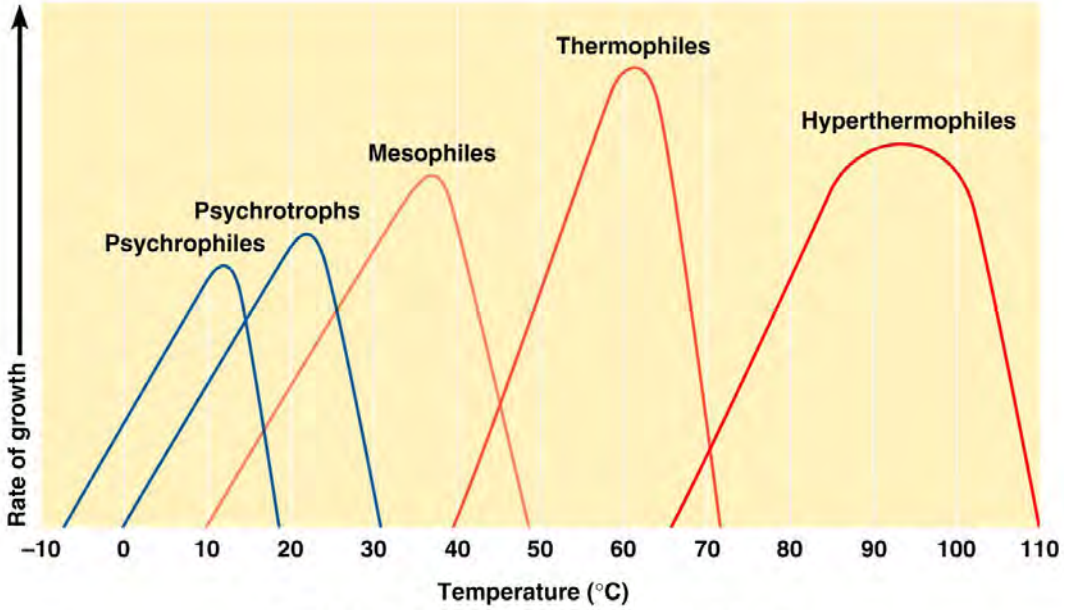
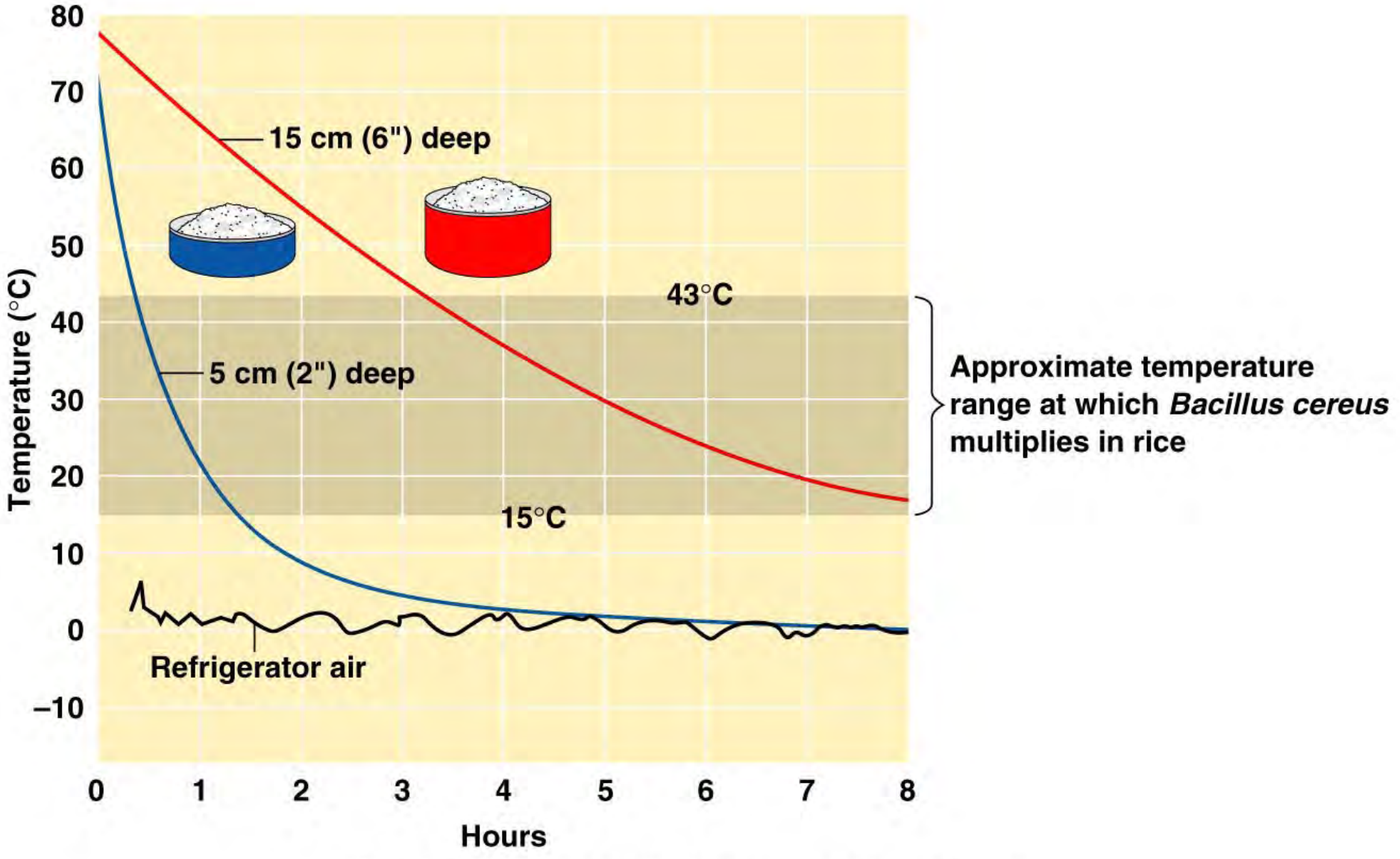


Fig : Effect of amount of food on its cooling rate



Physical Requirements for Growth:

pH and Osmotic Pressure

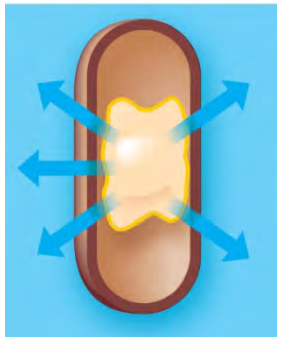
Most bacteria grow best between pH 6.5 and 7.5:
Neutrophils

Some bacteria are very tolerant of acidity or thrive in it: **Acidophiles** (preferred pH range 1 to 5)

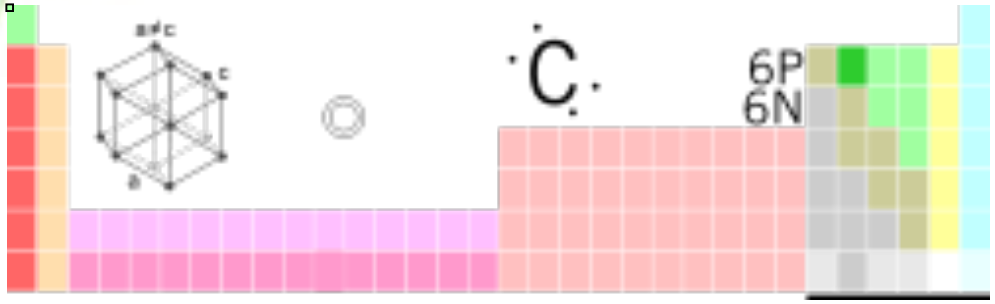
Molds and yeasts grow best between pH 5 and 6

Hypertonic environments (increased salt or sugar) cause **plasmolysis**

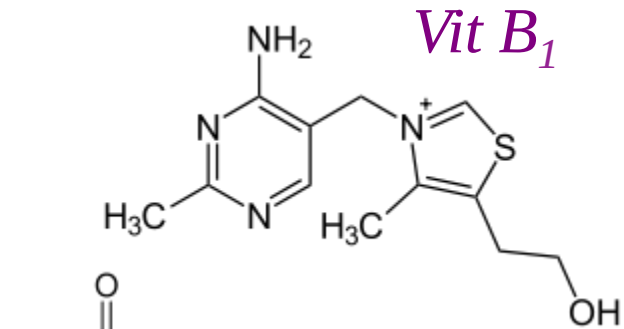
Obligate **halophiles** vs. facultative halophiles

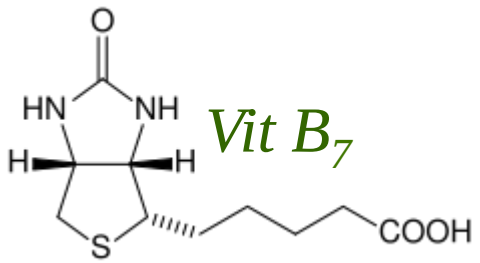


Chemical Requirements for Growth: **Carbon, N, S, P**, etc.



- Carbon
  - ~ Half of dry weight
  - Chemoheterotrophs use organic carbon sources
- Nitrogen, Sulfur, Phosphorus
  - Needed for ?
  - Found in amino acids and proteins (most bacteria decompose proteins)
  - S in thiamine and biotin
  - Phosphate ions (PO_4^{3-})

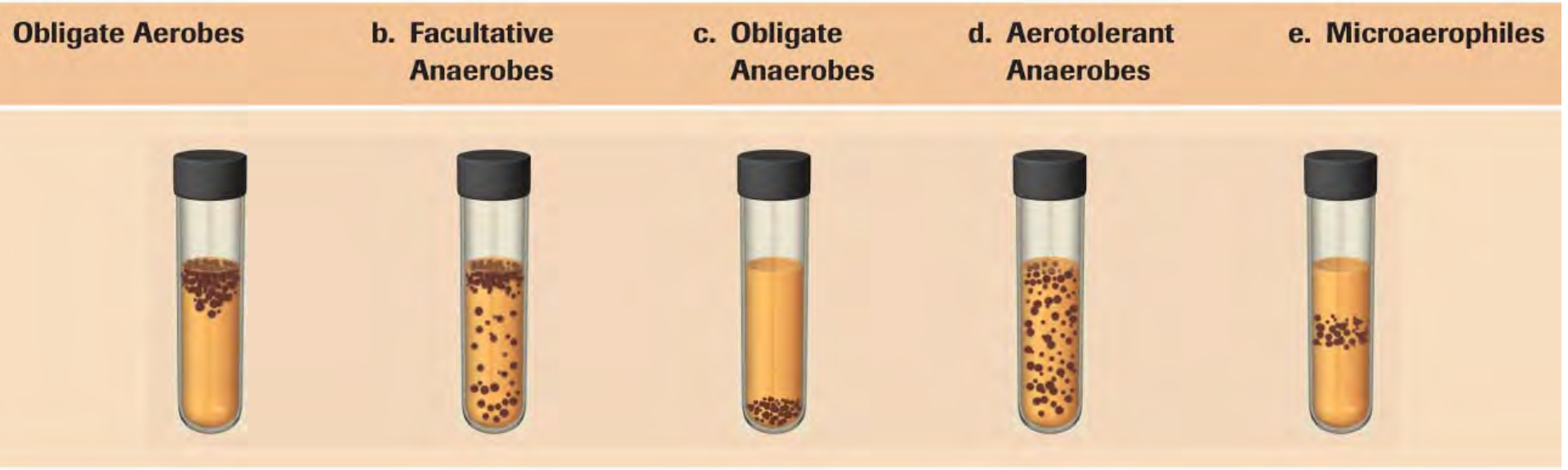




- Also needed K, Mg, Ca, trace elements (as cofactors), and organic growth factors

Chemical Requirements for Growth: **Oxygen**

O_2 requirements vary greatly



Toxic Forms of Oxygen

- Singlet oxygen: O_2 boosted to a higher-energy state
- Superoxide free radicals: O_2^-

$$O_2^- + 2\,H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

- Peroxide anion: O_2^{2-}

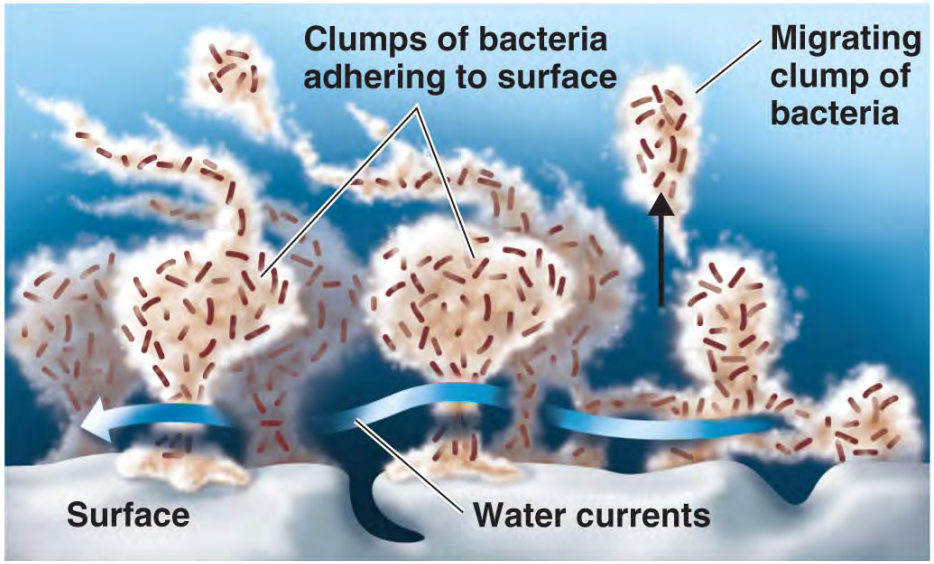
$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

$$H_2O_2 + 2\,O^+ \xrightarrow{\text{peroxidase}} 2\,H_2O$$

- Hydroxyl radical (OH•)

Biofilms

Fig 6.5



Microbial communities form slime or hydrogels

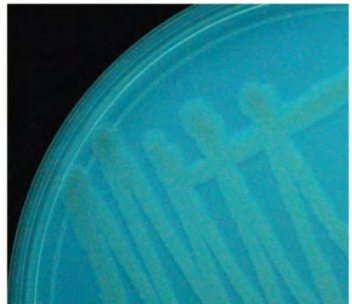
Starts via attachment of planctonic bacterium to surface structure.

Bacteria communicate by chemicals via **quorum sensing**

Sheltered from harmful factors (disinfectants etc.)

Cause of most nosocomial infections

Clinical Focus: Delayed Bloodstream Infection Following Catheterization



Culture Media

- Culture medium: Nutrients prepared for microbial growth
- Have to be sterile (not contain living microbes)
- **Inoculum**: Microbes introduced into medium
- Culture: Microbes growing in/on culture medium
- Chemically defined media: Exact chemical composition is known (for research purposes only)
- **Complex media**: Extracts and digests of yeasts, meat, or plants, *e.g.:*
  - Nutrient broth
  - Nutrient agar
  - Blood agar

Agar

- Complex polysaccharide
- Used as solidifying agent for culture media in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C

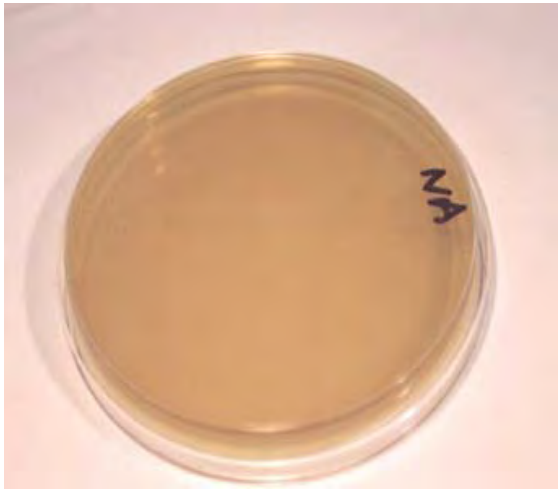
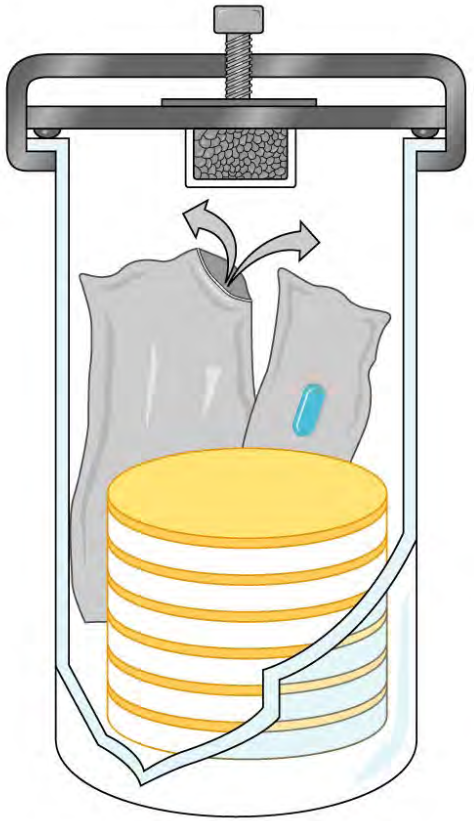


TABLE 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

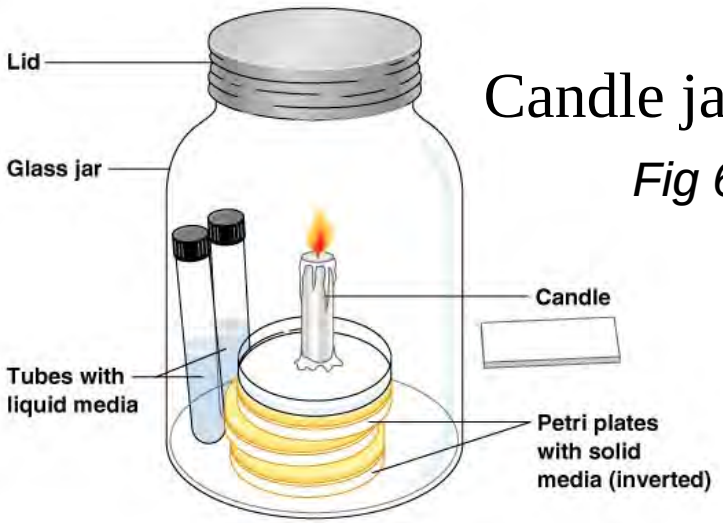
Anaerobic Culture Methods

- Use reducing media, containing chemicals (*e.g.:* thioglycollate) that combine with O_2
- Are heated shortly before use to drive off O_2
- Use anaerobic jar
- Novel method in clinical labs:
  Add **oxyrase** to growth media
  ⇒ OxyPlate (no need for anaerobic jar



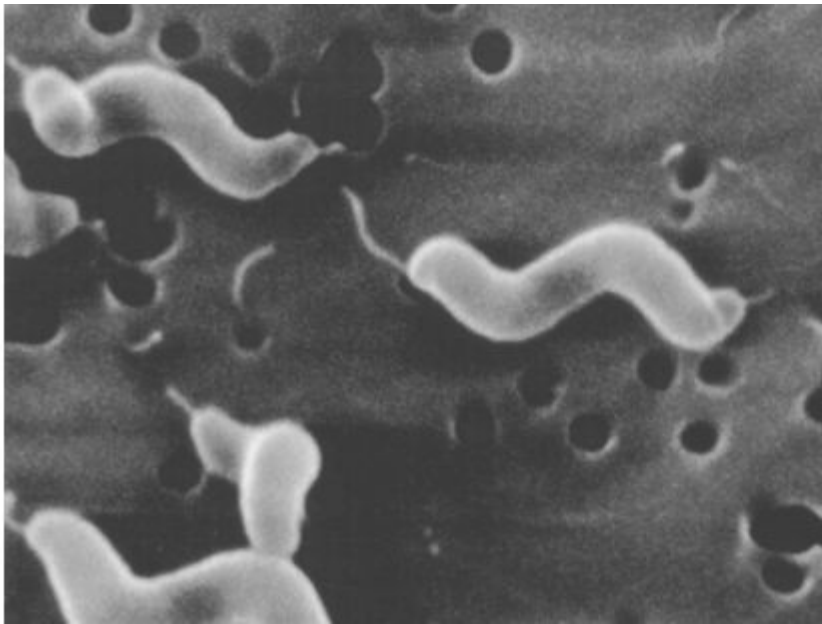
Capnophiles: Aerobic Bacteria Requiring High CO_2

- Low oxygen, high CO_2 conditions resemble those found in
  - intestinal tract
  - respiratory tract and
  - other body tissues where pathogens grow
- *E.g: Campylobacter jejuni*
- Use candle jar, CO_2-generator packets, or CO_2 incubators



Candle jar

Fig 6.7



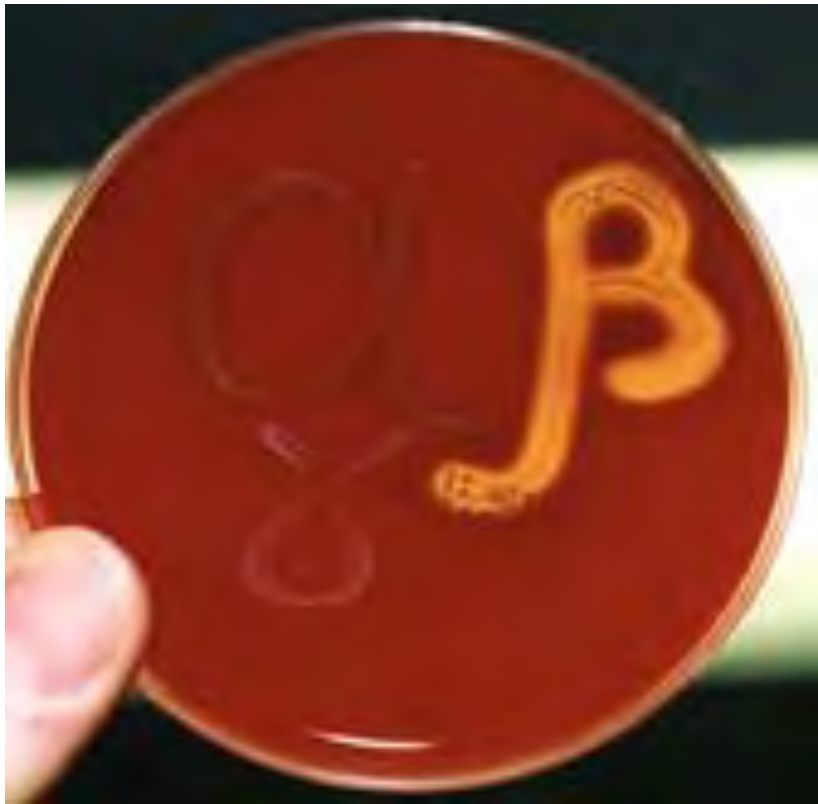
Selective Media and Differential Media

Selective medium:

Additives suppress unwanted and encourage desired microbes – *e.g.* EMB, mannitol salt agar etc.

Differential medium:

changed in recognizable manner by some bacteria ⇒ Make it easy to distinguish colonies of different microbes – *e.g.* α and β hemolysis on **blood agar**; MacConkey agar, EMB, mannitol salt agar etc.



Enrichment Media/Culture

- Encourages growth of desired microbe
- Example: *Assume soil sample contains a few phenol-degrading bacteria and thousands of other bacteria*
  - Inoculate phenol-containing culture medium with the soil and incubate
  - Transfer 1 ml to another flask of the phenol medium and incubate
  - Transfer 1 ml to another flask of the phenol medium and incubate
  - Only phenol-metabolizing bacteria will be growing

Pure Cultures

Contain only one species or strain.

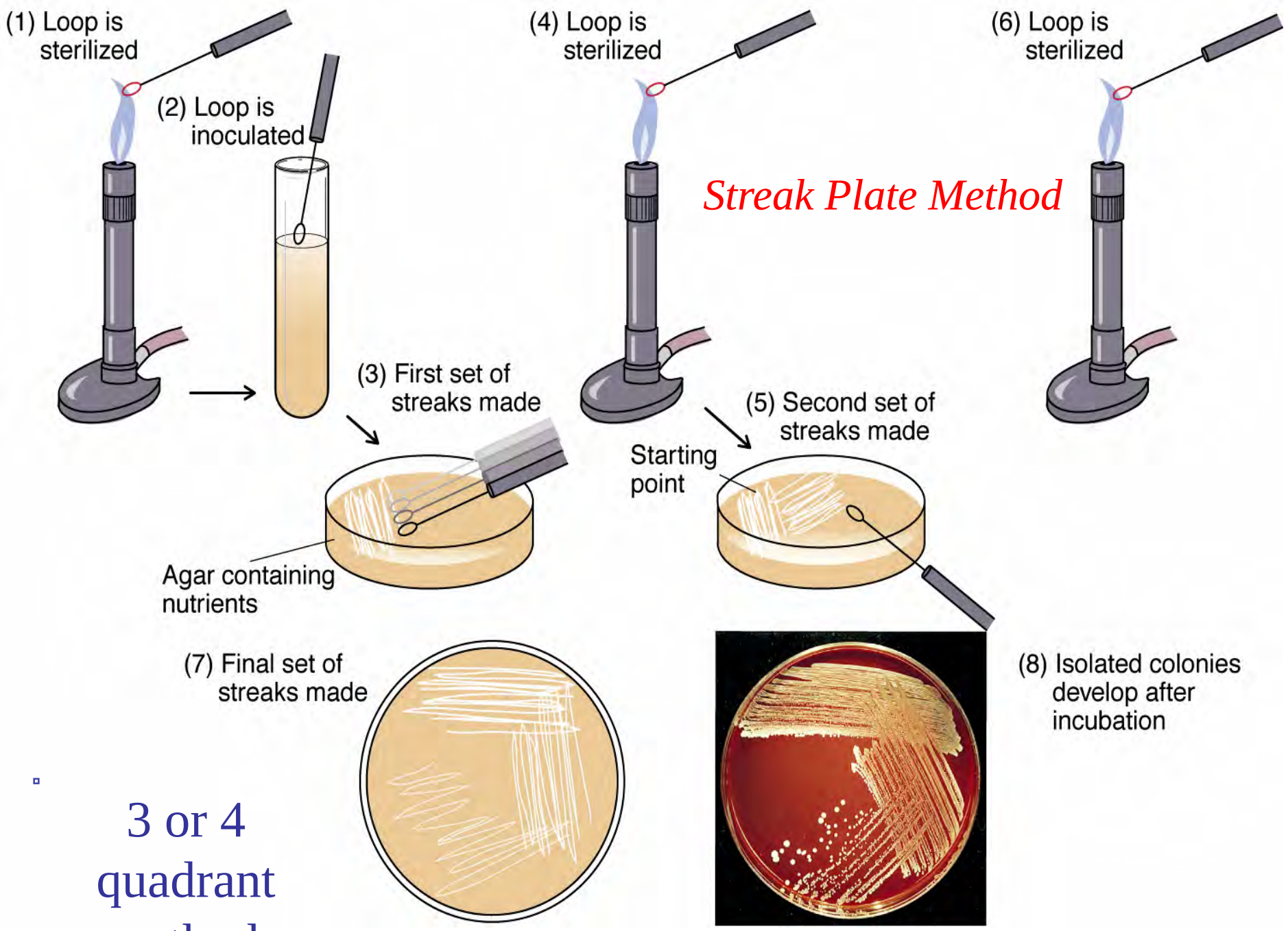
Most patient specimens and environmental samples contain several different kinds of bacteria

Streak-plate method is commonly used

Colony formation: A population of cells arising from a single cell or spore or from a group of attached cells (also referred to as **CFU**).

Only ~1% of all bacteria can be successfully cultured

Aseptic technique critical!



Streak Plate Method

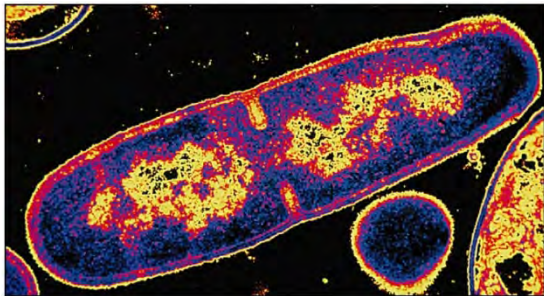
3 or 4 quadrant methods

Preserving Bacterial Cultures

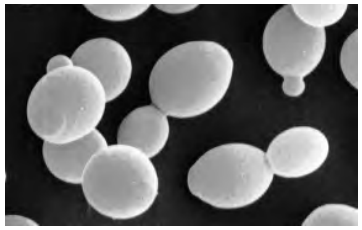
- **Deep-freezing**: Rapid cooling of pure culture in suspension liquid to –50°to –95°C. Good for several years.
- **Lyophilization** (freeze-drying): Frozen (–54° to –72°C) and dehydrated in a vacuum. Good for many years.

The Growth of Bacterial Cultures

Binary fission – exponential growth



Budding



Generation time – time required for cell to divide (also known as doubling time)

Ranges from 20 min *(E. coli)* to > 24h *(M. tuberculosis)*

Consider reproductive potential of *E. coli*

Fig 6.13

Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	$2^0 = 1$	0
5	$2^5 = 32$	1.51
10	$2^{10} = 1{,}024$	3.01
15	$2^{15} = 32{,}768$	4.52
16	$2^{16} = 65{,}536$	4.82
17	$2^{17} = 131{,}072$	5.12
18	$2^{18} = 262{,}144$	5.42
19	$2^{19} = 524{,}288$	5.72
20	$2^{20} = 1{,}048{,}576$	6.02

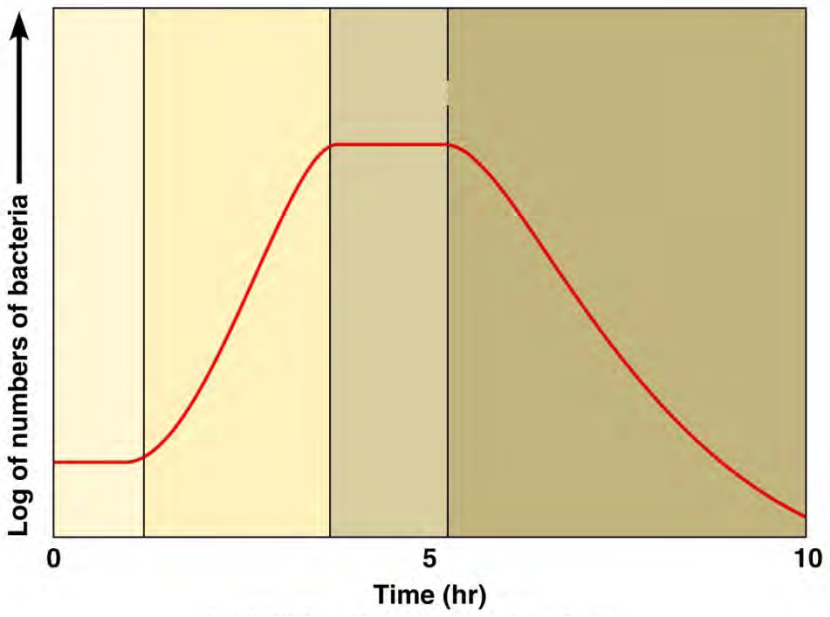
Bacterial Growth Curve

Illustrates the dynamics of growth

Foundation Fig 6.15

Phases of growth

- Lag phase
- Exponential or logarithmic (log) phase
- Stationary phase
- Death phase (decline phase)



Compare growth in liquid and on solid media

Bacterial Growth Curve: Arithmetic vs. Exponential Plotting

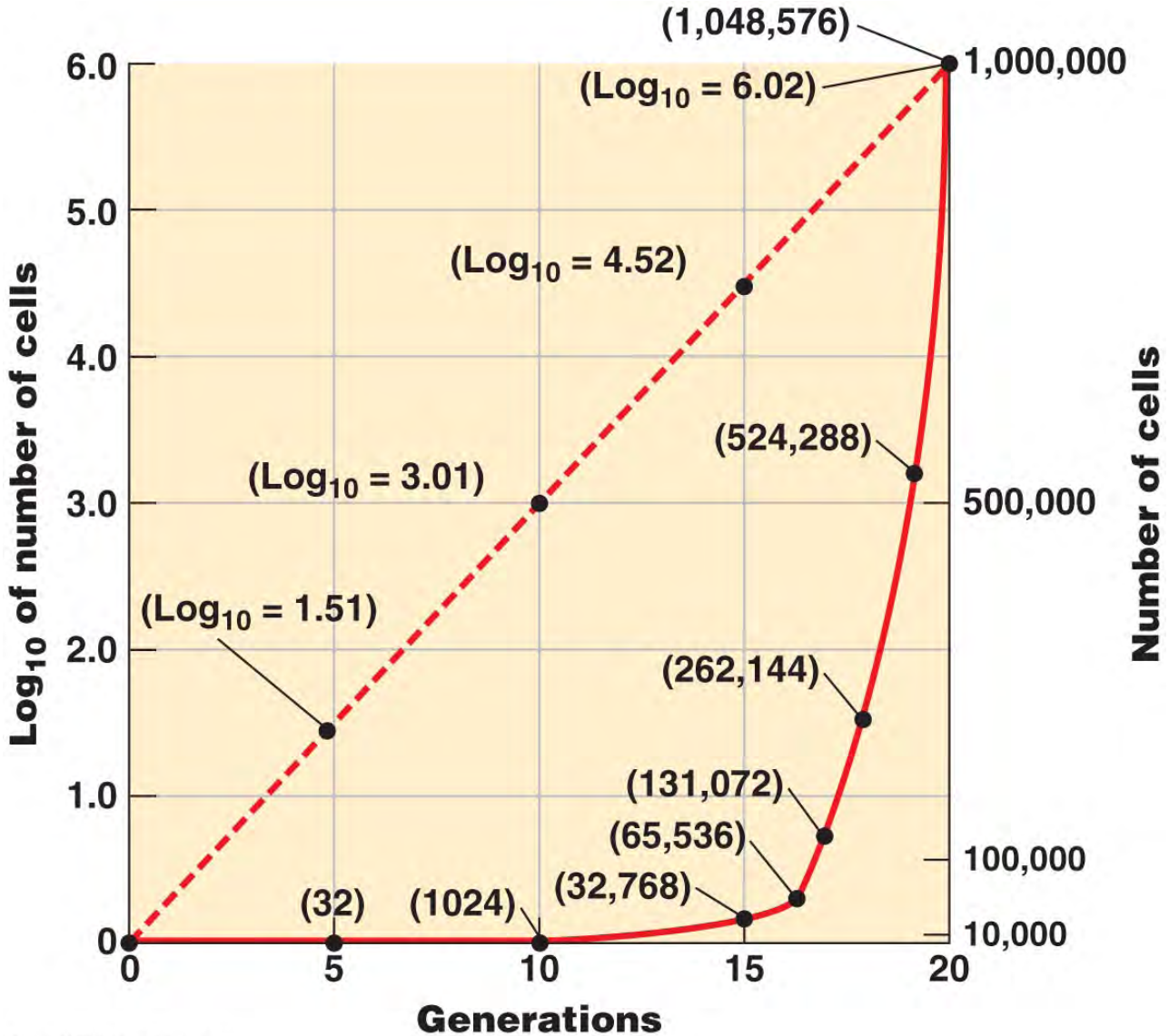


Fig 6.14

Direct Measurements of Microbial Growth

Viable cell counts: Plate counts: Serial dilutions put on plates→ **CFUs** form colonies

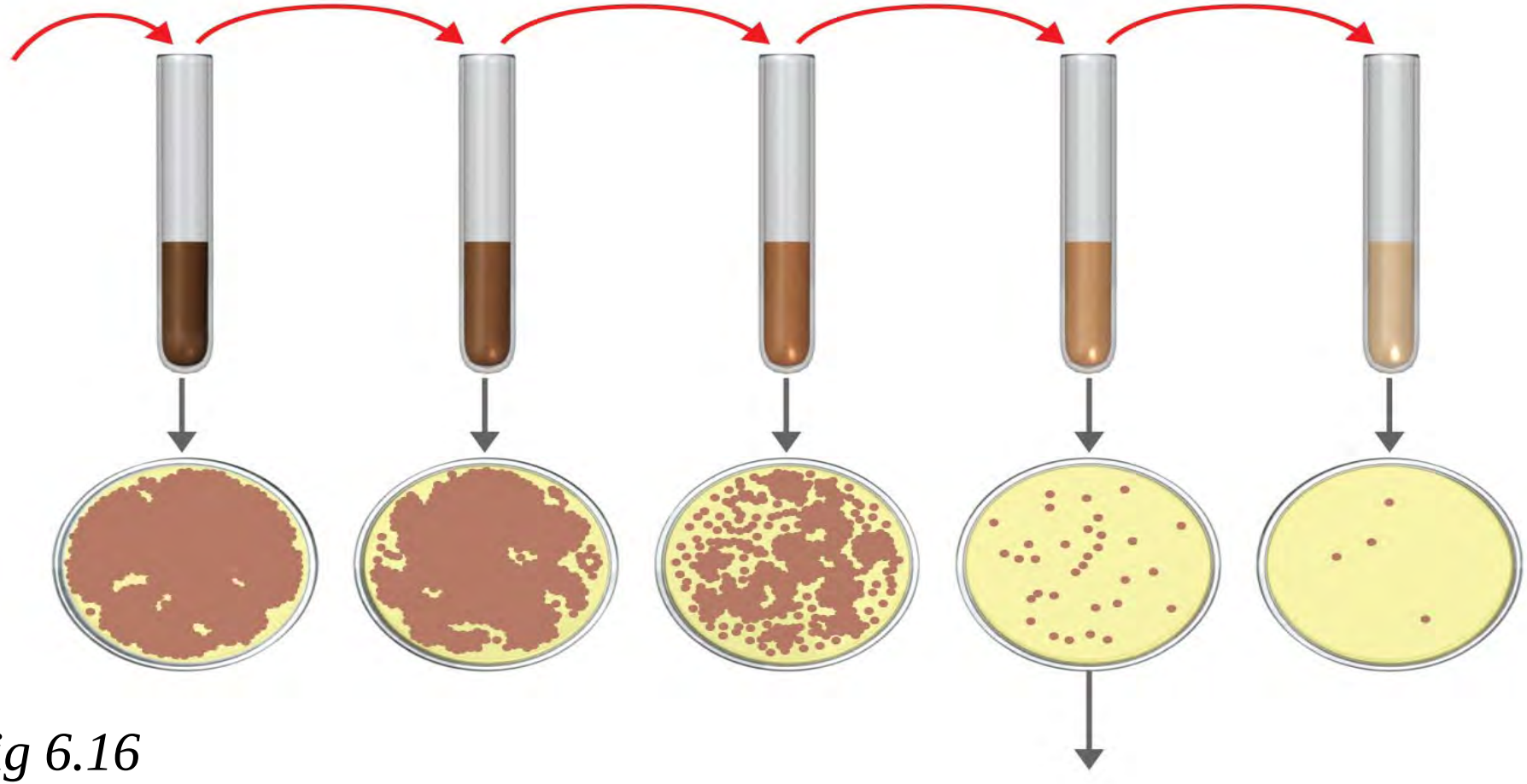


Fig 6.16

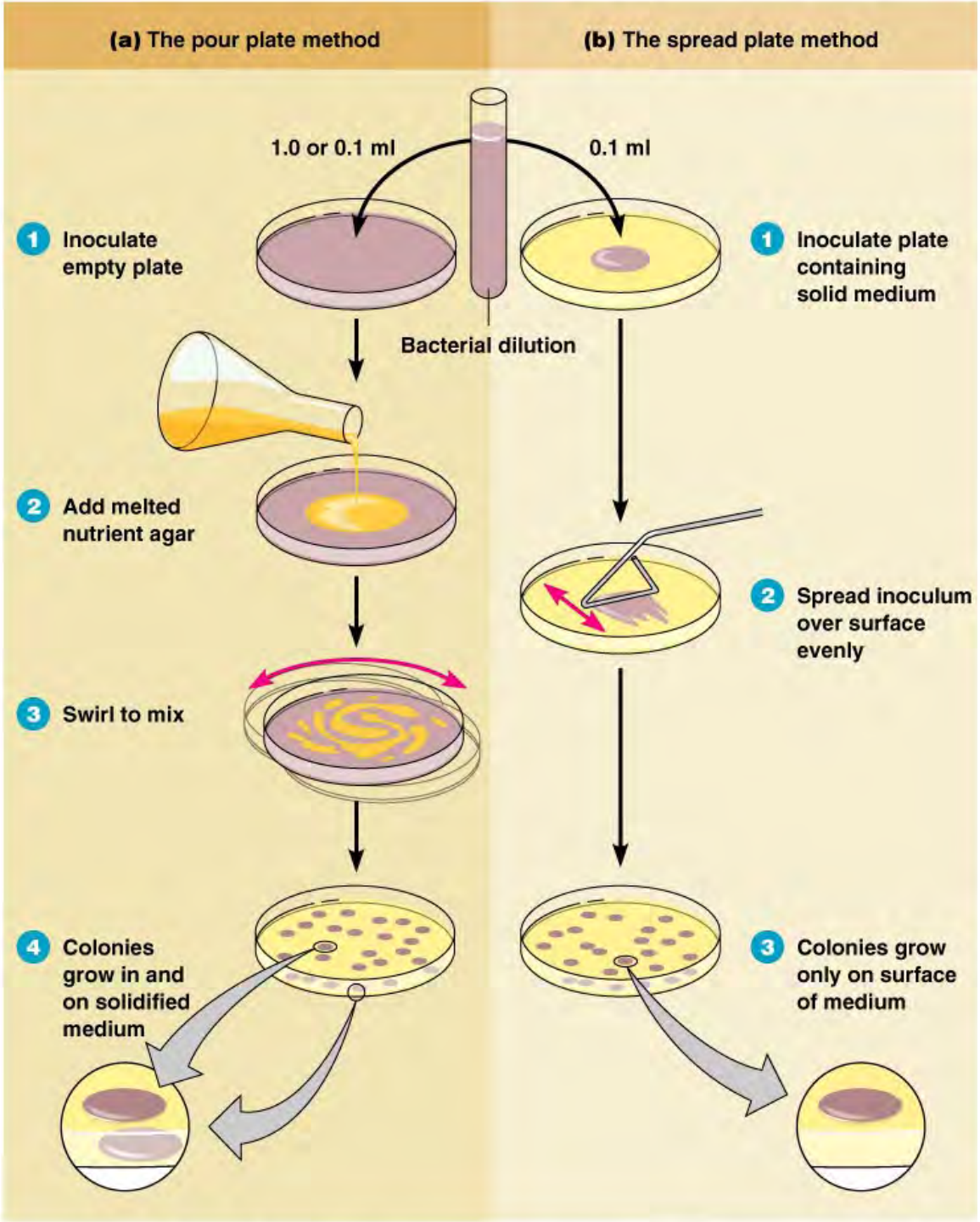
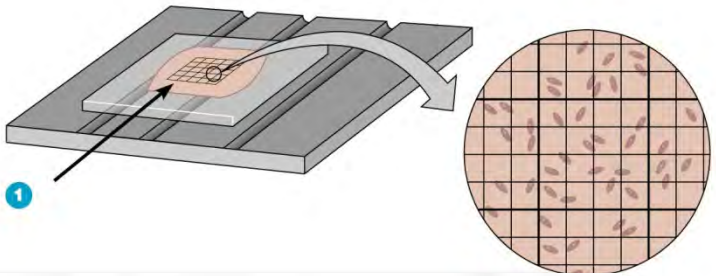

(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
Bacterial dilution
1 Inoculate plate containing solid medium
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium

Fig 6.17

Additional Direct Measurements

1. **Filtration** method of choice for low counts
2. Direct microscopic count: Counting chambers (slides) for microscope

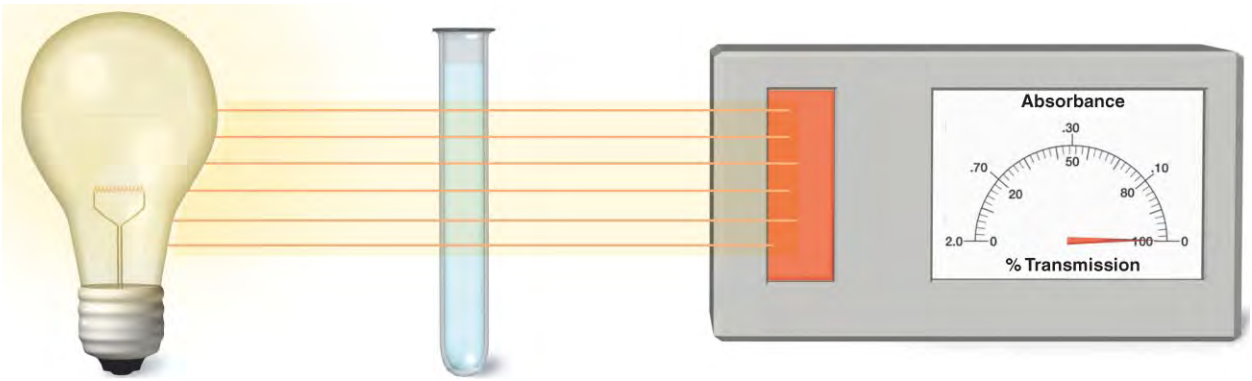
Fig 6.20



$$\text{Number of bacteria/ml} = \frac{\text{number of cells counted}}{\text{volume of area counted}}$$

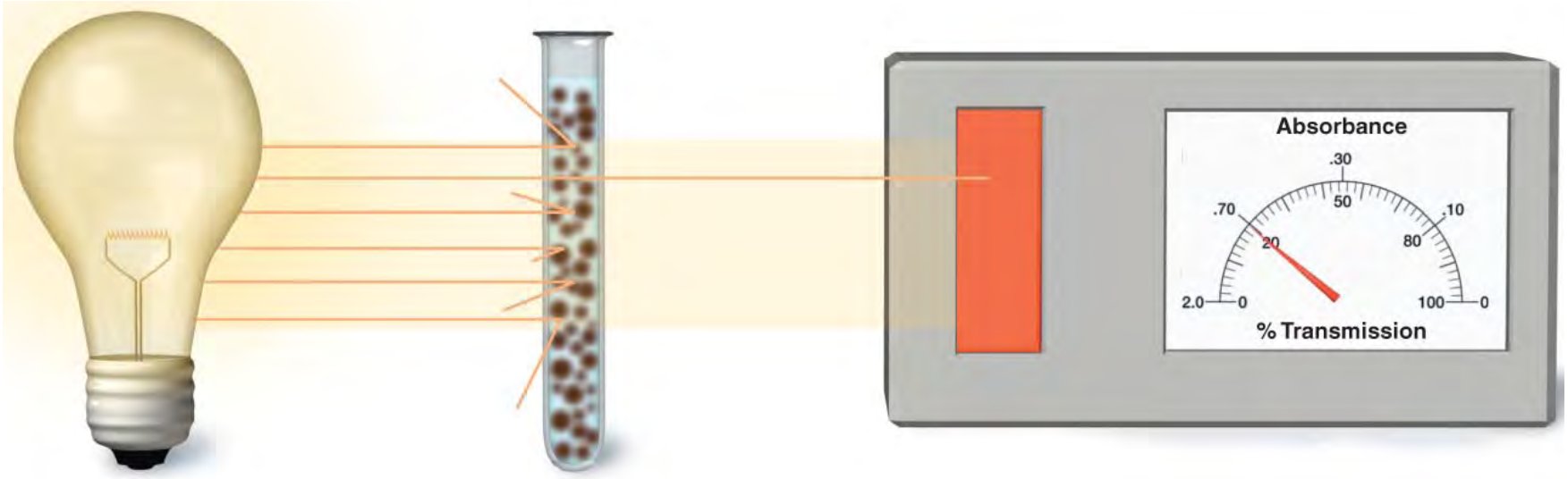
$$\frac{14}{8 \times 10^{-7}} = 17{,}500{,}000$$

Estimating Bacterial Numbers by Indirect Methods



Spectrophotometry to measure turbidity

OD is function of cell number



Measuring Microbial Growth - Overview

Direct Methods

- **Plate counts**
- **Filtration**
- *MPN*
- **Direct microscopic count**

Indirect Methods

- **Turbidity**
- *Metabolic activity*
- *Dry weight*

Control of Microbial Growth

SLOs

- Define sterilization, disinfection, antisepsis, sanitization, biocide, germicide, bacteriostasis, and asepsis.
- Describe the microbial death curve.
- Describe the effects of microbial control agents on cellular structures.
- Compare effectiveness of moist heat (autoclaving, pasteurization) vs .dry heat.
- Describe how filtration, low temperature, high pressure, desiccation, and osmotic pressure suppress microbial growth.
- Explain how radiation kills cells.
- List the factors related to effective disinfection.
- Interpret results of use-dilution tests and the disk-diffusion method.
- Identify some methods of action and preferred uses of chemical disinfectant
- Differentiate between halogens used as antiseptics and as disinfectants.
- Identify the appropriate uses for surface-active agents.
- List the advantages of glutaraldehyde over other chemical disinfectants.
- Identify the method of sterilizing plastic labware.
- Explain how microbial control is affected by the type of microbe.

Terminology

(a) Antiseptic

(b) Disinfectant

- **Sepsis**: microbial contamination.
- **Asepsis**: absence of significant contamination.
- Aseptic surgery techniques prevent microbial contamination of wounds.
- Antimicrobial chemicals, expected to destroy pathogens but not to achieve sterilization
  - **Disinfectant:** used on objects
  - **Antiseptic:** used on living tissue
- **Nosocomial**

. . . More Terminology

- **Sterilization**: Removal of all microbial life (heat, filtration)
- For food: **Commercial sterilization** to kill *C. botulinum* endospores
- **Sanitization**: reduces microbial numbers to safe levels (*e.g.:* eating utensils)
- **Bacteriostatic**: Inhibits bacterial reproduction
- **Bactericidal**: Kills bacteria
- **Fungicide, sporicide, germicide, biocide**

Rate of Microbial Death

Bacterial populations subjected to heat or antimicrobial chemicals die at a constant rate.

Microbial Death Curve, plotted logarithmically, shows this constant death rate as a straight line.

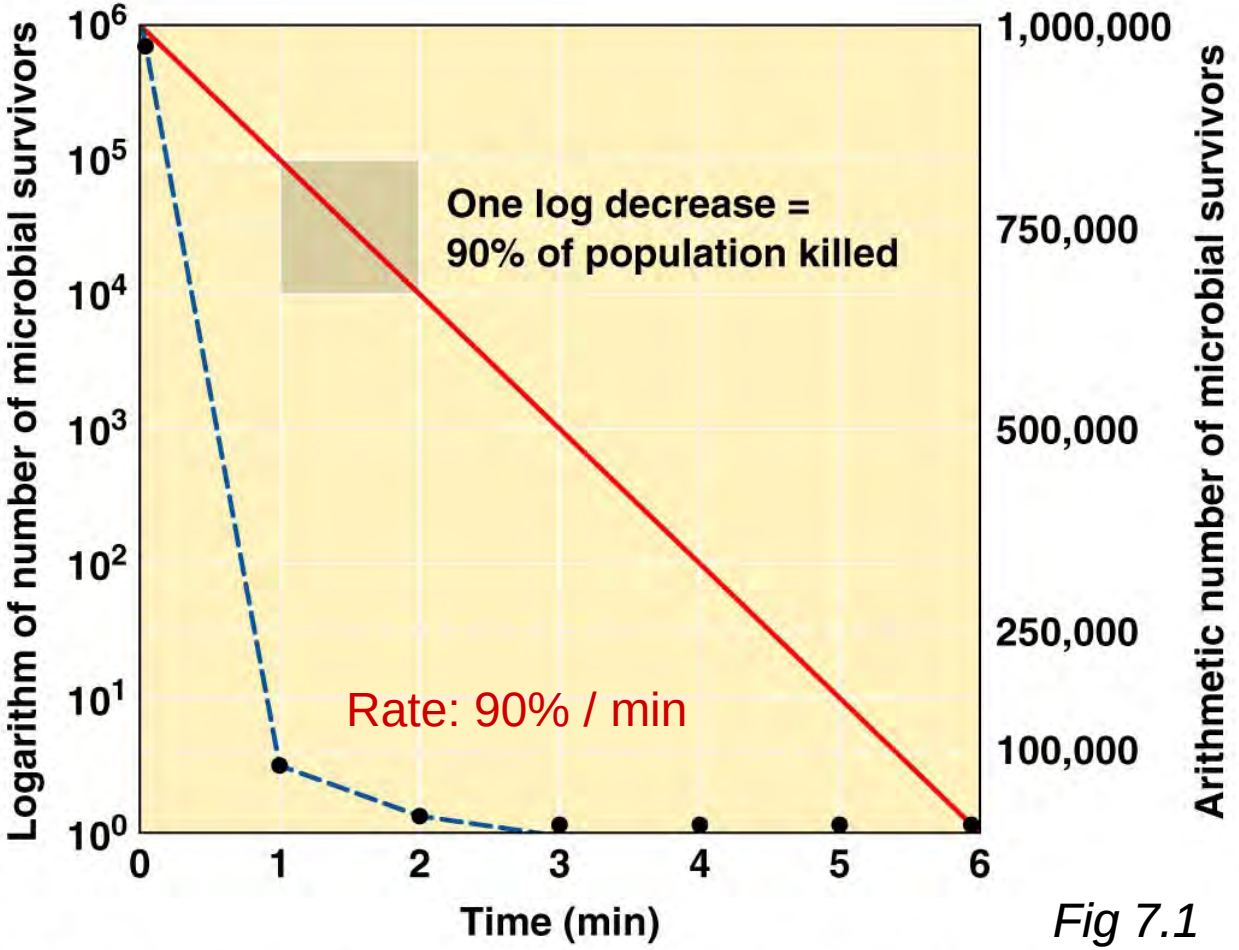


Fig 7.1

Effectiveness of Antimicrobial Treatment Depends on

- Time it takes to kill a microbial population is proportional to **number of microbes**.
- Microbial species and life cycle phases (*e.g.:* endospores) have **different susceptibilities** to physical and chemical controls.
- **Organic matter** may interfere with heat treatments and chemical control agents.
- **Exposure time**: Longer exposure to lower heat produces same effect as shorter time at higher heat.

Actions of Microbial Control Agents

- Alternation of membrane permeability
- Damage to proteins
- Damage to nucleic acids

Physical Methods of Microbial Control

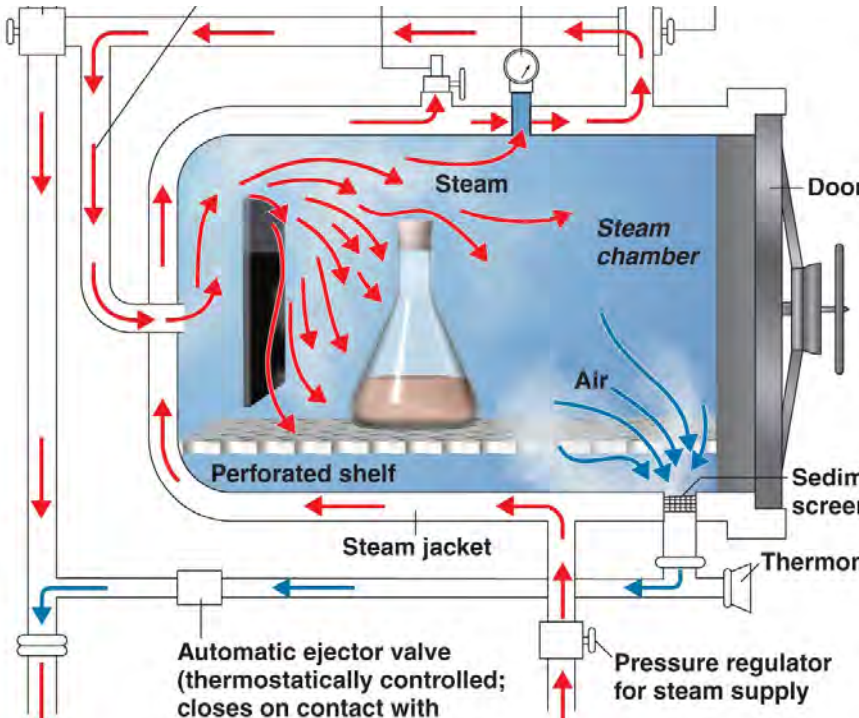
- Heat is very effective (fast and cheap).
- **Thermal death point (TDP):** Lowest temperature at which all cells in a culture are killed in 10 min.
- **Thermal death time (TDT):** Time to kill all cells in a culture
- **Decimal Reduction Time (DRT):** Minutes to kill 90% of a population at a given temperature

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

Moist Heat Sterilization

- Denatures proteins
- **Autoclave**: Steam under pressure
- Most dependable sterilization method
- **Steam must directly contact** material to be sterilized.
- Pressurized steam reaches higher temperatures.
- Normal autoclave conditions: **121.5°C for 15 min.**
- Prion destruction: 132°C for 4.5 hours
- Limitations of the autoclave



Pasteurization

- Significant number reduction (esp. spoilage and pathogenic organisms) → does not sterilize!
- Historical goal: destruction of *M. tuberculosis*
- **Classic holding method**: 63°C for 30 min
- **Flash pasteurization** (**HTST**): 72°C for 15 sec.
  Most common in US.
  Thermoduric organisms survive
- **Ultra High Temperature** (**UHT**):
  140°C for < 1 sec.
  Technically not pasteurization because it sterilizes.

Dry heat sterilization kills by oxidation

- Flaming of loop
- Incineration of carcasses
  - Anthrax
  - Foot and mouth disease
  - Bird flu
- Hot-air sterilization

::

	Hot-air	Autoclave
Equivalent treatments	170˚C, 2 hr	121˚C, 15 min

Filtration

- **Air filtration** using high efficiency particulate air (HEPA) filters. Effective to 0.3 µm
- **Membrane filters** for fluids.
  - Pore size for bacteria: 0.2 – 0.4 µm
  - Pore size for viruses: 0.01 µm

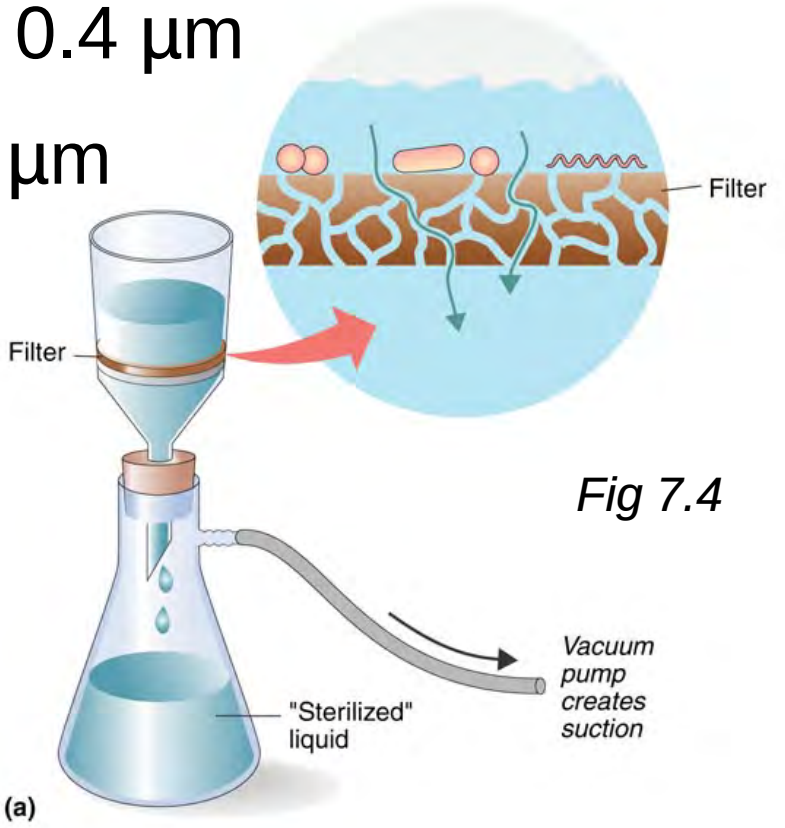


Fig 7.4

Low Temperature

- Slows enzymatic reactions ⇒ inhibits microbial growth
- Freezing forms ice crystals that damage microbial cells
- Refrigeration (watch out for _____!, deep freezing, lyophilization

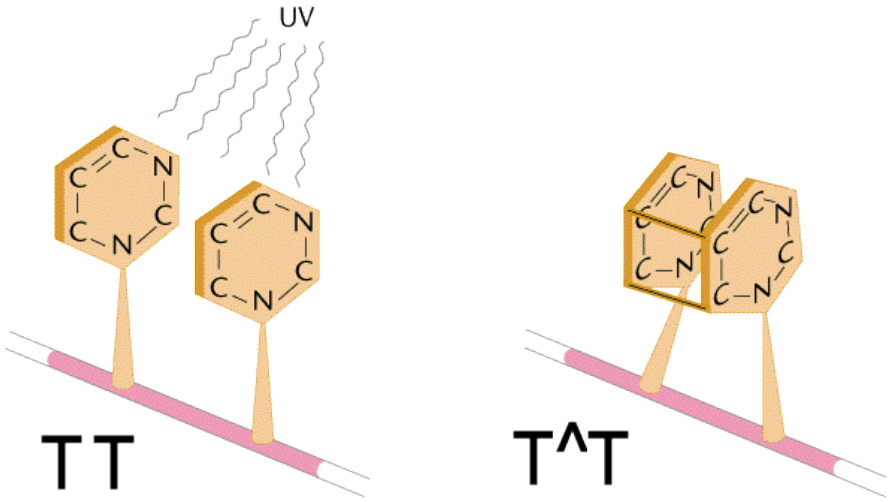
Various Other Methods

- High pressure in liquids denatures bacterial proteins and preserves flavor
- Desiccation prevents metabolism
- Osmotic pressure causes plasmolysis

Ionizing Radiation

- X-rays, γ-rays, electron beams $\rightarrow$ dislodge e- from atoms $\rightarrow$ production of free radicals and other highly reactive molecules
- Commonly used Cobalt-60 radioisotope
- *Salmonella* and *Pseudomonas* are particularly sensitive
- Sterilization of heat sensitive materials: drugs, vitamins, herbs, suture material
- Also used as “cold pasteurization” of food $\Rightarrow$ Consumer fears!?

Nonionizing Radiation: UV light

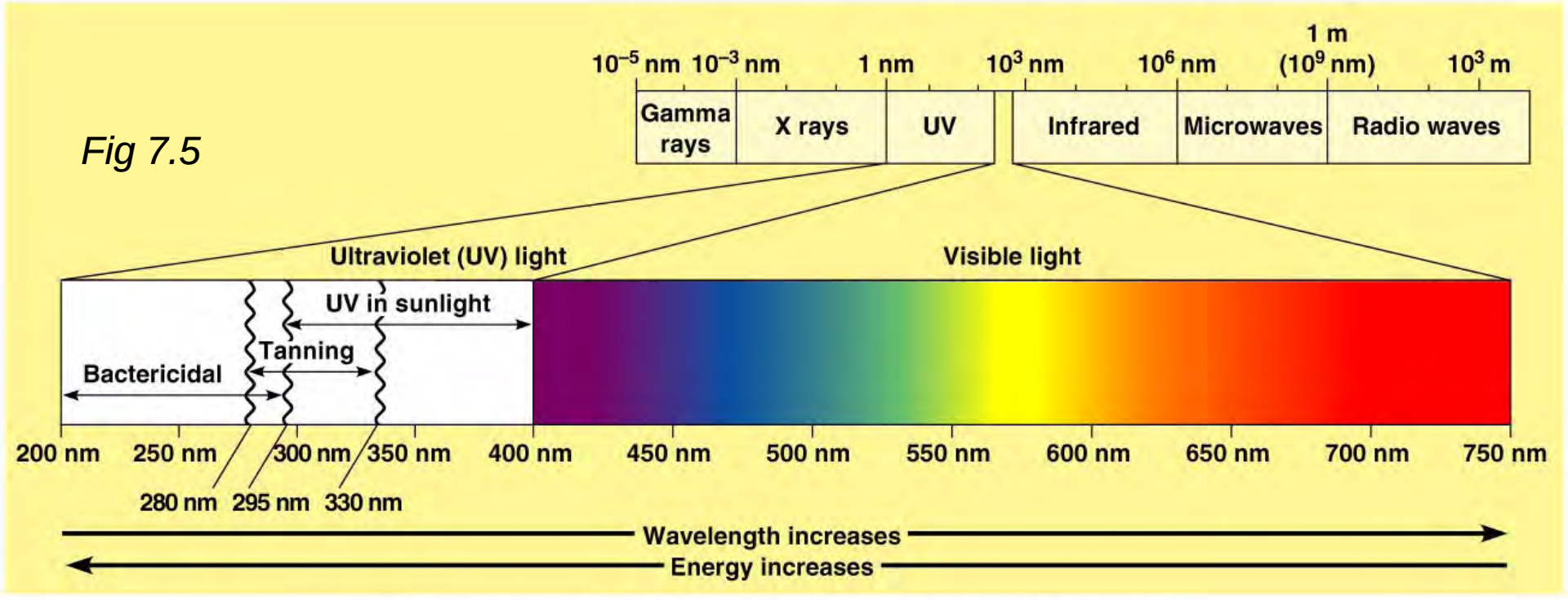


- Most effective wave legnth ~ 260 nm
- Effect: **thymine dimers**
- Actively dividing organisms are more sensitive because thymine dimers cause . . . .?
- Used to limit air and surface contamination. Use at close range to **directly exposed microorganisms**
  - *E.g.:* germicidal lamps in OR, cafeteria, and our lab ??

Nonionizing Radiation: Microwave

- Wavelength: 1 mm – 1m
- H_2O quickly absorbs energy release as heat to environment
- Indirect killing of bacteria through heat
- Solid food heats unevenly, why?

Fig 7.5

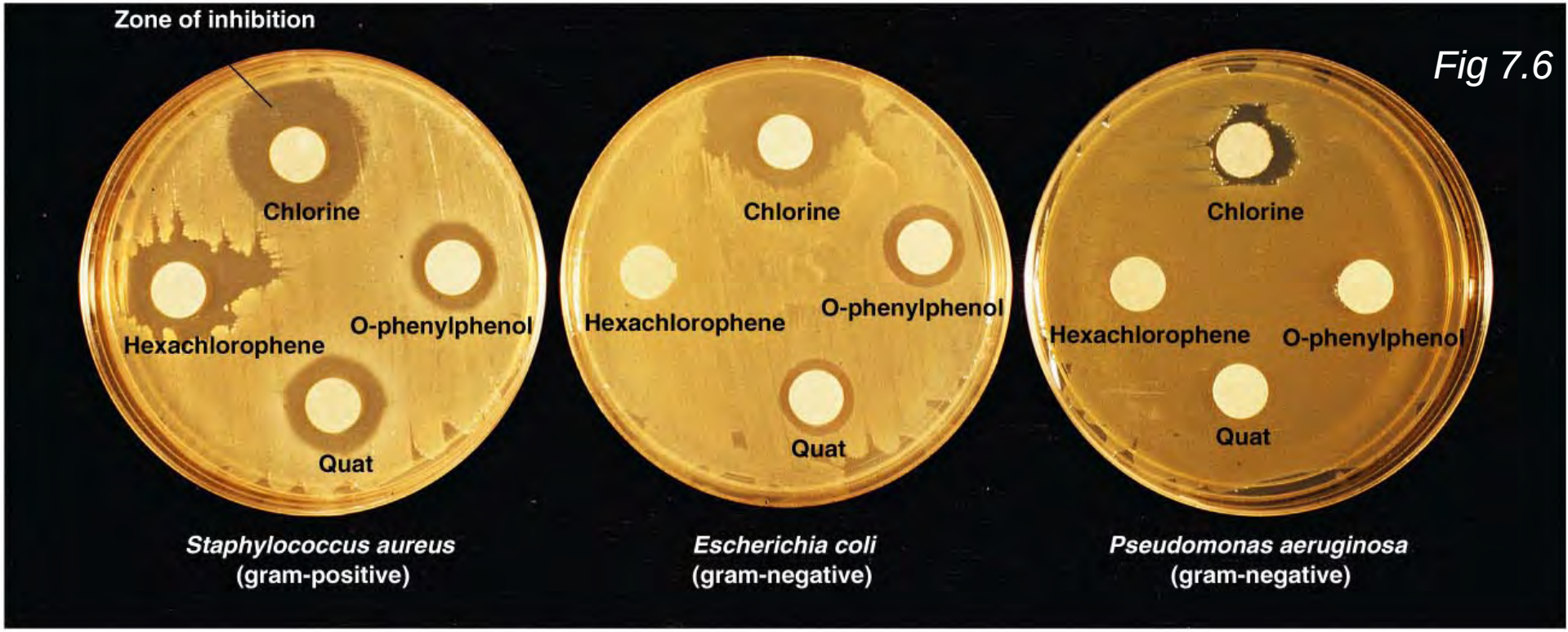


Chemical Methods of Microbial Control

- Few chemical agents achieve sterility.
- Consider presence of organic matter, degree of contact with microorganisms, and temperature
- Disinfectants regulated by EPA
  Antiseptics regulated by FDA
- **Use-dilution test**
  1. Metal rings dipped in test bacteria are dried.
  2. Dried cultures of *S. choleraesuis*, *S. aureus*, and *P. aeruginosa* are placed in disinfectant for 10 min at 20°C.
  3. Rings are transferred to culture media to determine whether bacteria survived treatment.

Disk-diffusion Method

Disk of filter paper is soaked with a chemical and placed on an inoculated agar plate; a zone of inhibition indicates effectiveness.



Types of Disinfectants

- **Phenol = carbolic acid** (historic importance)
- **Phenolics**: Cresols (Lysol) - disinfectant
- **Bisphenols**
  - **Hexachlorophene** (pHisoHex, prescription), hospitals, surgeries, nurseries
  - Triclosan (toothpaste, antibacerial soaps, etc.)

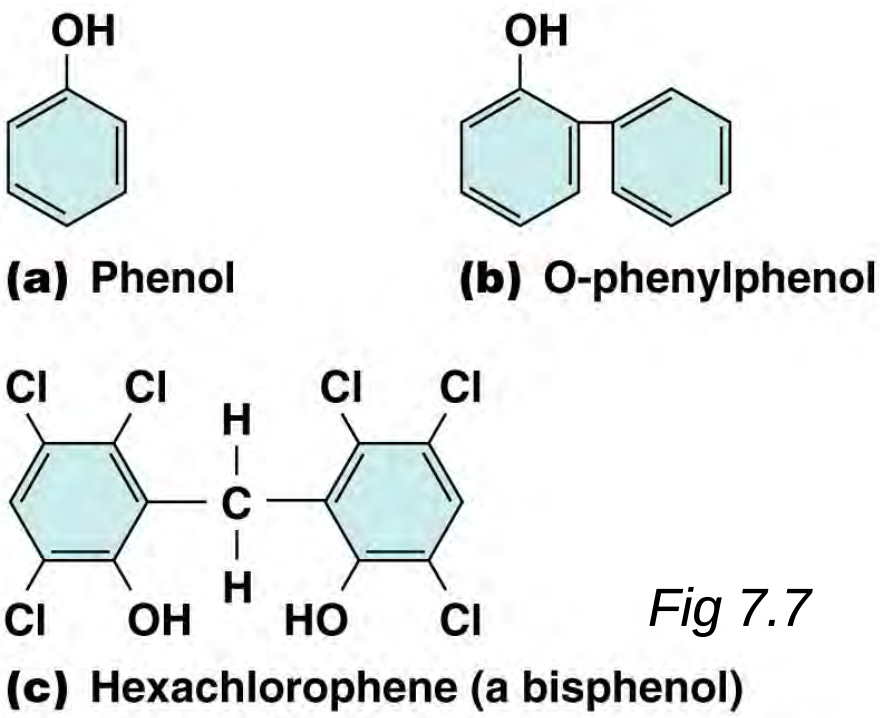
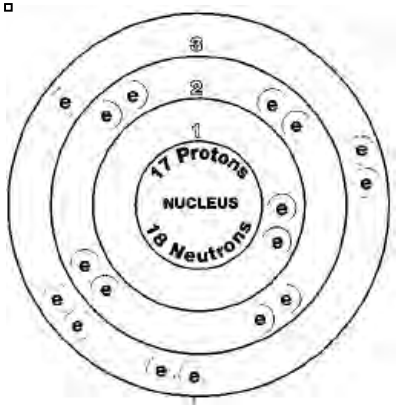


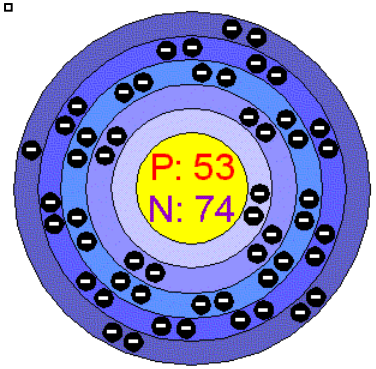
Fig 7.7

Phenol and derivatives disrupt plasma membranes (lipids!) and lipid rich cell walls (??)

Remain active in presence of organic compoundsP

Halogens





Chlorine

- Oxidizing agent
- Widely used as disinfectant
- Forms bleach (hypochlorous acid) when added to water.
- Broad spectrum, not sporicidal (pools, drinking water)

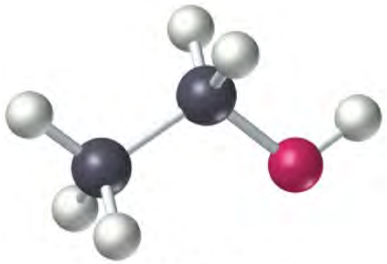
Iodine

More reactive, more germicidal. Alters protein synthesis and membranes.

Tincture of iodine (solution with alcohol) → wound antiseptic

Iodophors combined with an organic molecule → iodine detergent complex (e.g. Betadine®). Occasional skin sensitivity, partially inactivated by organic debris, poor sporicidal activity.

Alcohols



- **Ethyl** (60 – 80% solutions) and **isopropyl alcohol**
- Denature proteins, dissolve lipids
- No activity against spores and poorly effective against viruses and fungi
- Easily inactivated by organic debris
- Also used in hand sanitizers and cosmetics

TABLE 7.6 **Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution Against *Streptococcus pyogenes***

Concentration of Ethanol (%)	Time (sec) 10	20	30	40	50
100	–	–	–	–	–
95	+	+	+	+	+
90	+	+	+	+	+
80	+	+	+	+	+
70	+	+	+	+	+
60	+	+	+	+	+
50	–	–	+	+	+
40	–	–	–	–	–

NOTE: A minus sign indicates no biocidal action (bacterial growth); a plus sign indicates biocidal action (no bacterial growth). The highlighted area represents bacteria killed by biocidal action.

Heavy Metals

Oligodynamic action: toxic effect due to metal ions combining with sulfhydryl (—SH) and other groups ⇒ proteins are denatured.

- **Mercury** ($HgCl_2$, Greeks & Romans for skin lesions); Thimerosal
- **Copper** against chlorophyll containing organisms → Algicides
- **Silver** ($AgNO_3$): Antiseptic for eyes of newborns
- Zinc ($ZnCl_2$) in mouthwashes, ZnO in antifungal in paint

Surface Acting Ingredients / Surfactants

- Soaps and Detergents
- Major purpose of soap: Mechanical removal and use as wetting agent
- Definition of detergents
  - **Acidic-Anionic detergents**→ Anion reacts with plasma membrane. Nontoxic, non-corrosive, and fast acting. Laundry soap, dairy industry.
  - **Cationic detergents** → Quarternary ammonium compounds (**Quats**). Strongly bactericidal against against wide range, but esp. Gram+ bacteria

Soap	Degerming
Acid-anionic detergents	Sanitizing
Quarternary ammonium compounds (cationic detergents)	Strongly bactericidal, denature proteins, disrupt plasma membrane

Chemical Food Preservatives

Sulfur dioxide

wine

Organic acids

Inhibit metabolism

Sorbic acid, benzoic acid, and calcium propionate

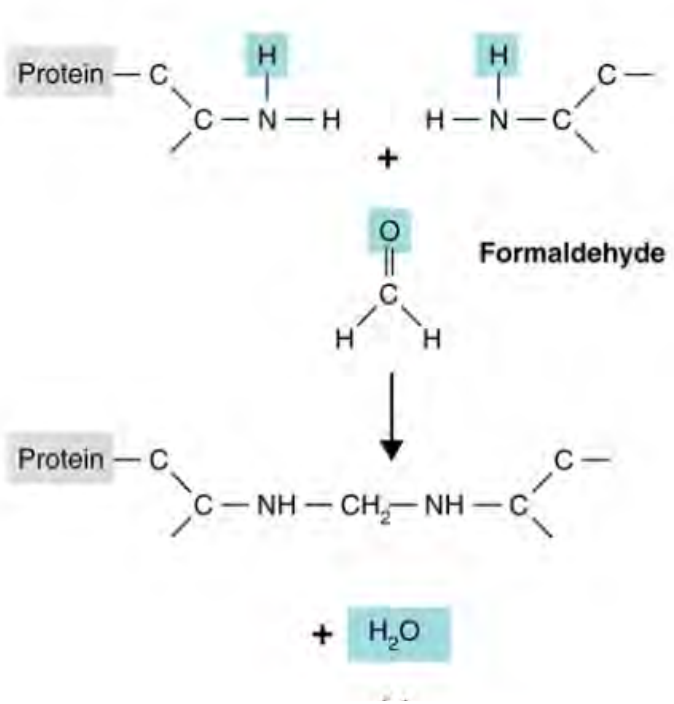
Control molds and bacteria in foods and cosmetics

Sodium nitrate **and nitrite** prevents endospore germination.
In meats. Conversion to nitrosamine (carcinogenic)

Aldehydes and Chemical Sterilants

Aldehydes (alkylating agents)

- Inactivate proteins by cross-linking with functional groups ($–NH_2$, –OH, –COOH, –SH)
- **Glutaraldehyde**: Sterilant for delicate surgical instruments (Kills *S. aureus* in 5, *M. tuberculosis* in 10 min)
- **Formaldehyde**: Virus inactivation for vaccines

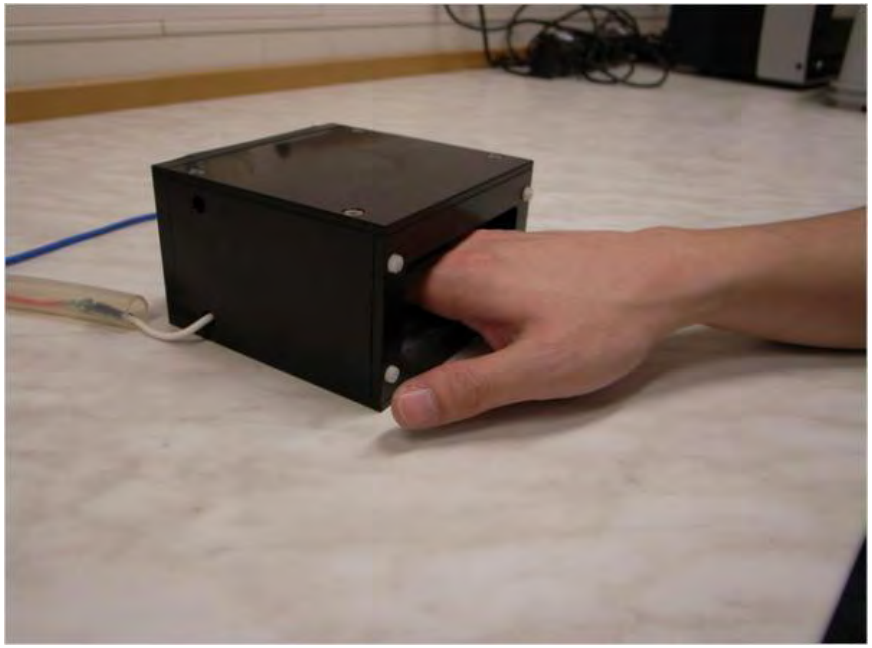


Chemical Sterilants for heat sensive material

- Denature proteins
- Ethylene oxide

Plasma

- Luminous gas with free radicals that destroy microbes
- Use: Tubular instruments, hands, etc.



Hydrogen Peroxide: Oxidizing agent

Inactivated by catalase ⇒

Not good for open wounds

Good for inanimate objects; packaging for food industry (containers etc.)

3% solution (higher conc. available)

Esp. effective against anaerobic bacteria (e.g.:

Effervescent action, may be useful for wound cleansing through removal of tissue debris

Microbial Characteristics and Microbial Control

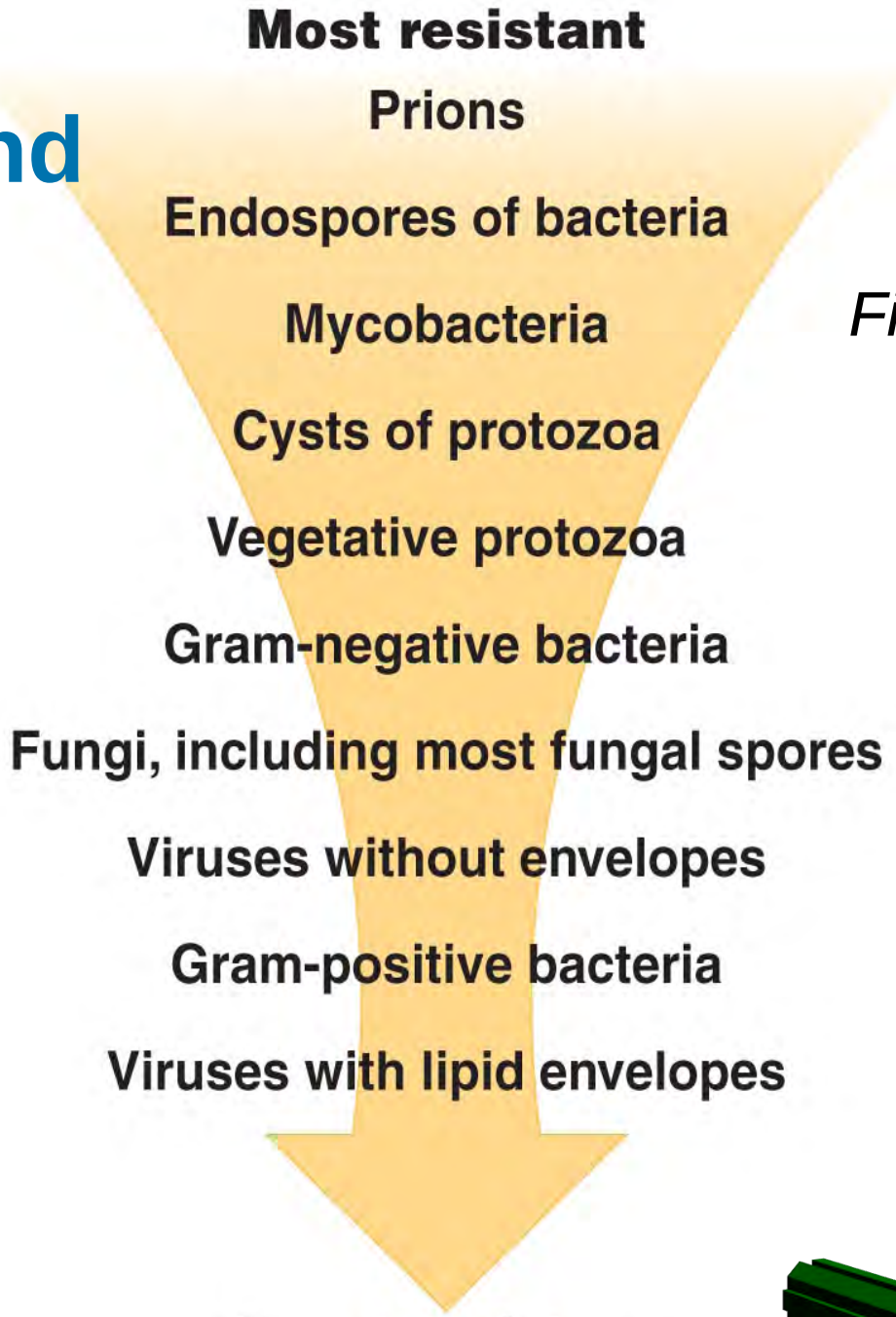


Fig 7.11

TABLE 7.7 The Effectiveness of Chemical Antimicrobials Against Endospores and Mycobaceria

Chemical Agent	Endospores	Mycobacteria
Mercury	No activity	No activity
Phenolics	Poor	Good
Bisphenols	No activity	No activity
Quaternary ammonium compounds	No activity	No activity
Chlorines	Fair	Fair
Iodine	Poor	Good
Alcohols	Poor	Good
Glutaraldehyde	Fair	Good
Chlorhexidine	No activity	Fair

Virus and Phages

Student Learning Outcomes

Differentiate a virus from a bacterium.

Explain the difference between enveloped and nonenveloped viruses.

Define viral species.

Describe how bacteriophages and animal viruses are cultured.

Compare and contrast the lytic and lysogenic cycles of bacteriophages.

Define oncogene and transformed cell.

Discuss the relationship between viruses and cancer.

Explain latent viral infections and give an example.

Discuss how a proteins can be infectious.

Foundations of Virology

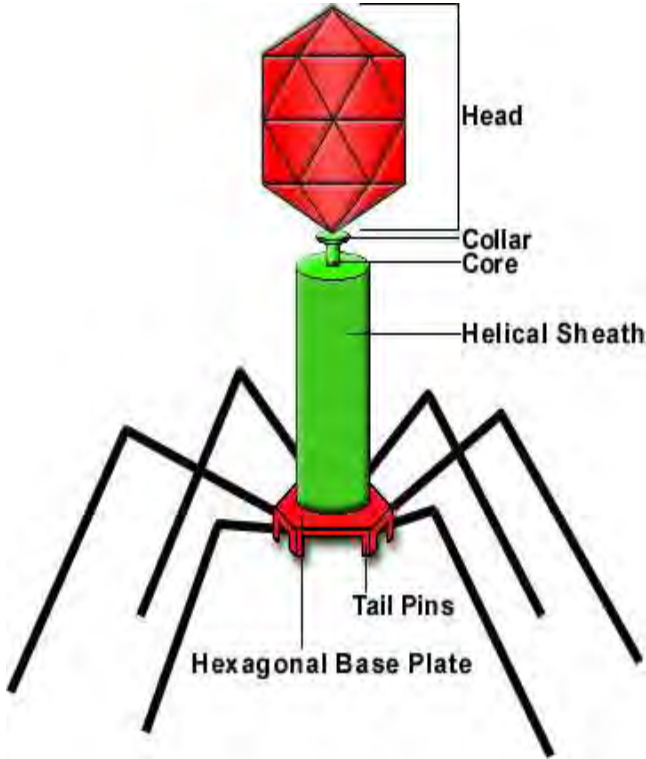
Non-living agents that infect all life forms

(phages vs. animal viruses)

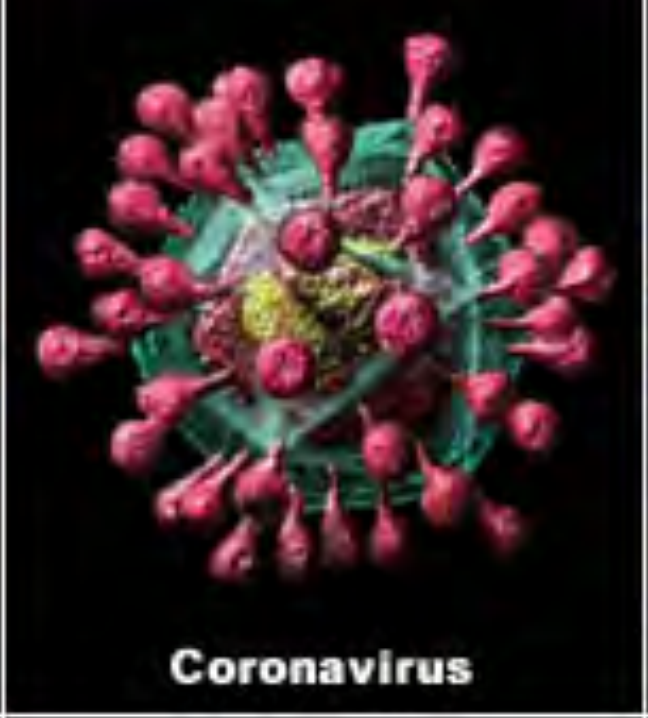
Viral cultivation differs from bacterial cultivation

~ 1,500 known viruses (estimates: ~ 400,000 exist)

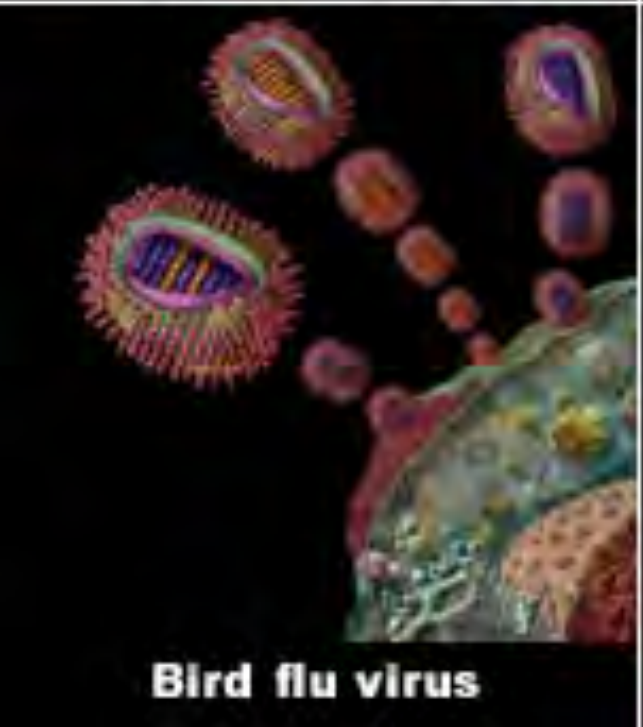
Advent of EM allowed for visualization of viruses




Hepatitis C virus


Coronavirus


Herpes virus

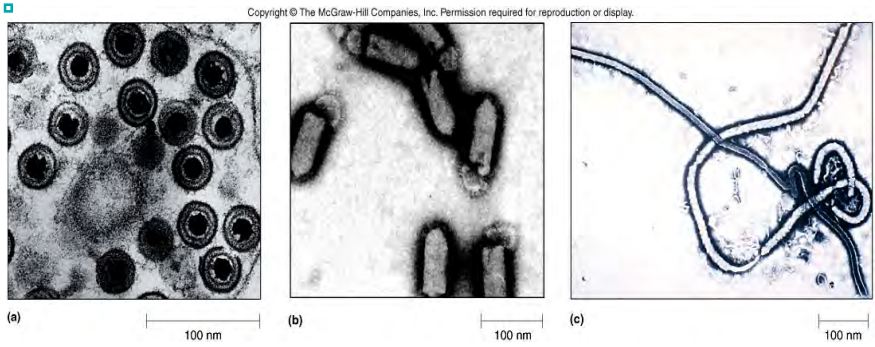

Bird flu virus


Smallpox virus


Influenza virus

General Characteristics of Viruses

- **Obligatory intracellular parasites**
- Filterable
- Virus = Latin for poison
- Contain DNA **or** RNA



- Contain a protein coat = **capsid** made up of **capsomeres**. Various shapes
- Some are enclosed by an envelope (naked vs. enveloped)
- Some viruses have spikes (COH/protein)
- Most viruses are tissue specific
- **Host range** is determined by specific host attachment sites and cellular factors

Virus Shapes and Sizes

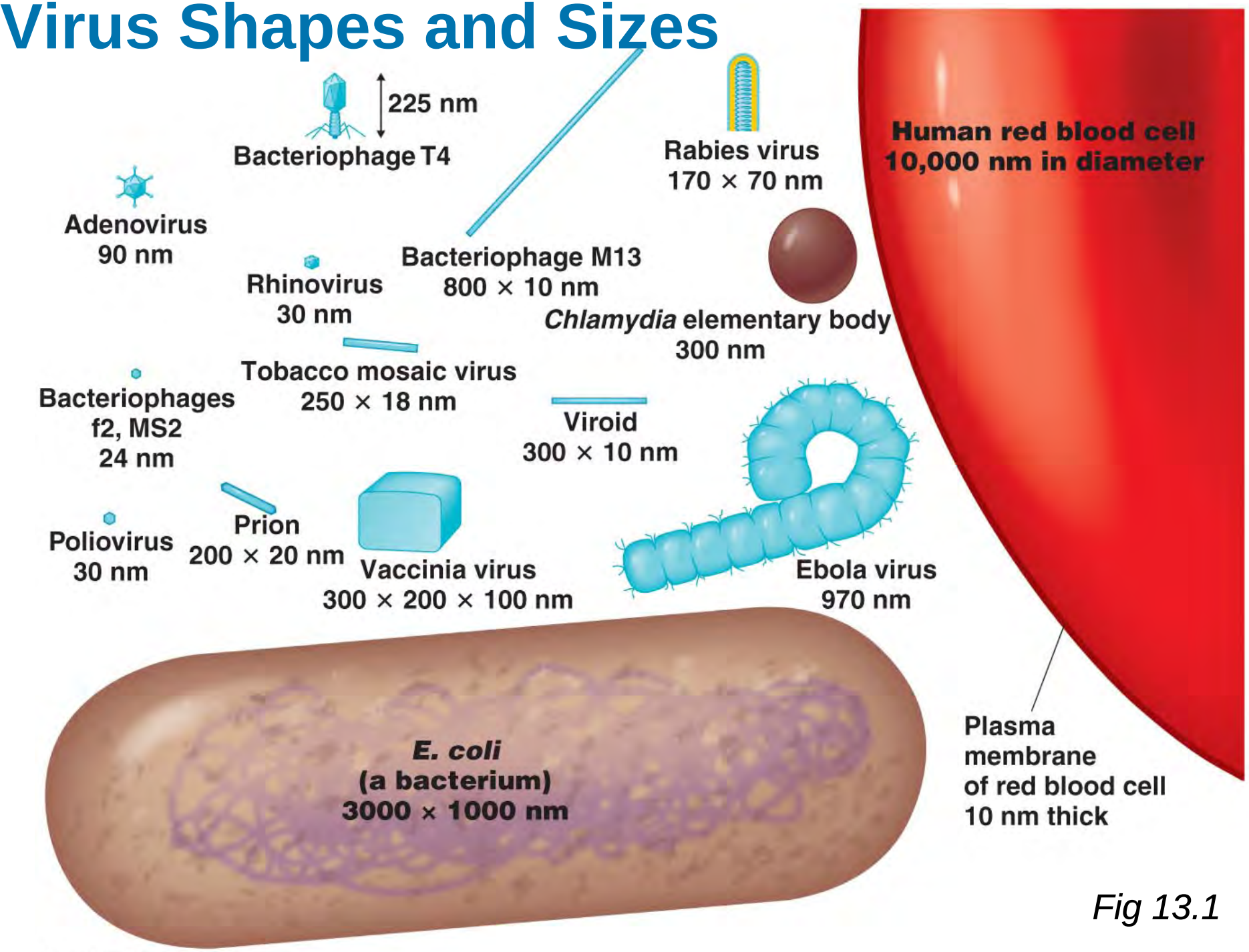
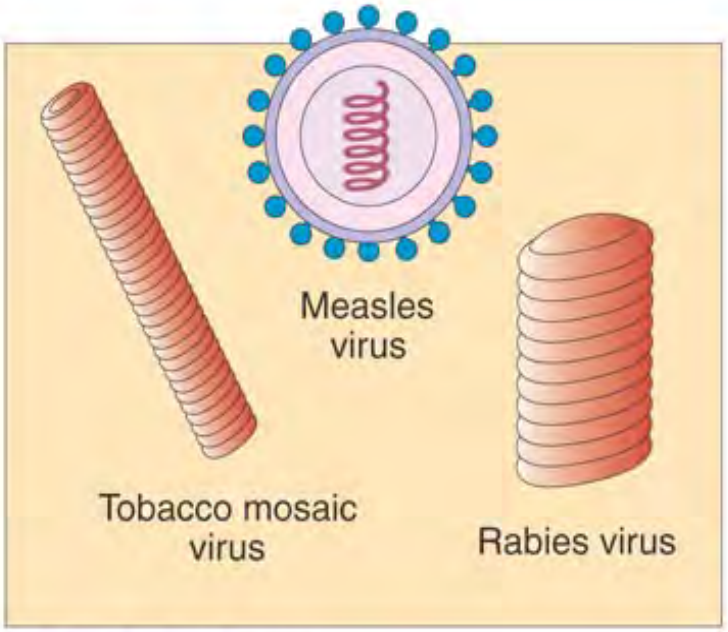
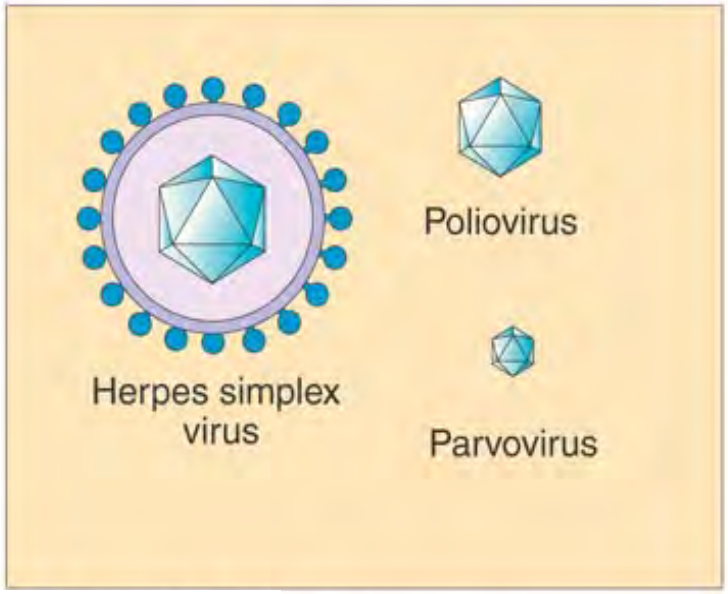


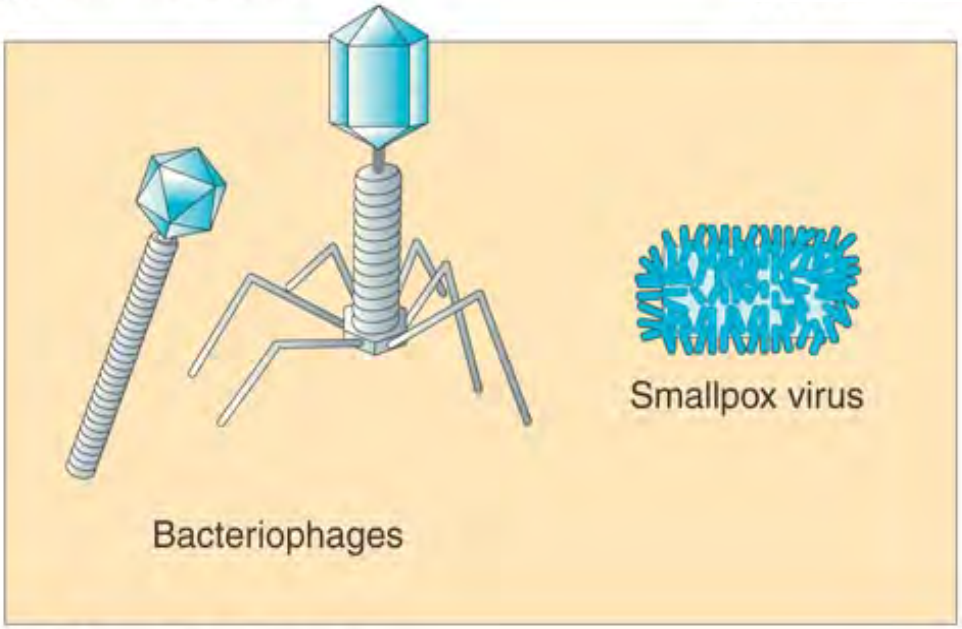
Fig 13.1



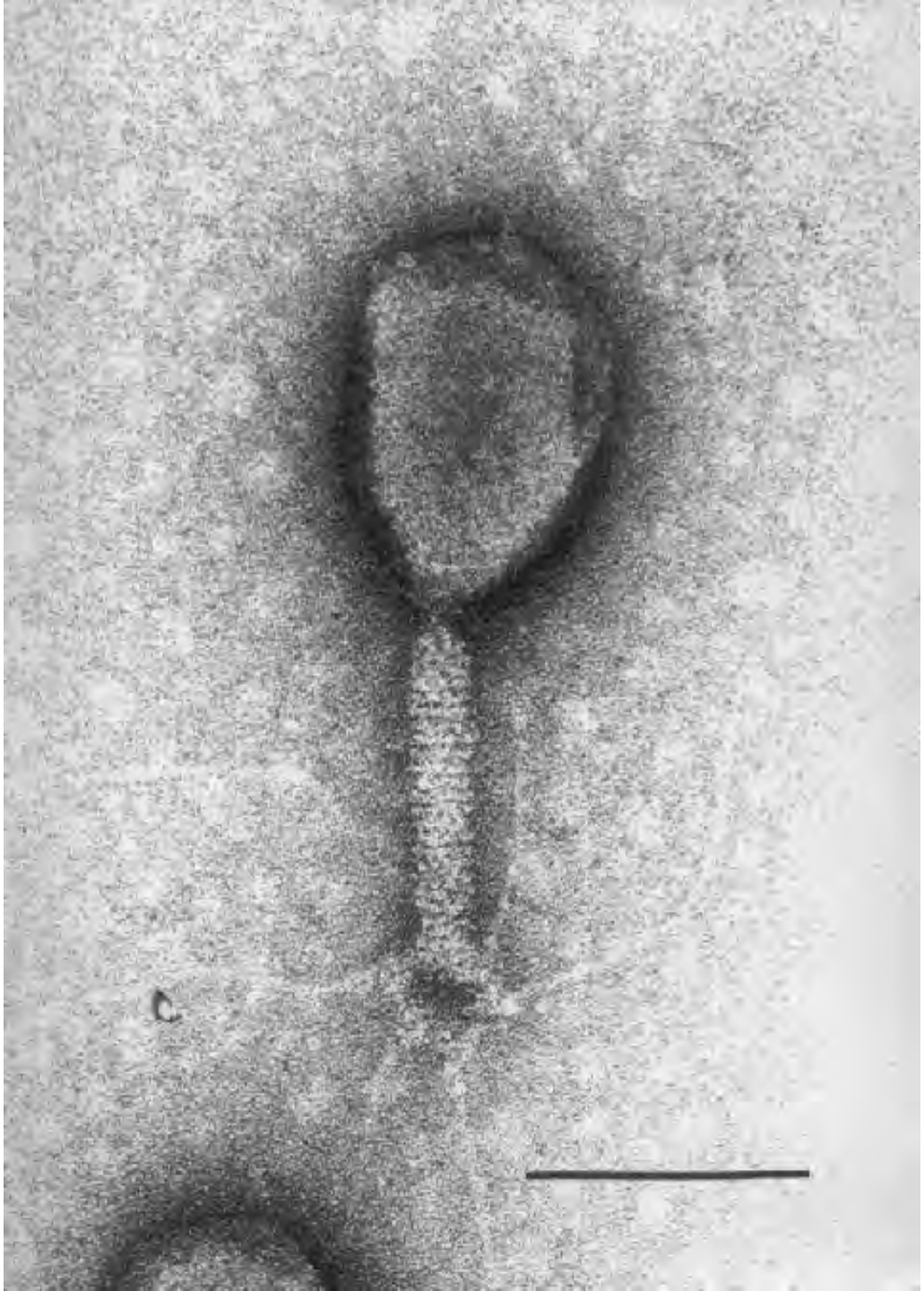
(a) Helical viruses



Polyhedral viruses



(c) Complex viruses



Electron micrograph of Aeromonas virus 31, an unassigned virus in the family *Myoviridae*

photograph by Dr Hans Ackermann.

Morphology of an enveloped helical virus

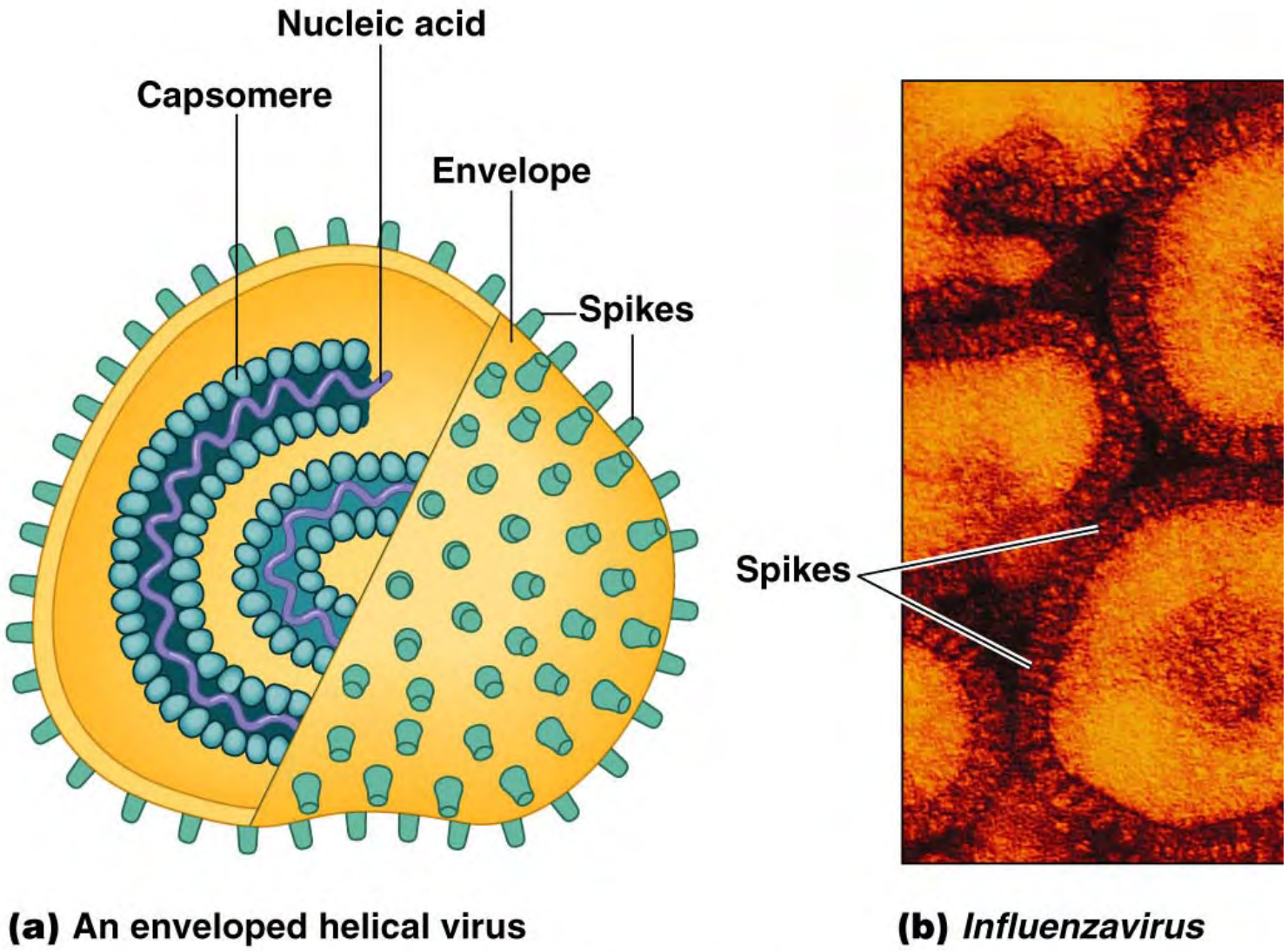
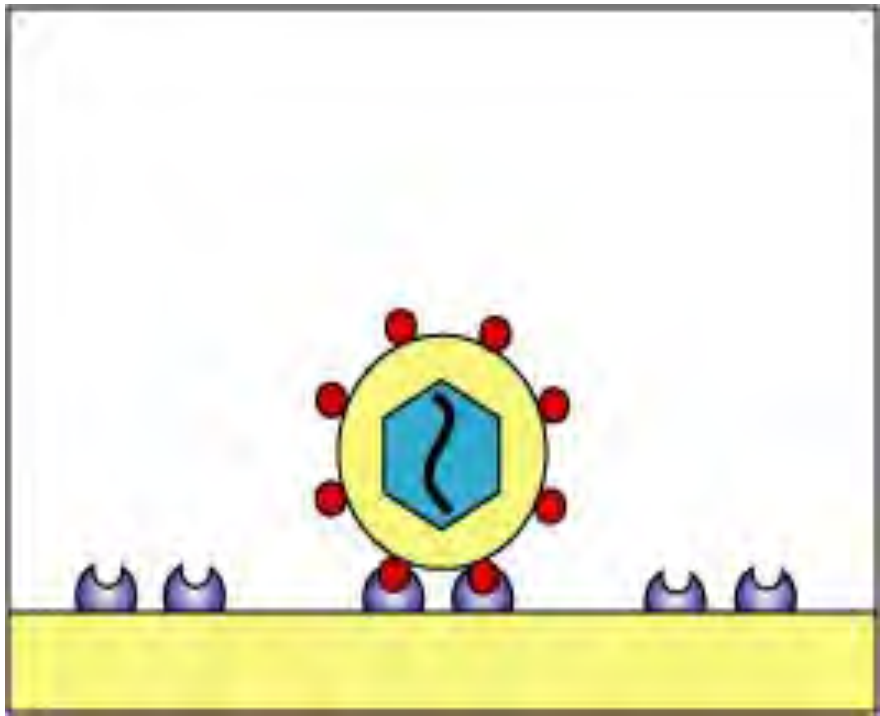


Fig 13.3

Host Range and Specificity

Virus / host cell interaction usually very specific (narrow host range) – *due to?*

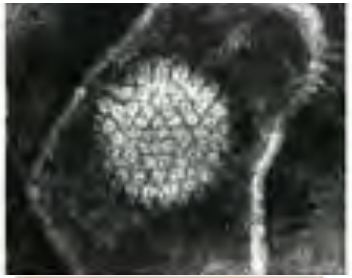
Tissue tropism

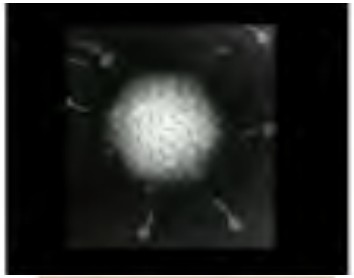


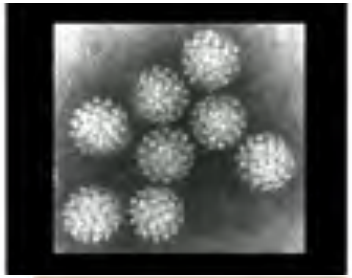
Taxonomy of Viruses

- No evidence for common viral ancestor.
- Classification based on type of NA, strategy for replication, and morphology.
  - **Family** names end in ***–viridae***
  - **Genus** and **species** names end in ***-virus***.
- **Viral species**: A group of viruses sharing the same genetic information and ecological niche (host). Common names are used for species.
- Subspecies are designated by a number.


Poxviridae


Herpesviridae


Adenoviridae


Papovaviridae

Taxonomy of Viruses

- Herpesviridae
- *Herpesvirus*
- Human herpes virus HHV-1, HHV-2, HHV-3

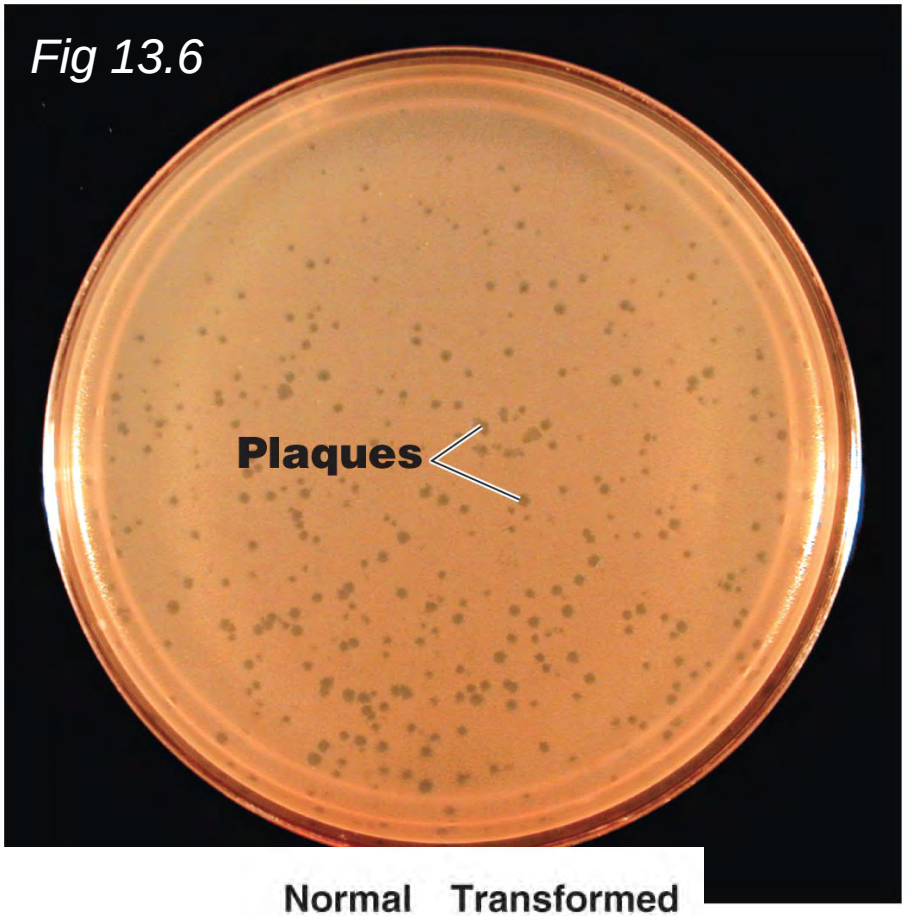
- Retroviridae
- *Lentivirus*
- Human immunodeficiency virus HIV-1, HIV-2

Isolation, Cultivation, and Identification of Viruses

- Viruses must be grown in living cells

  Bacteriophages form plaques on a lawn of bacteria

  Animal viruses may be grown in cell culture, embryonated eggs, or living animals



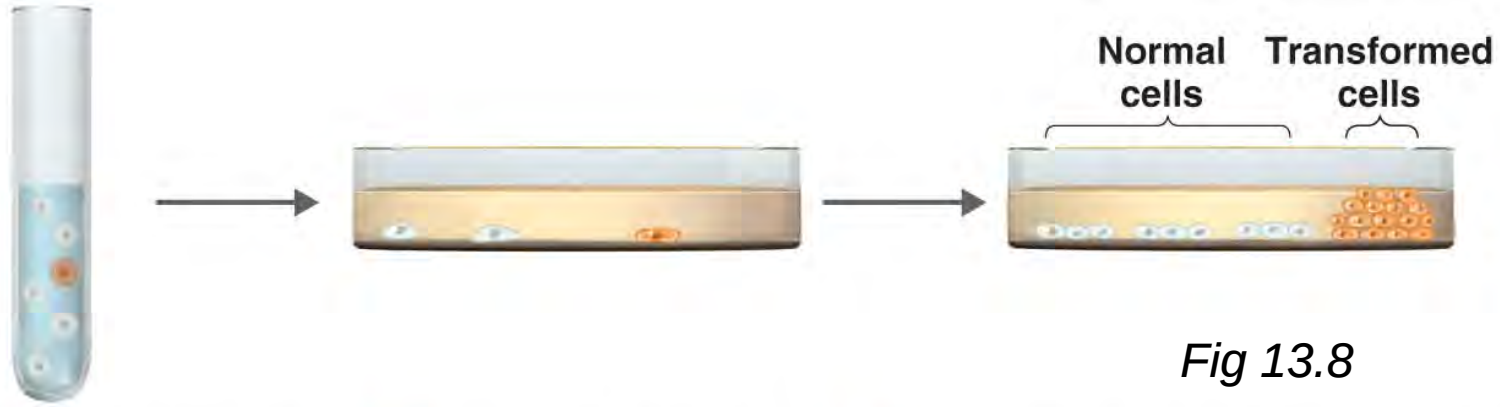
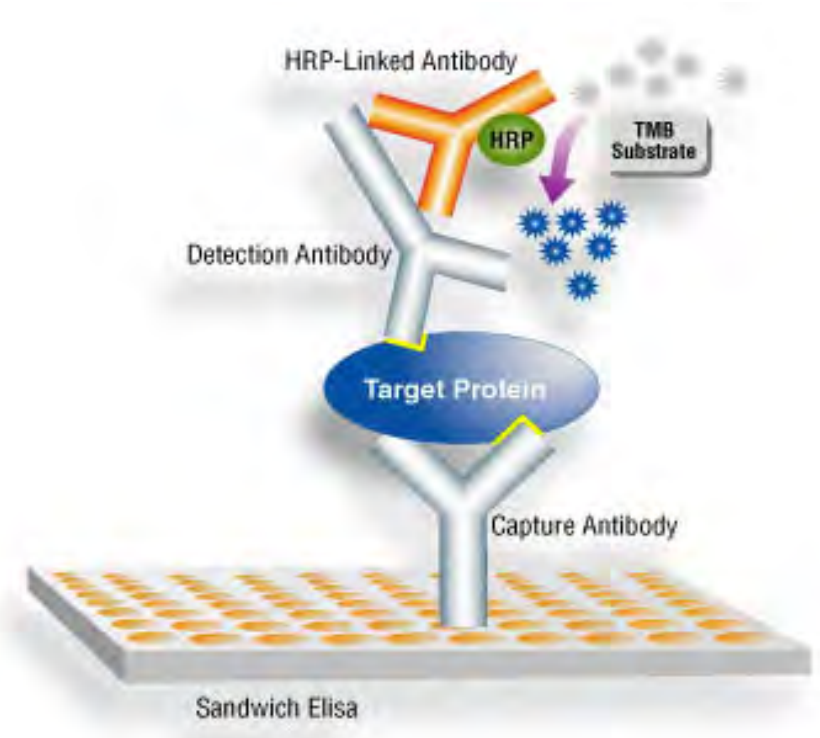


Fig 13.8

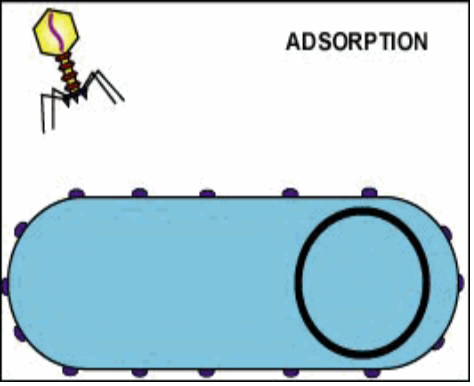
Virus Identification

- Cytopathic effects
- Serological tests
  - Detect antibodies against viruses in a patient
  - Use antibodies to identify viruses in neutralization tests, viral hemagglutination, and Western blot
- Nucleic acids
  - RFLPs
  - PCR
- Novel methods such as Biophotonics

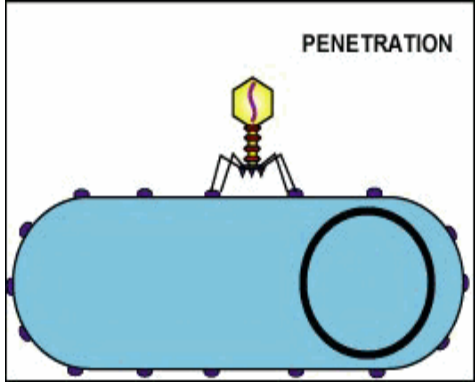


Viral Replication

- **Obligate intracellular parasites using host cell machinery**
- **Very limited number of genes encode proteins for**
  - **Capsid formation**
  - **Viral nucleic acid replication**
  - **Movement of virus into and out of cell**
- **Kill or live in harmony within the host cell – Outside the cell, viruses are inert**



Bacteriophage: The Lytic Cycle



1. **Attachment** to cell surface receptors (chance encounter – no active movement)
2. **Penetration** – only genome enters
3. **Biosynthesis** – Production of phage DNA and proteins
4. **Maturation** – assembly to form intact phage
5. **Release** due to phage induced lysozyme production

See Fig 13.11

Lytic Cycle of a T-Even Bacteriophage

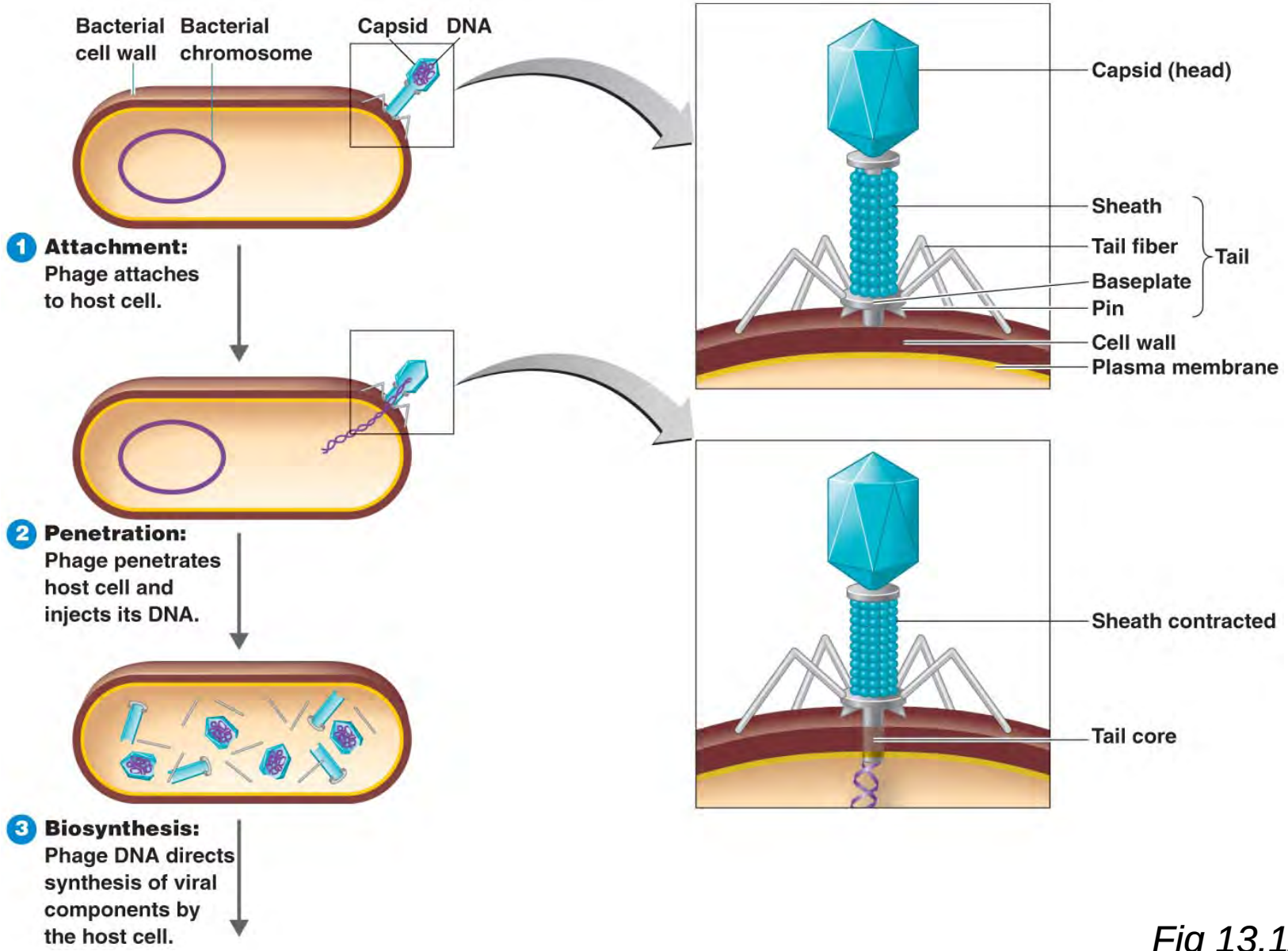


Fig 13.11

Lytic Cycle of a T-Even Bacteriophage

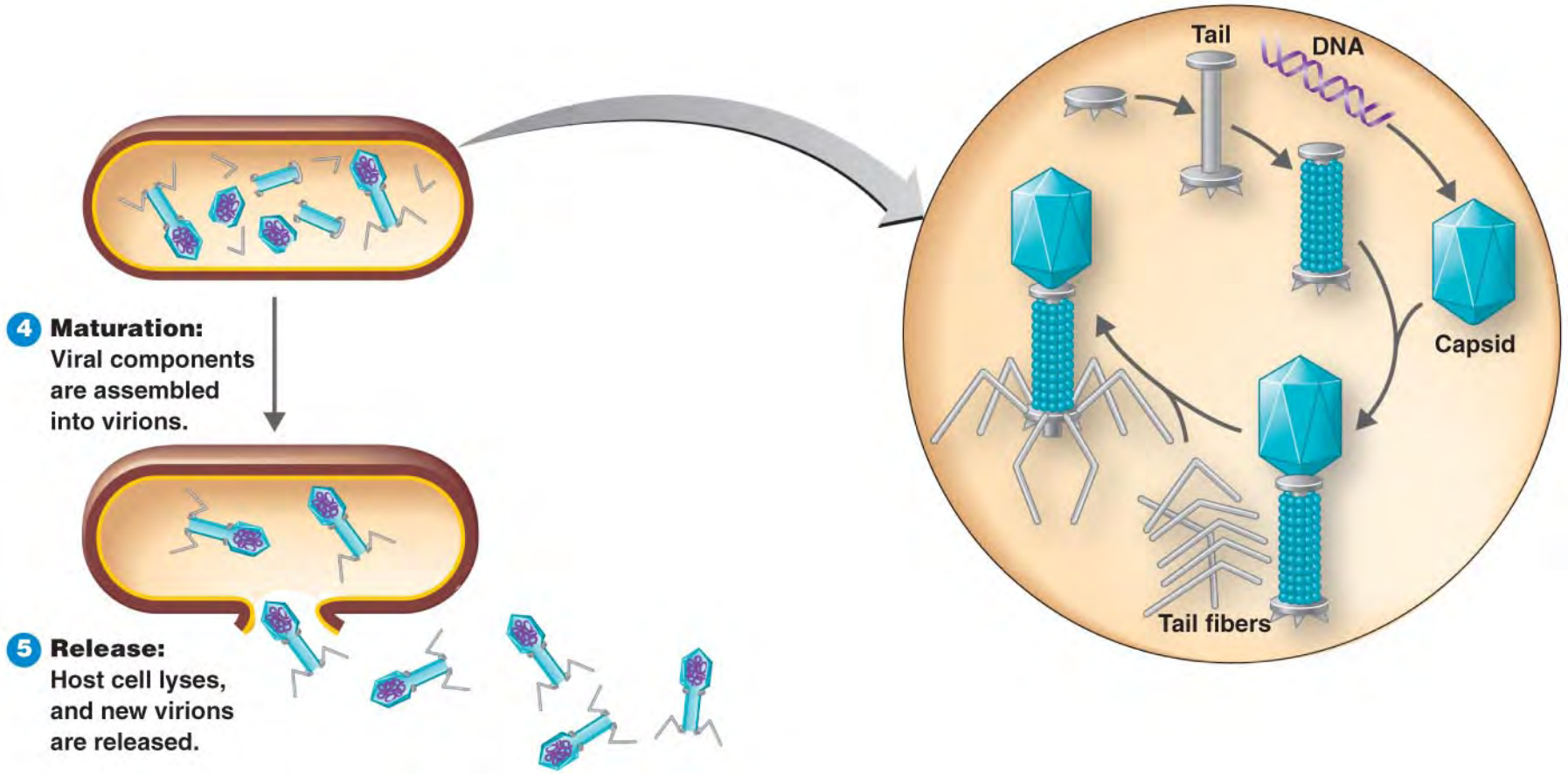


Fig 13.11

Results of Multiplication of Bacteriophages

- **Lytic cycle**
  - Lytic or virulent phage
  - Phage causes lysis and death of host cell
- **Lysogenic cycle**
  - Lysogenic or temperate phage
  - Phage DNA incorporated in host DNA $\Rightarrow$ **Prophage**
  - Phage conversion
  - Specialized transduction

Lytic and Lysogenic Cycles

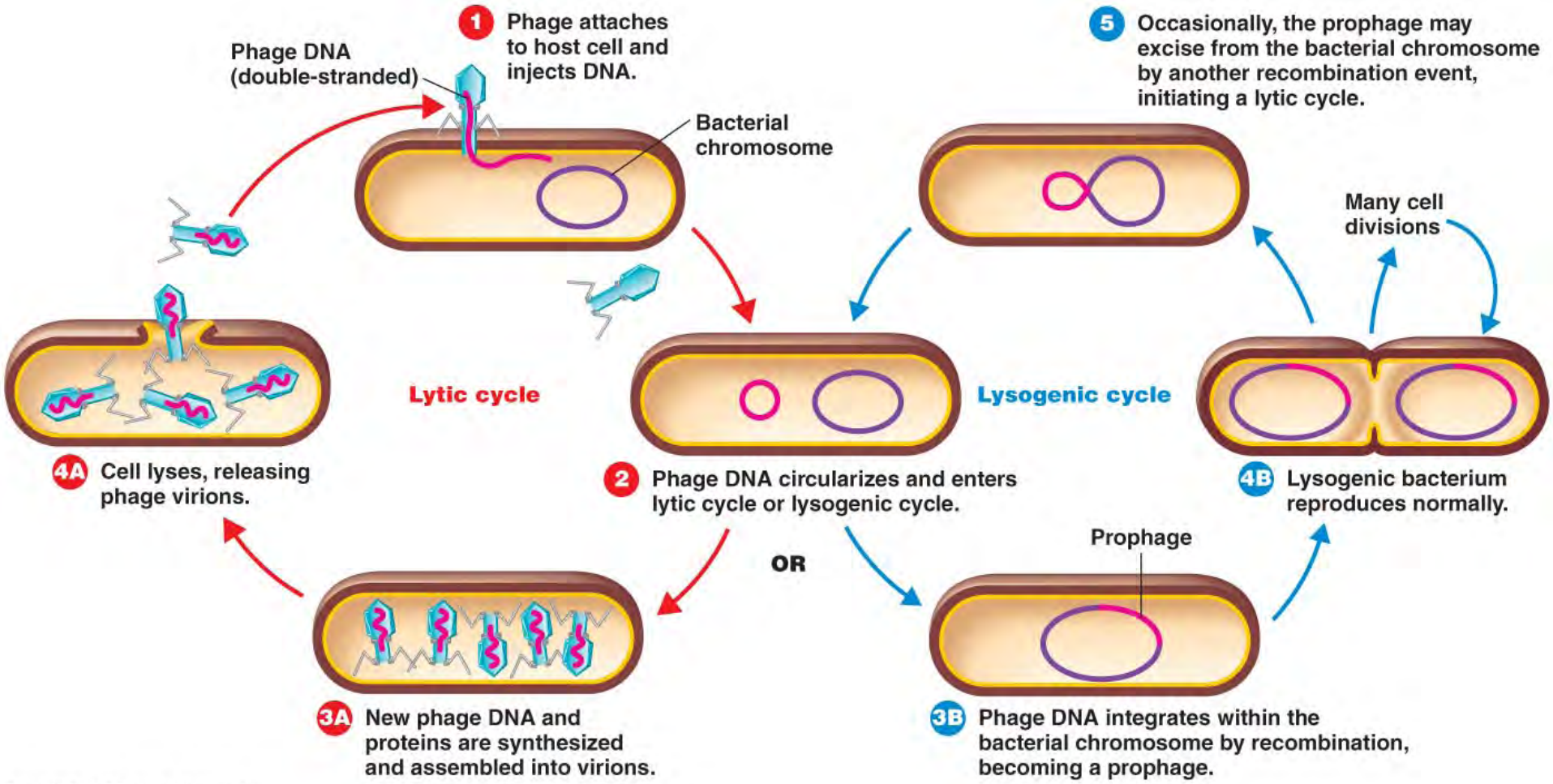
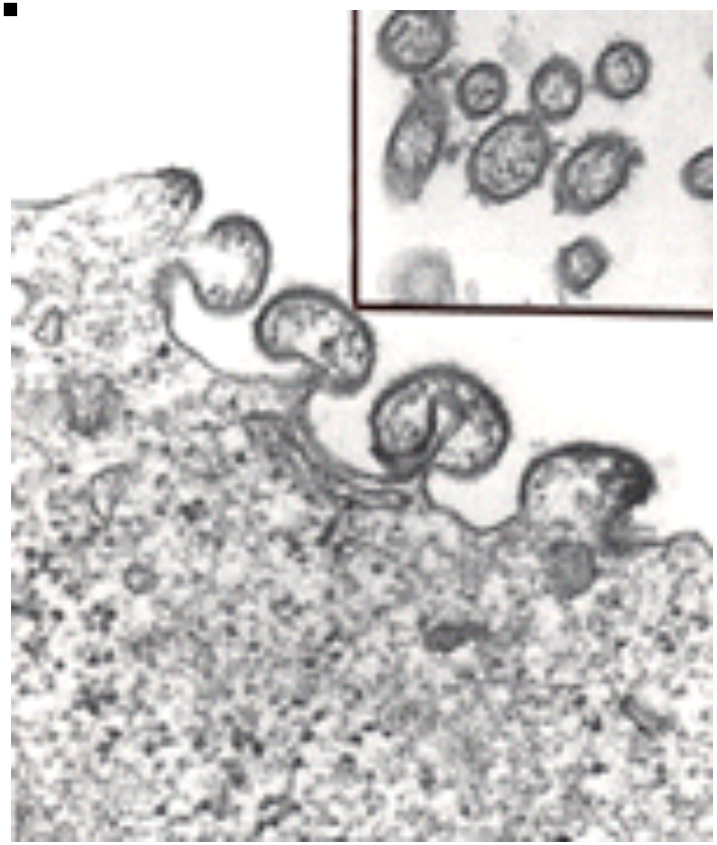
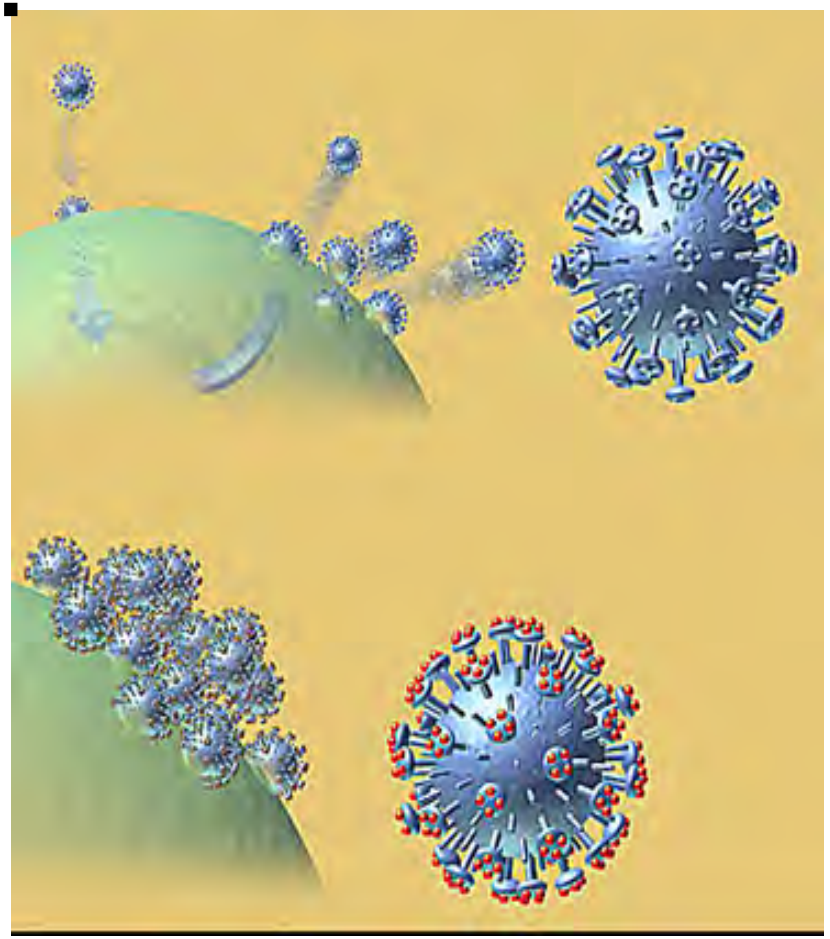


Fig 13.12

Some animal viruses exit the host cells via budding.

HSV envelopment and release

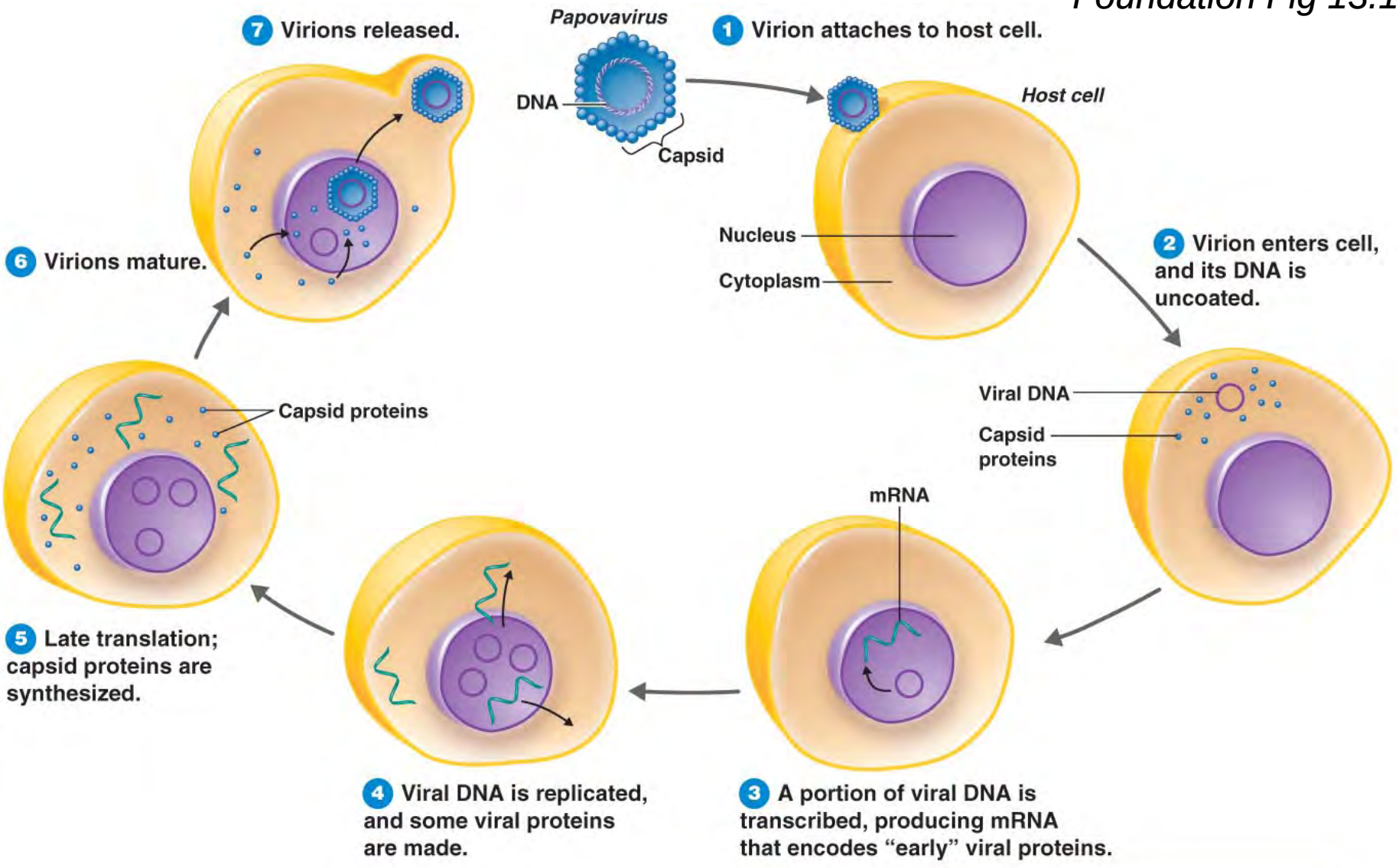




Compare to Fig. 13.20

Multiplication of DNA Viruses

Foundation Fig 13.15



Multiplication of RNA Viruses

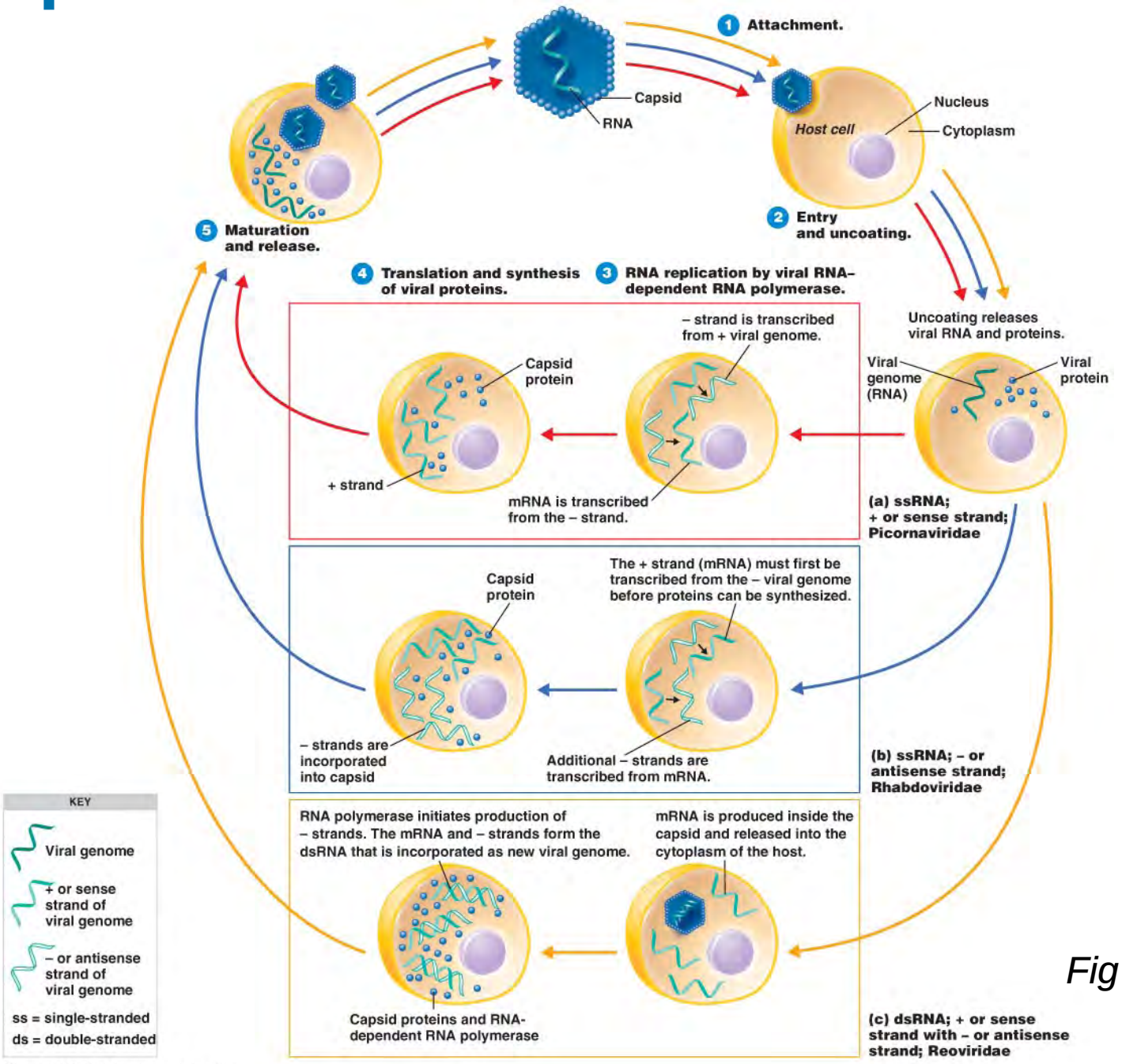


Fig 13.17

Multiplication of a Retrovirus

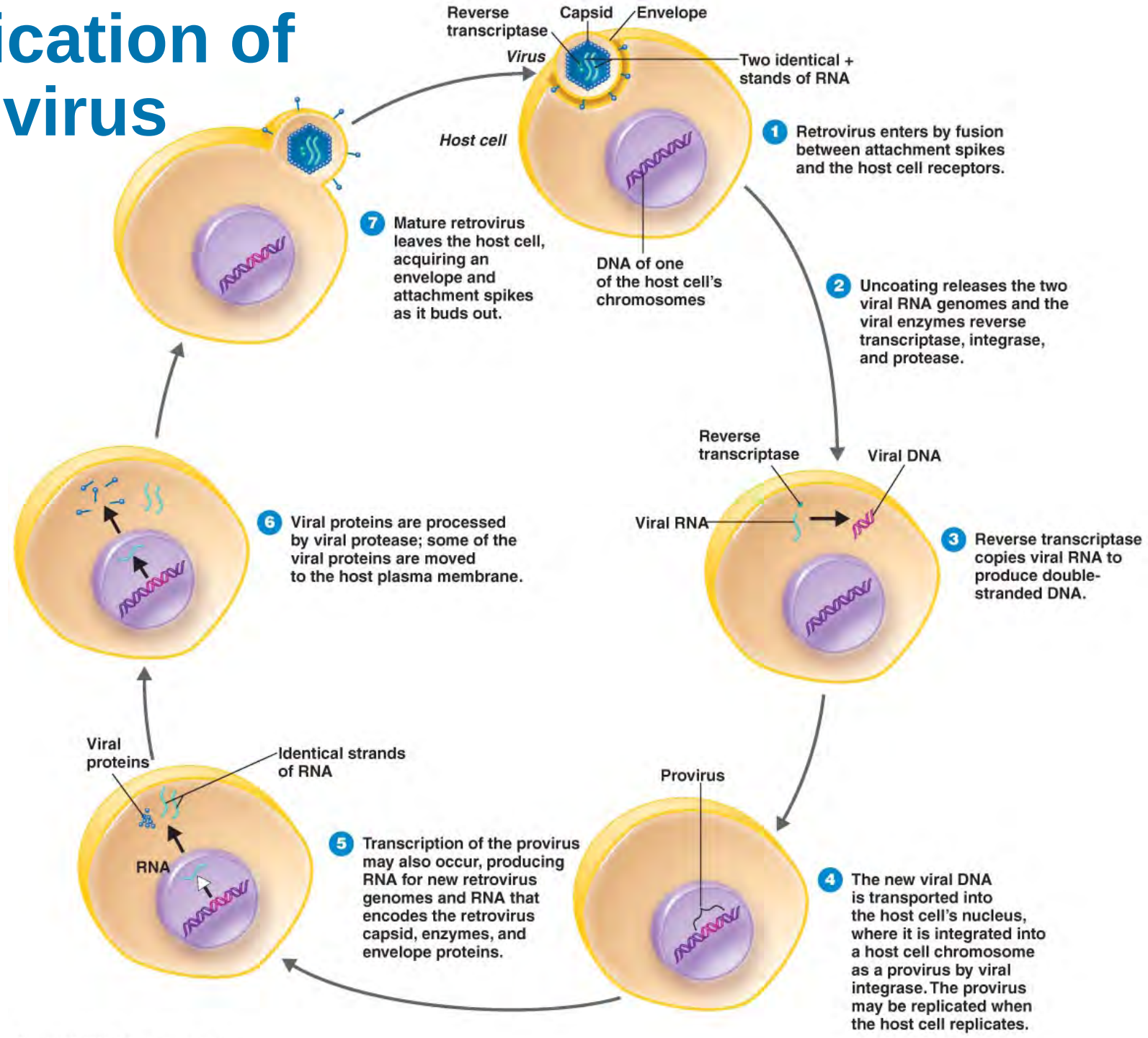


Fig 13.19

Cancer

Cancer → uncontrolled mitotic divisions

Benign vs. **malignant** tumors

Oncology

3 important characteristics of cancer cells:

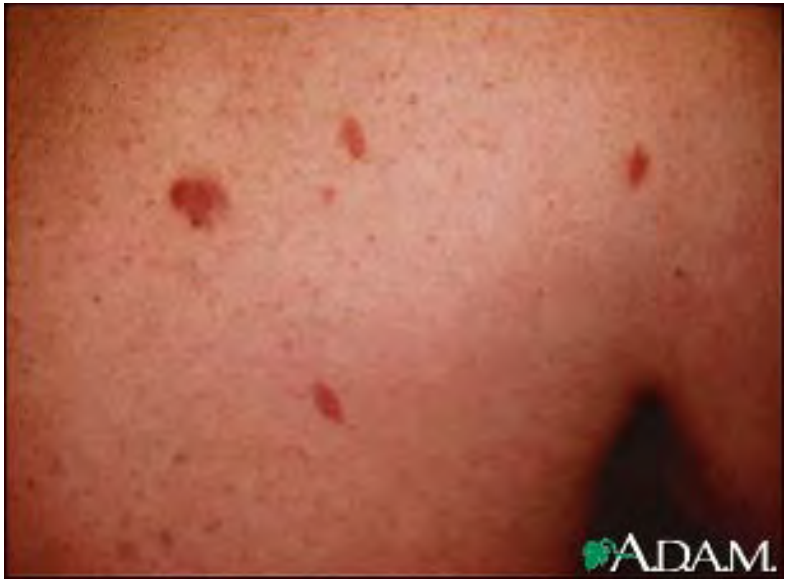
1. Rapid cell division
2. Loss of anchoring junctions and contact inhibition → metastasis
3. Dedifferentiation of cells

Viruses and Cancer

- The genetic material of oncogenic viruses becomes integrated into the host cell's DNA (→provirus).
- Conversion of **proto-oncogenes** to **oncogenes**
- Activated oncogenes transform normal cells into cancerous cells
- **Transformed cells** have increased growth, loss of contact inhibition, **tumor-specific transplant antigens**, and **T antigens**
- Oncogenic Viruses are responsible for ~10 % of human cancers

Oncogenic DNA Viruses and RNA Viruses

- Papilloma virus (HPV) → cervical cancer
- Epstein-Barr virus (EBV) → Burkitt's lymphoma
- HV8 → Kaposi's sarcoma
- Hepatitis B virus (HBV) → liver cancer
- Hepatitis C virus (HCV) → liver cancer
- human T-cell leukemia virus (HTLV-1)



Latent and Persistent Viral Infections

Latent:

Virus remains in asymptomatic host cell for long periods

Persistent:

Disease processes occurs over a long period; generally is fatal