Nutrition, Growth and Metabolism of Bacteria

Nutritional Requirement of Bacteria

- Bacteria needs of nutrients and obtain from their environment.
- The essential nutritional requirements of bacteria include:
  - Chemical requirements and
  - Physical requirements

- **nutrients** -the chemicals and elements that are utilized for bacterial growth
  - consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn
  - are found in the form of water, inorganic ions, small molecules, and macromolecules
  - serve either a structural or functional role in the cells

1. Carbon and Energy source for bacterial growth

Nutritional Type	Energy Source	Carbon Source	Examples
Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
Chemoautotrophs or Lithotrophs (Lithoautotrophs)	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	CO_2	A few Bacteria and many Archaea
Chemoheterotrophs or Heterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

- 2. Nitrogen - obtained from
  - *inorganic* source
    - e.g. Nitrogen gas (N_2), Nitrate (NO_3), Nitrite(NO_2), and Ammonia (NH_3)
  - *organic* source
    - e.g. Proteins, broken down to amino acids
- Phosphorus and sulphur for synthesis of ATP, DNA, RNA and co-enzymes

3. Inorganic ions (trace elements) are metal ions required by certain cells in such small amounts

- usually act as cofactors for essential enzymatic reactions in the cell.

–K, Ca, Mg, Fe, Co, Mn, Mo, Zn

4. **growth factors-** are essential substances that the organism is unable to synthesize from available nutrients

Vitamins, amino acids, purine or pyrimidine bases, heme, meat, yeast extract, milk protein hydrolysates, blood, grow in egg yolks, etc.

Physical and Environmental Requirements

- Depending on the O_2 requirement, bacteria are divided into :

1. **Strict (Obligate) Aerobes** – require O_2 for growth e.g. *Pseudomonas aeruginosa*

2. **Strict (Obligate) Anaerobes** – grow in the absence of O_2 & may even die on exposure to O_2 e.g. *Bacteroides fragilis*

3. **Microaerophilic** – grow best in the presence of low oxygen levels
   e.g. *Campylobacter* spp, *Helicobacter* spp

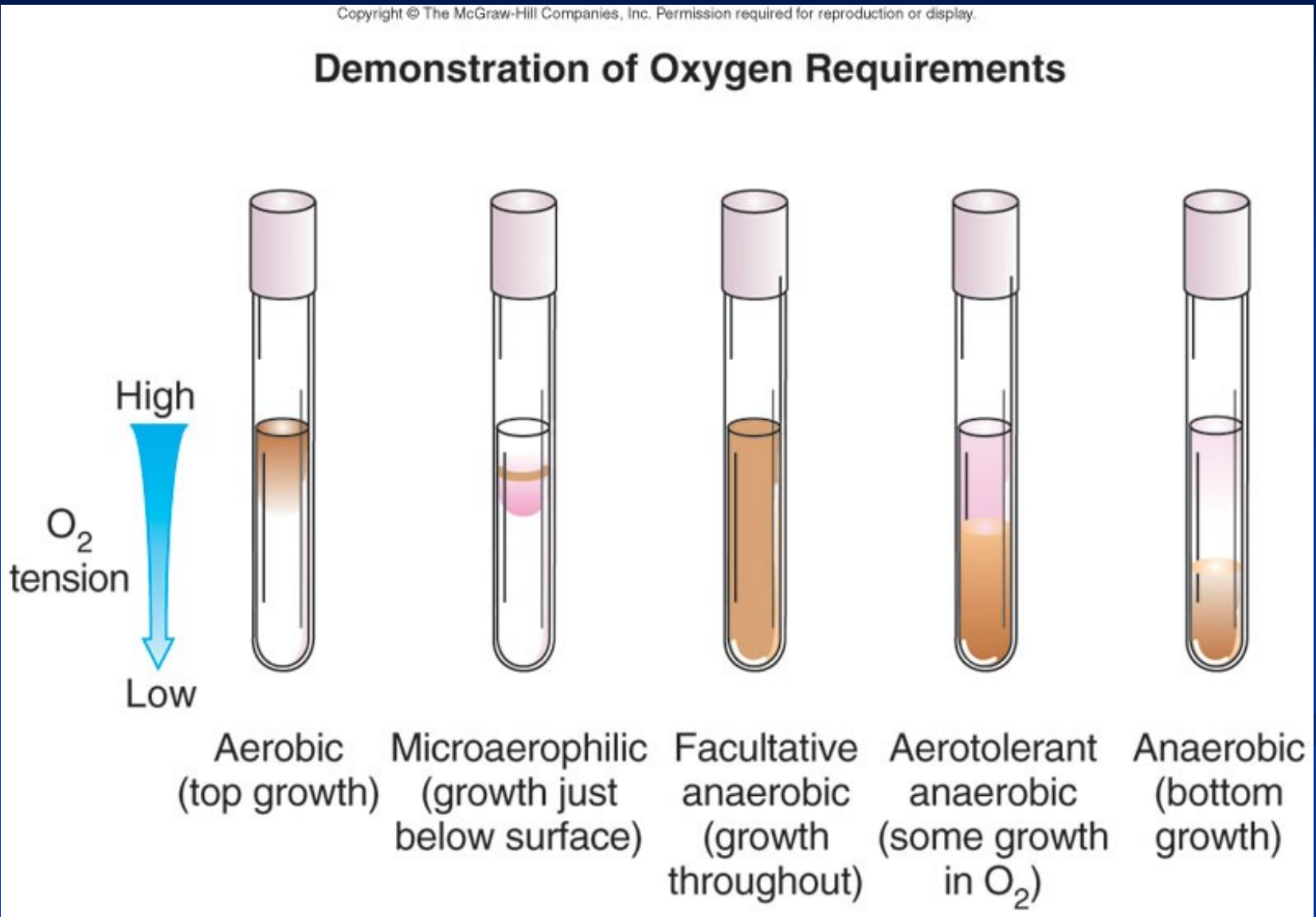
4. **Facultative anaerobe** – aerobic but can also grow in the absence of O_2
   e.g. *Staphylococcus* spps

5. **Aerotolerant anaerobe** – anaerobic, but tolerates exposure to O_2
   e.g. *Clostridium perfringens*

6. **Capnophilic organism** – requires high CO_2 levels
   e.g. *Neisseria* spps

Oxygen requirements

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Temperature

- Mesophilic – grows best between 25ºC and 40ºC.
  e.g. most bacterial pathogens

- Psychrophilic (cold loving) – grows best below 20ºC
  e.g. Flavobacterium spps

- Thermophilic – grows best at high temp, 55- 58ºC
  e.g. Bacillus stereothermophilus

PH

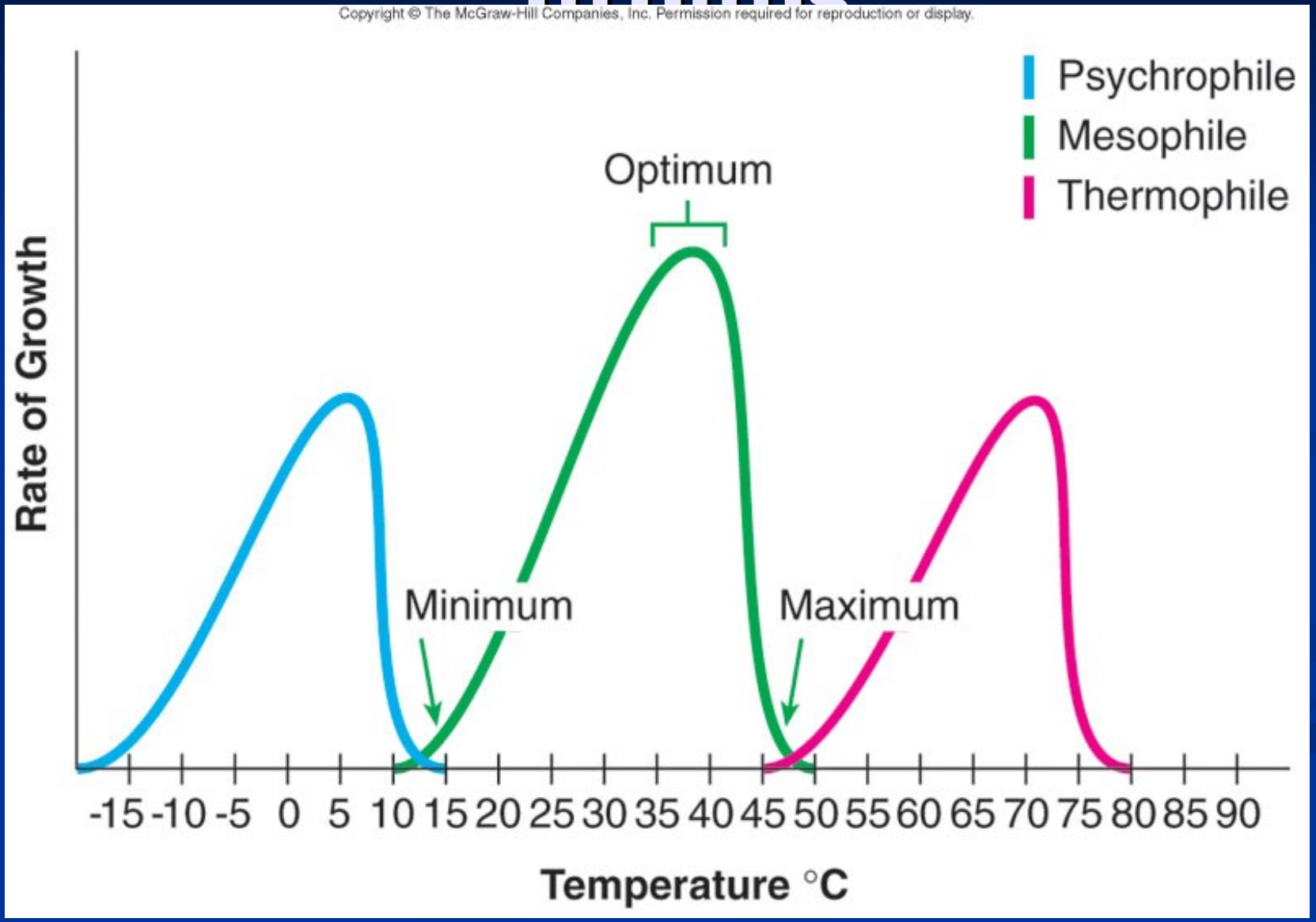
- Neutral or slightly alkaline pH (7.2 – 7.6) – majority of pathogenic bacteria grow best.

- Lactobacilli – acidic pH

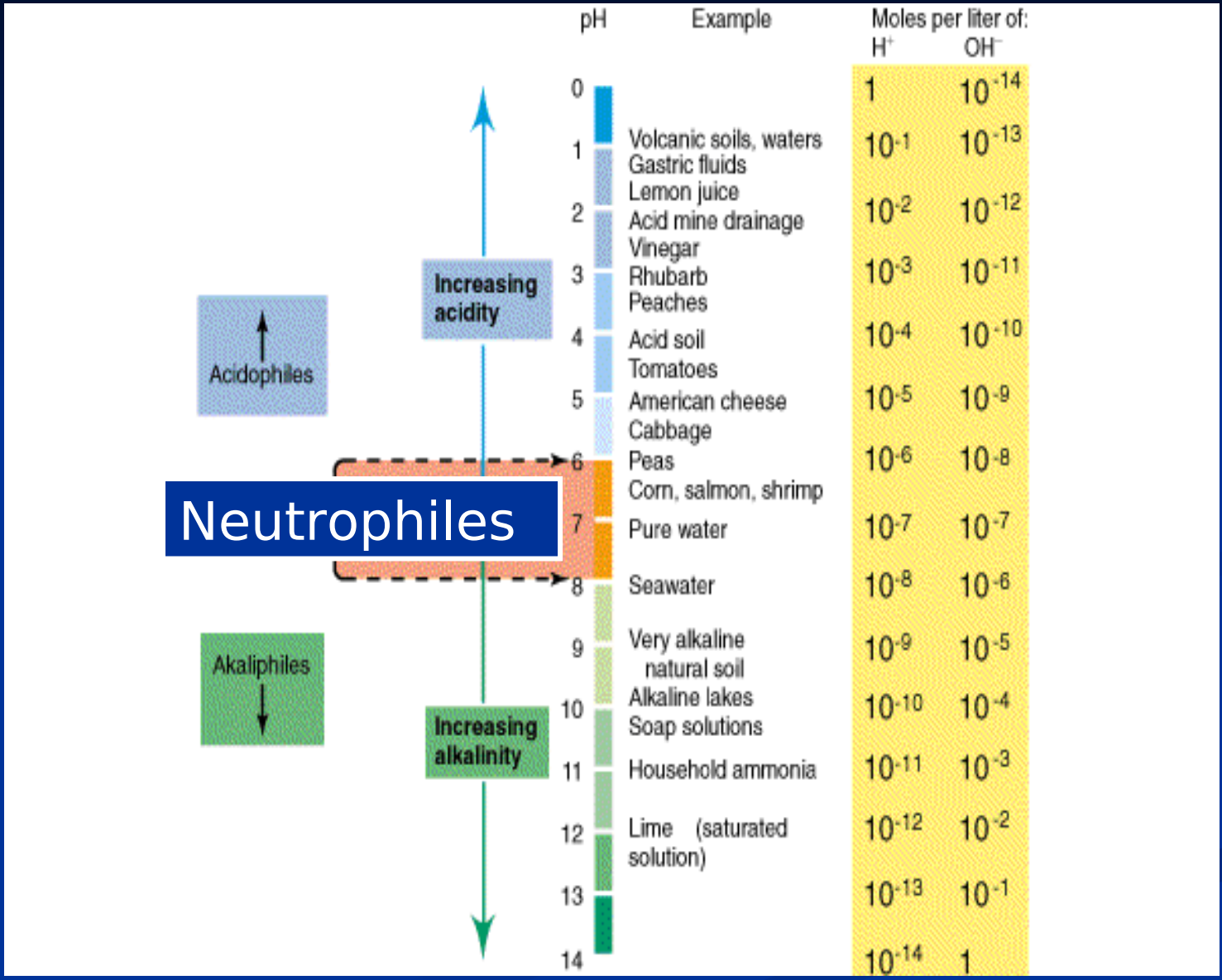
- *Vibrio cholerae* – alkaline pH

Water – essential ingredient of bacterial protoplasm. Hence drying is lethal to cells

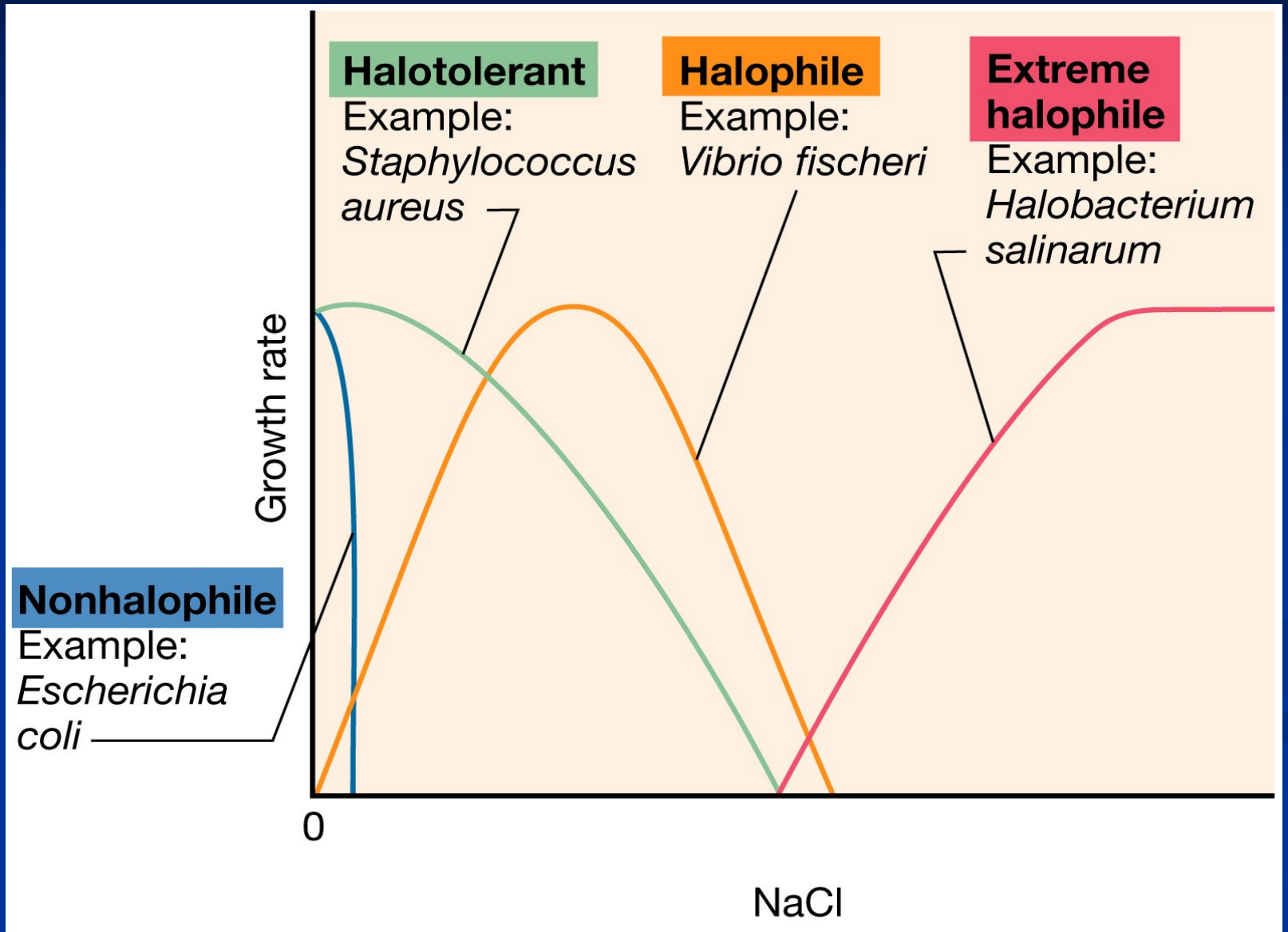
Temperature adaptation groups



pH Optima


pH
Example
Moles per liter of:
H+
OH-
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Volcanic soils, waters
Gastric fluids
Lemon juice
Acid mine drainage
Vinegar
Rhubarb
Peaches
Acid soil
Tomatoes
American cheese
Cabbage
Peas
Corn, salmon, shrimp
Pure water
Seawater
Very alkaline natural soil
Alkaline lakes
Soap solutions
Household ammonia
Lime (saturated solution)
1
10-14
10-1
10-13
10-2
10-12
10-3
10-11
10-4
10-10
10-5
10-9
10-6
10-8
10-7
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10-6
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10-4
10-11
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1
Increasing acidity
Acidophiles
Neutrophiles
Akaliphiles
Increasing alkalinity

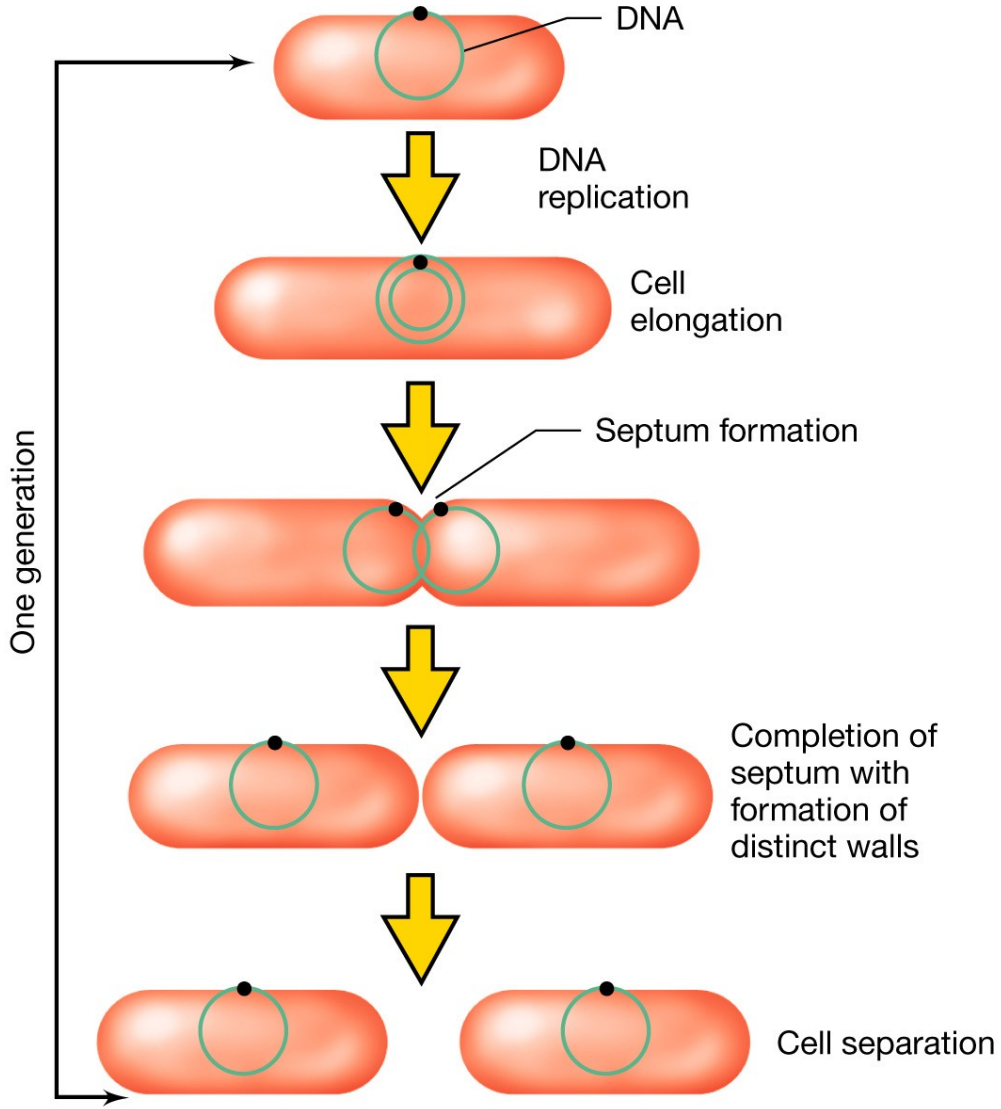
Effect of salt on growth



Bacterial Growth

- It is an increase in all the cell components, which ends in multiplication of cell leading to an increase in population.
- It involves - an increase in the size of the cell & an increase in the number of individual cells.
- Bacteria divide by binary fission.
  - forming two equal size progeny
  - genetically identical offspring
  - cells divide in a geometric progression doubling cell number

Bacterial Growth – binary fission



Generation time

- Interval of time between two cell divisions

  OR

- The time required for a bacterium to give rise to 2 daughter cells under optimum conditions

- Also called population doubling time.

Table 2. Generation times for some common bacteria under optimal conditions of growth.

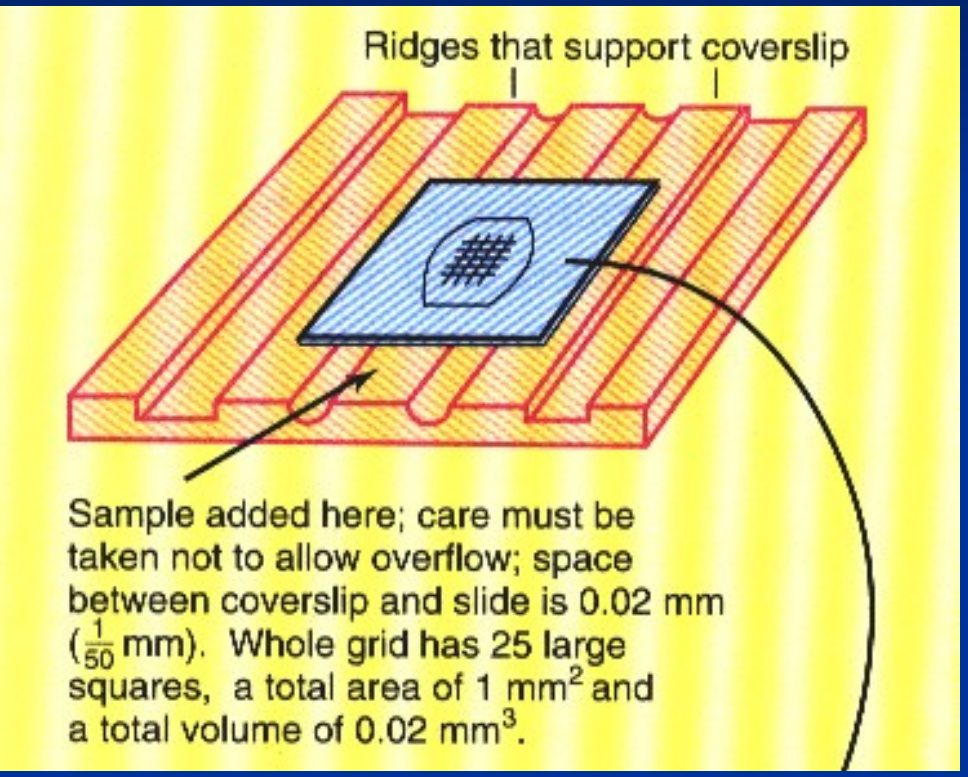
Bacterium	Medium	Generation Time (minutes)
Escherichia coli	Glucose-salts	17
Bacillus megaterium	Sucrose-salts	25
Streptococcus lactis	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast extract	344-461
Mycobacterium tuberculosis	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

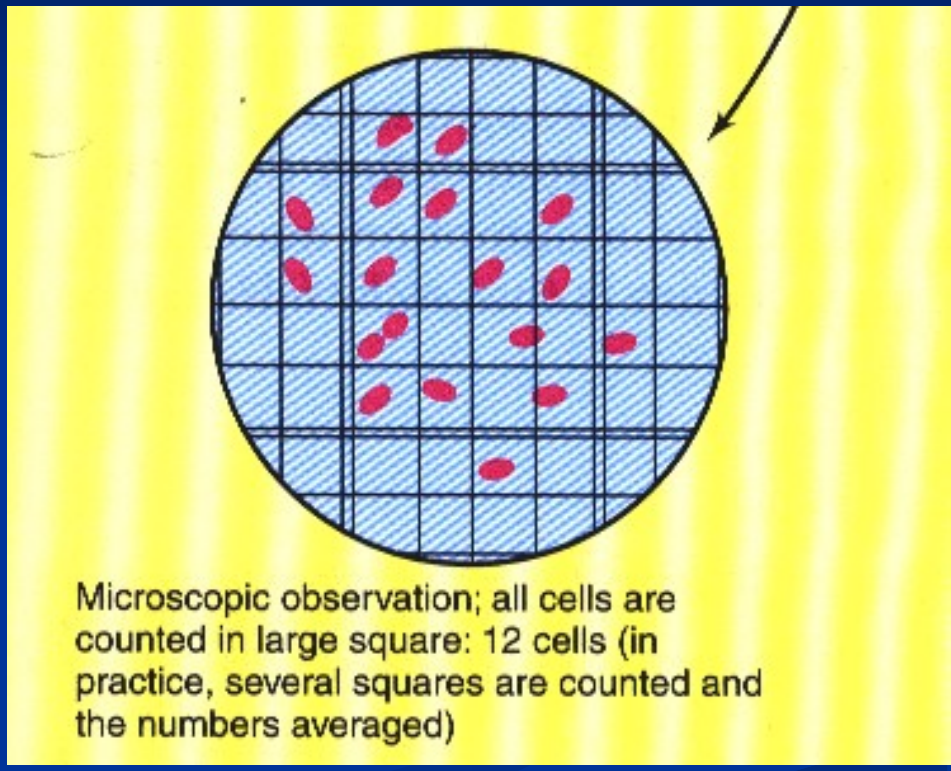
Methods used for the measurement of Growth

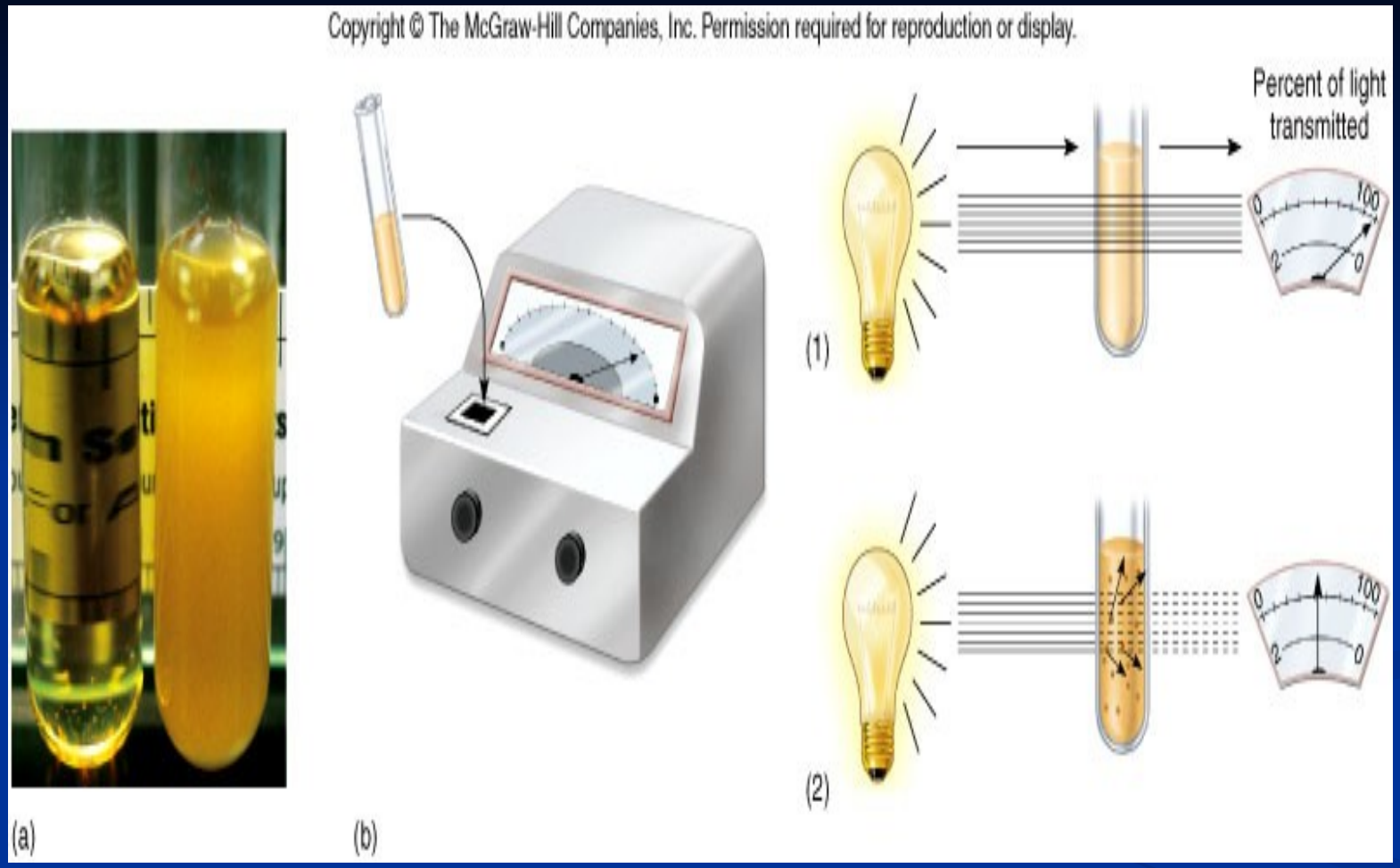
It includes various methods like:

- Cell count: This method is done by using microscope and caliberated slide
- Cell mass: measuring total weight of population of cells, which gives the direct relationship of total cells,
- Cell density: This can be known by the formation of turbidity in the liquid media. As the number of cells increases the turbidity in the media increases, which can be detected by a device called Spectrophotometer.
- Metabolic activity: This is done for the rapid detection of microorganisms, by detecting the ATP and CO_2 produced by bacteria

DIRECT COUNT







Turbidimetric measurements as indicators of bacterial growth.
The greater the turbidity the larger the population density.

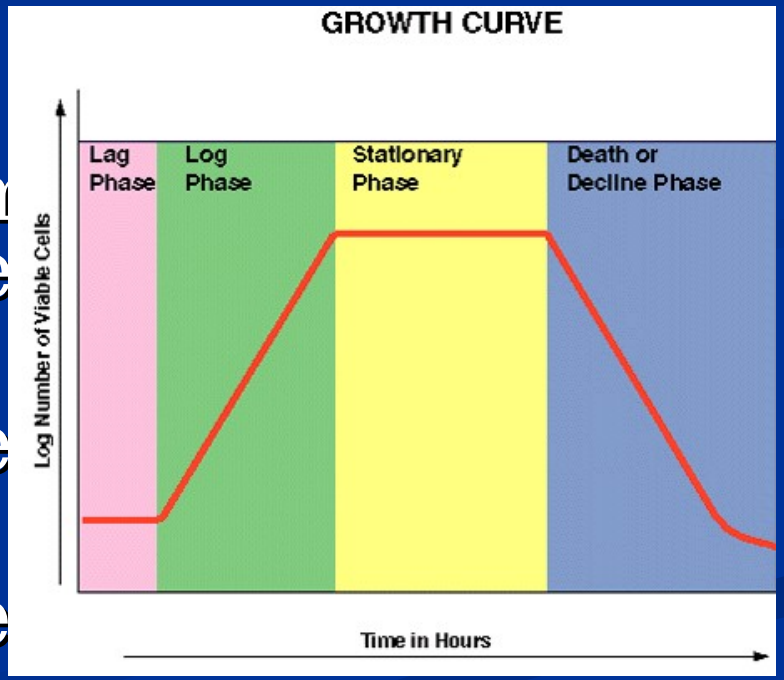
Bacterial Growth Curve

- When a bacterium is added to a suitable liquid medium & incubated, its growth follows a definite course.

- If bacteria counts are made at intervals after inoculation & plotted in relation to time, a growth curve is obtained.

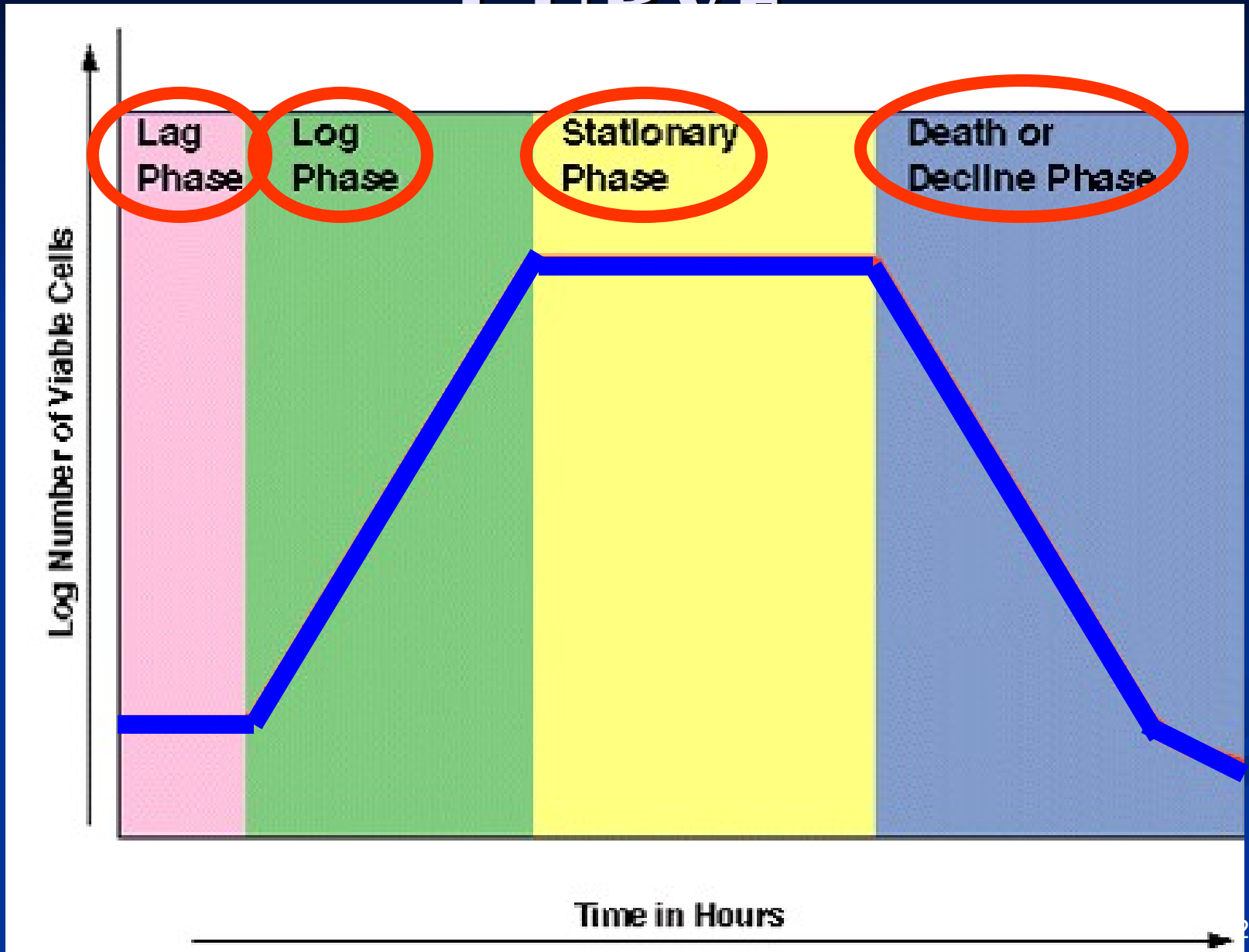
BACTERIAL GROWTH CURVE

- Growth of culture goes through four phases with time
- 1) Lag phase
- 2) Log or Logarithm phase
- 3) Stationary phase
- 4) Death or Decline phase



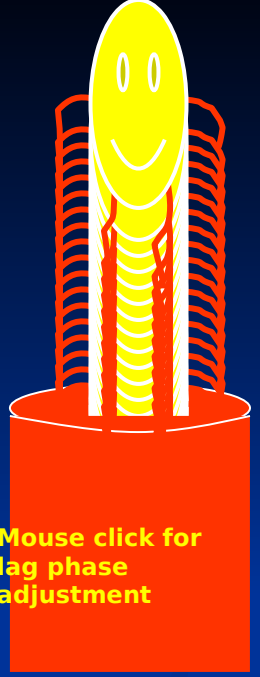
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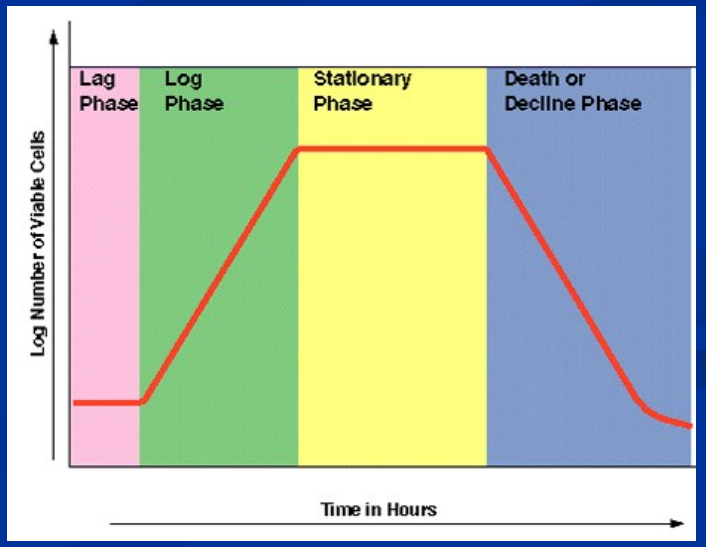
BACTERIAL GROWTH CURVE



LAG PHASE

- **Lag (preparatory) phase**
- Adjustment period to the new environment
- High cellular metabolism
- Rapid biosynthesis of macromolecules and enzymes
- Primary metabolites are essential for growth
- maximum cell size towards the end of lag phase.
- The length of lag phase depends upon:
  - Type of bacteria, and medium used.
  - The phase of the culture from which inoculation is taken.
  - Size of inoculums
  - Environmental factors like temperature

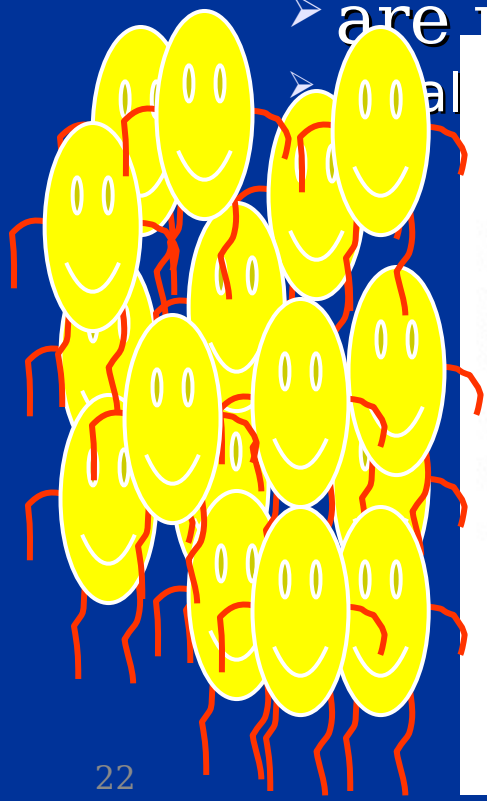


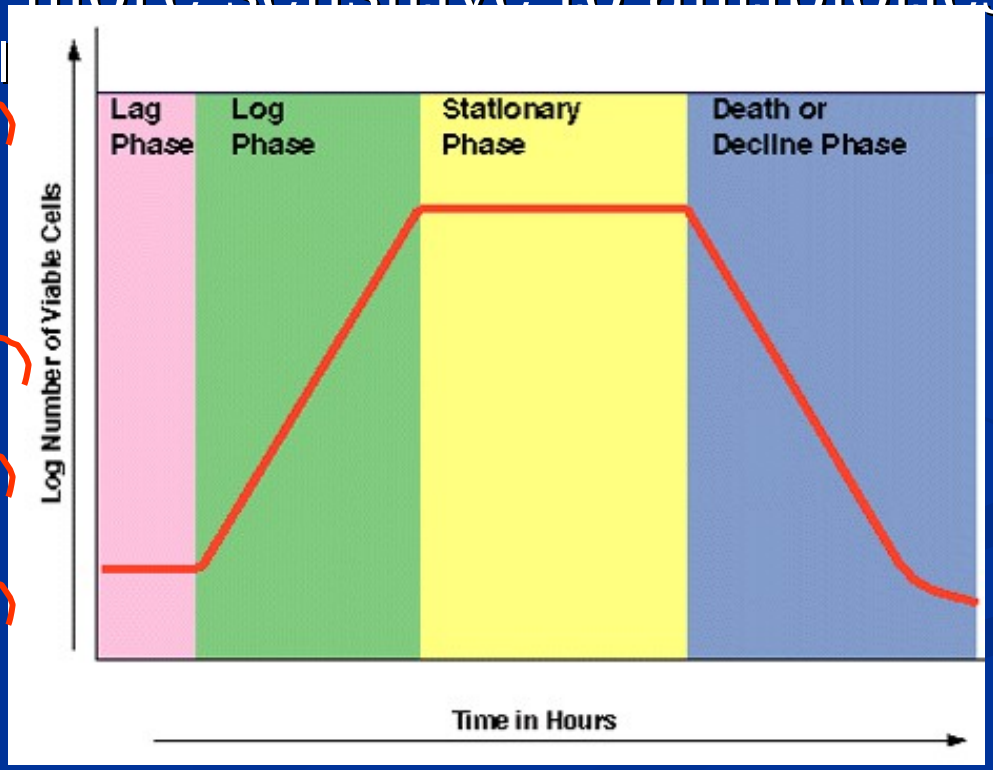


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LOGARITHMIC PHASE

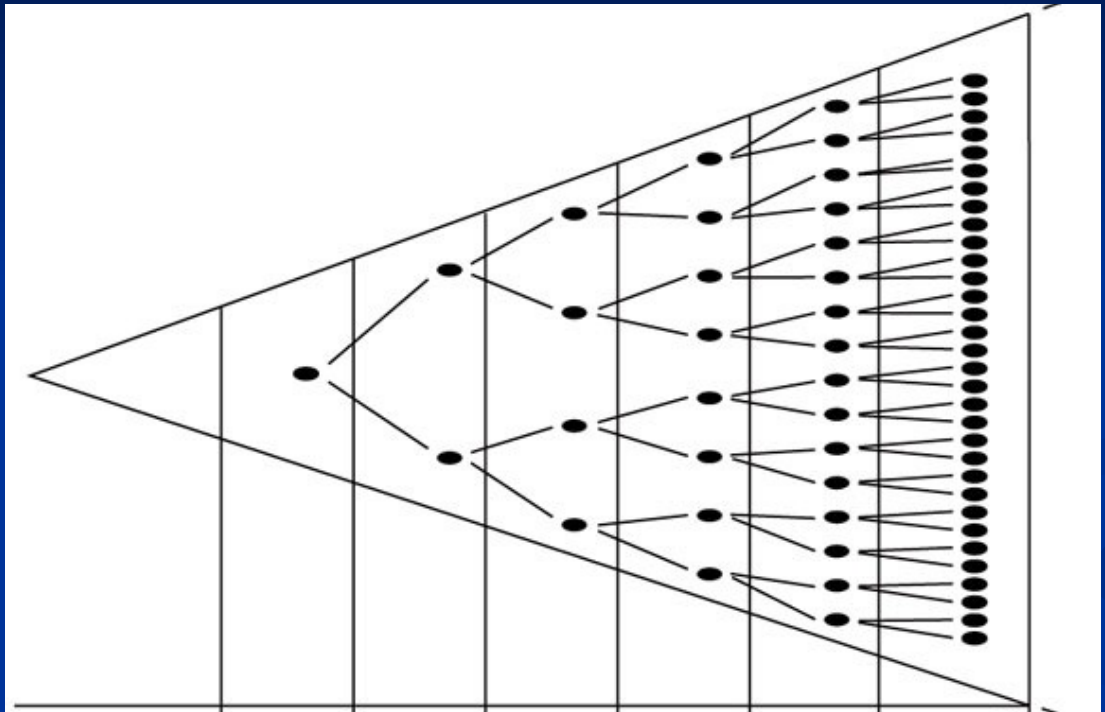
- Growth rate death rate
- Cell grow exponentially
- Maximum rate of cell division takes place
- are more sensitive to antibiotics
- al





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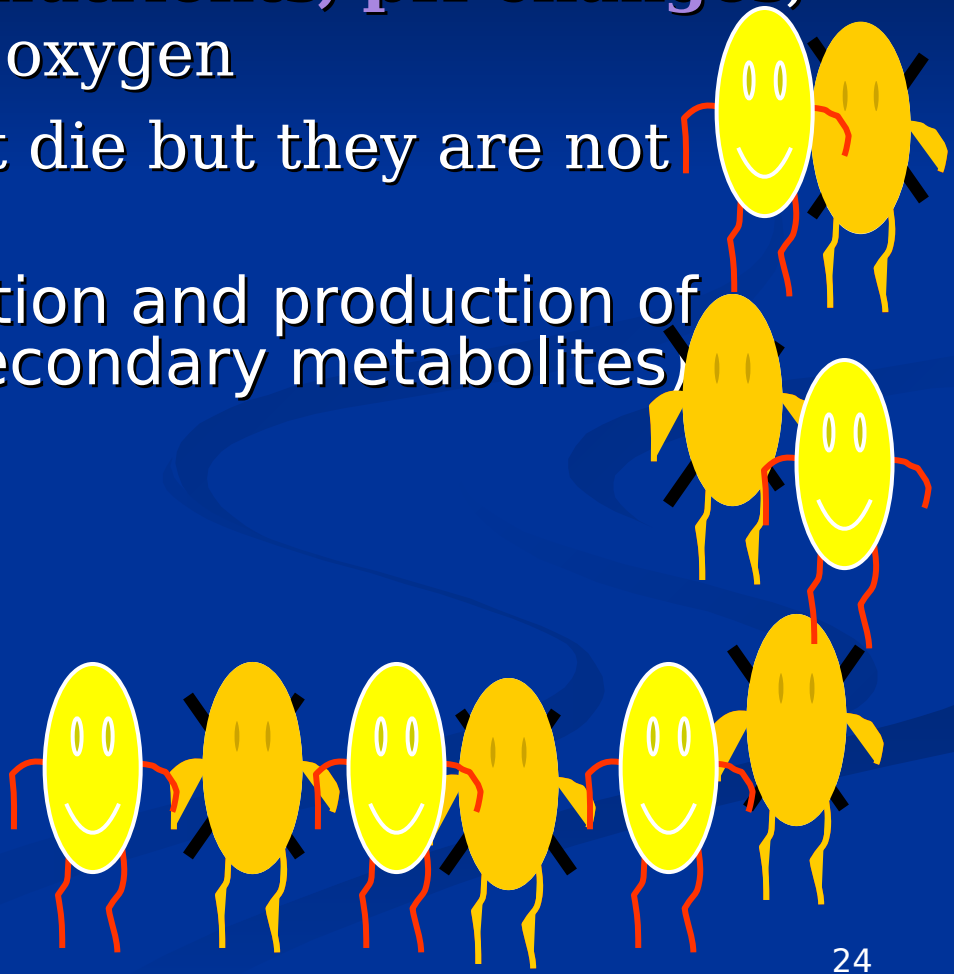
Exponential Growth



Number of cells	1	2	4	8	16	32
Number of generations		1	2	3	4	5
Exponential value		2^1 (2×1)	2^2 (2×2)	2^3 (2×2×2)	2^4 (2×2×2×2)	2^5 (2×2×2×2×2)

STATIONARY PHASE

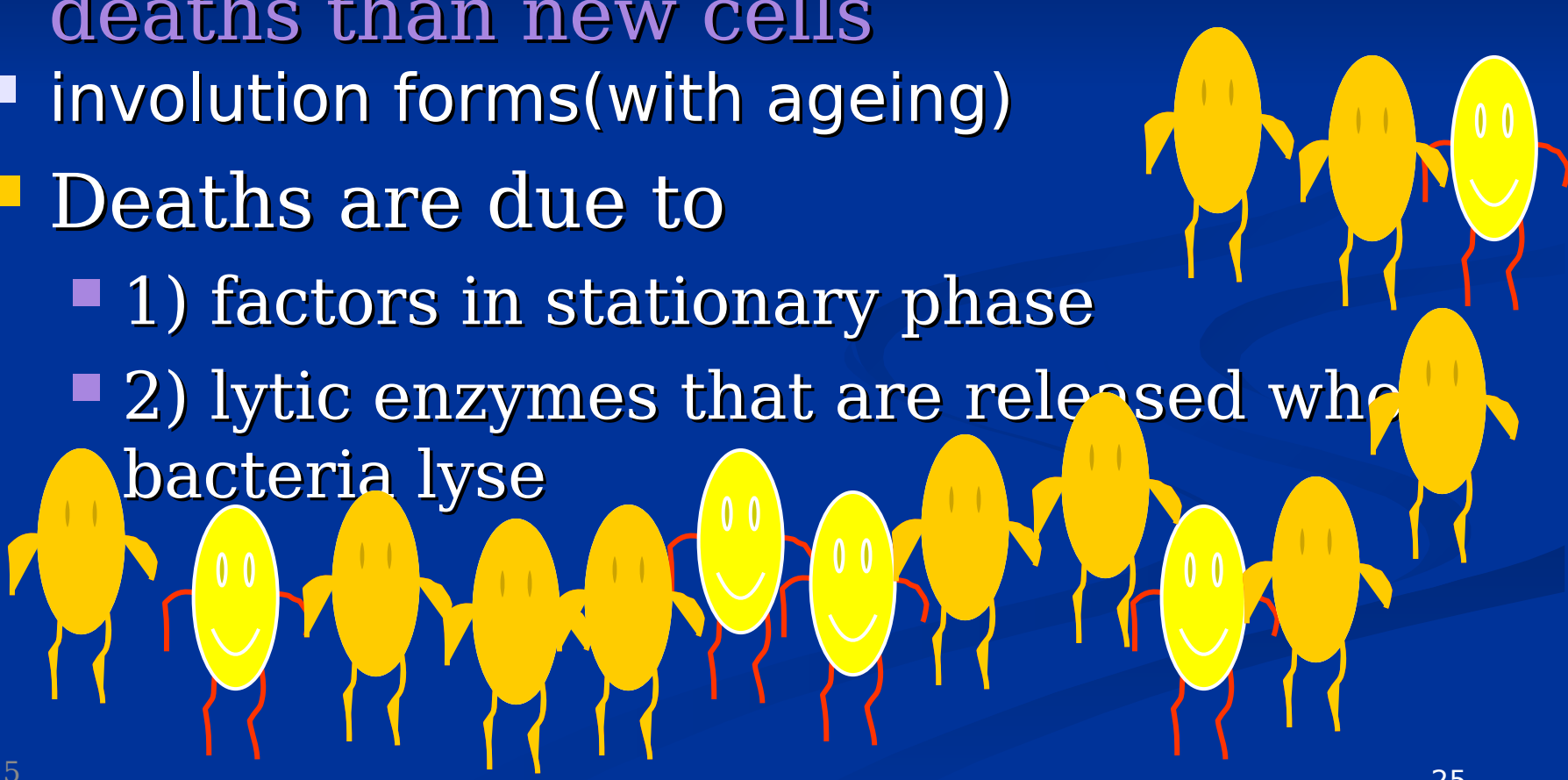
- Dying and dividing organisms are at an equilibrium (death rate = growth rate)
- Death is due to **reduced nutrients, pH changes**, **toxic waste** and reduced oxygen
- In some cases cells do not die but they are not multiplying
- irregular staining, sporulation and production of exotoxins & antibiotics (secondary metabolites)



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DEATH PHASE

- The population is dying in a geometric fashion so there are more deaths than new cells
- involution forms(with ageing)
- Deaths are due to
  - 1) factors in stationary phase
  - 2) lytic enzymes that are released when bacteria lyse



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Mutation & gene transfer

- “Acquiring genes through gene transfer provides new genetic information to microorganisms, which may allow them to survive changing environments.”
- “The major source of variation within a bacterial species is mutation.”
- **Mutations** are heritable changes in the structure of genes
- “In mutations, usually only a single gene changes at any one time.”

- “In contrast, gene transfer results in many genes being transferred simultaneously, giving the recipient cell much more additional genetic information.”

Mutation: Terms & concepts

- Wild Type refers to the microorganism as isolated from the wild (the normal, usually active, form of a gene)
- Auxotroph- a mutated microorganism that has lost a metabolic function
- The **wild-type** parent to an auxotroph is called a **Prototroph**.
- A **Mutation** is found in a **gene;** a **mutant** is an **organism** harboring a Mutation.
- Mutants can be spontaneous or induced by a Mutagen; an agent that causes DNA to mutate.

Types of Mutation

- **Point mutation** = single base is substituted.
- **Missense mutation** = base change changes single amino acid to different amino acid.
- **Nonsense mutation** = base change changes single amino acid to stop codon.
- **Deletion or insertion mutation** = change in number of bases making up a gene.
- **Frameshift mutation** = insertion or deletion of something other than multiples of three bases.
- **Reversion mutation** = mutated change back to that of wild type.
- **Silent mutation** – alters a base but does not change the amino acid

Genetic recombination and Transfer

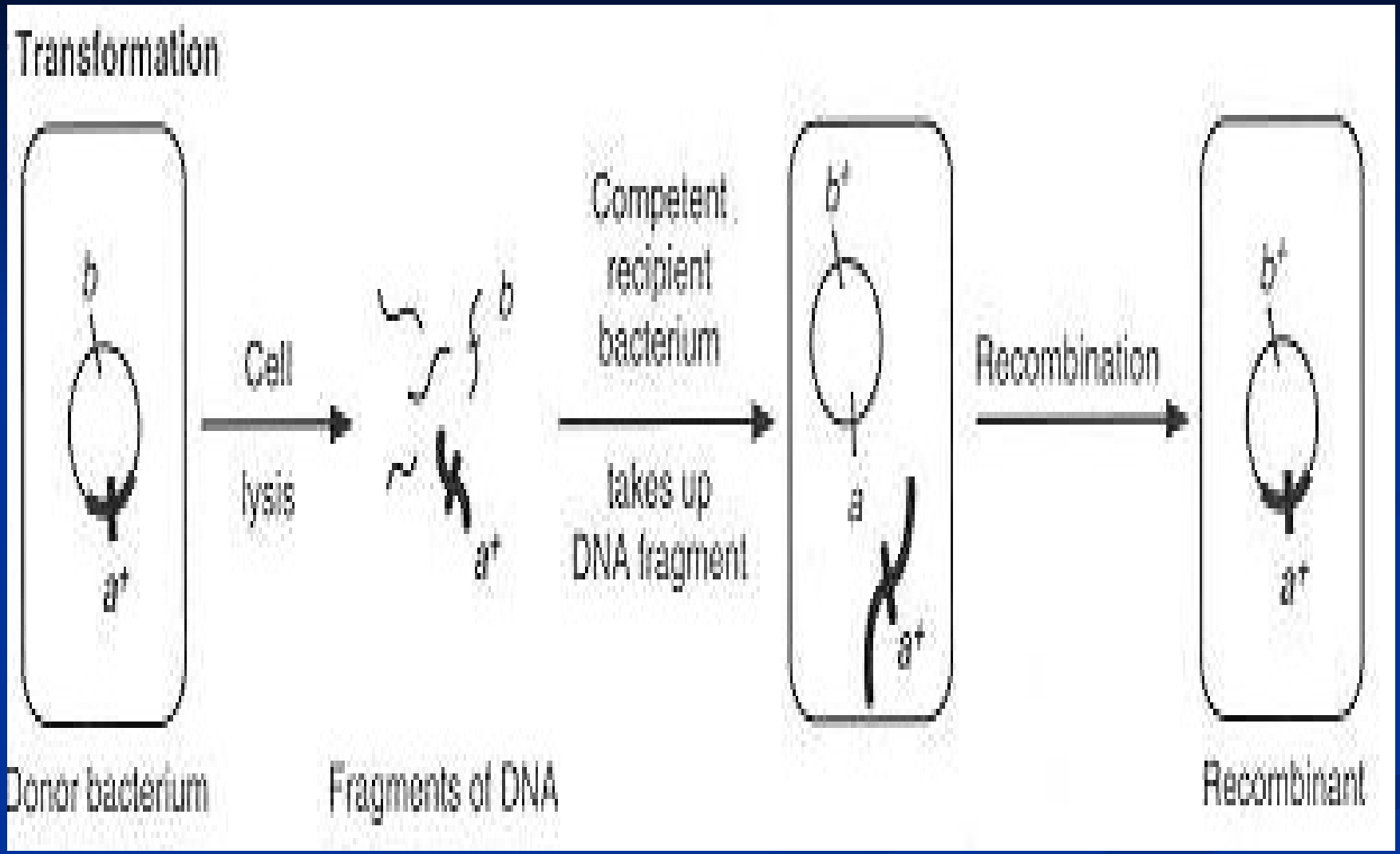
- Genetic recombination occurs when genes are exchanged between two different organisms to form the same or closely related species
  - Three methods for recombination in bacteria

conjugation	requires the attachment of two related species & formation of a bridge that can transport DNA
transformation	transfer of naked DNA
transduction	DNA transfer mediated by bacterial virus

Transformation –

- When a bacterial cell dies, the cell will lysis out and the DNA is be released into the environment
- A recipient cell can take up some of the DNA from the environment
- A cell has to be **competent** to take up some of the DNA from the environment
- A cell can become competent by altering it's cell wall and cytoplasmic membrane
- Transformation is common among many pathogens e.g E. coli

fig. 5

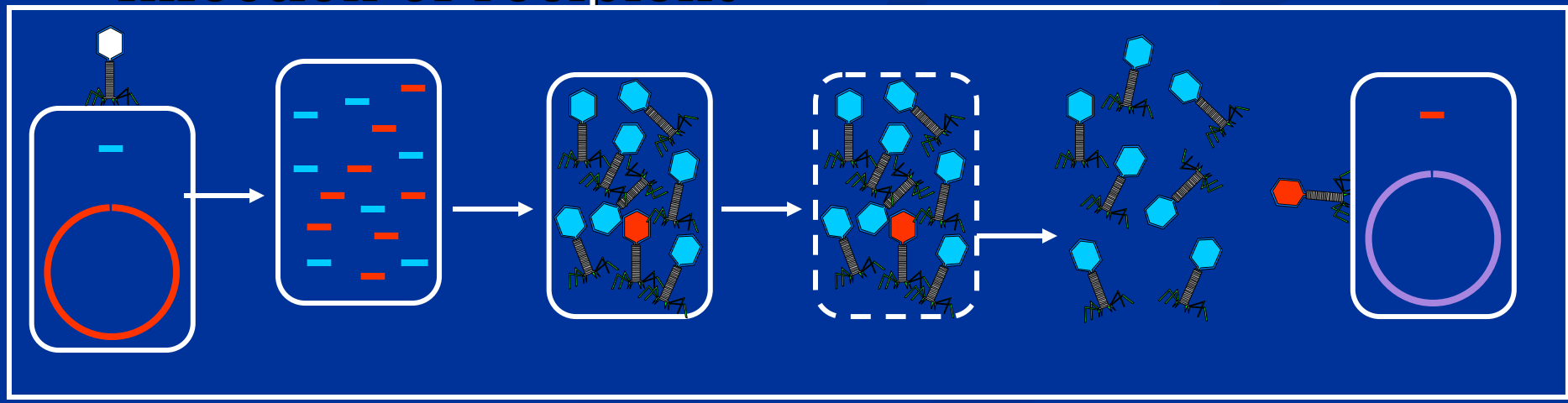

Transformation
b
a+
Donor bacterium
Cell
lysis
b
a+
Fragments of DNA
Competent
recipient
bacterium
takes up
DNA fragment
b+
a
a+
Recombination
b+
a+
Recombinant

Transduction

- is virus-mediated (bacteriophages) transfer of genetic information from donor to recipient cell
- Types of transduction
  - Generalized Transduction : in which potentially any donor bacterial gene can be transferred
  - Specialized Transduction : in which only certain donor genes can be transferred

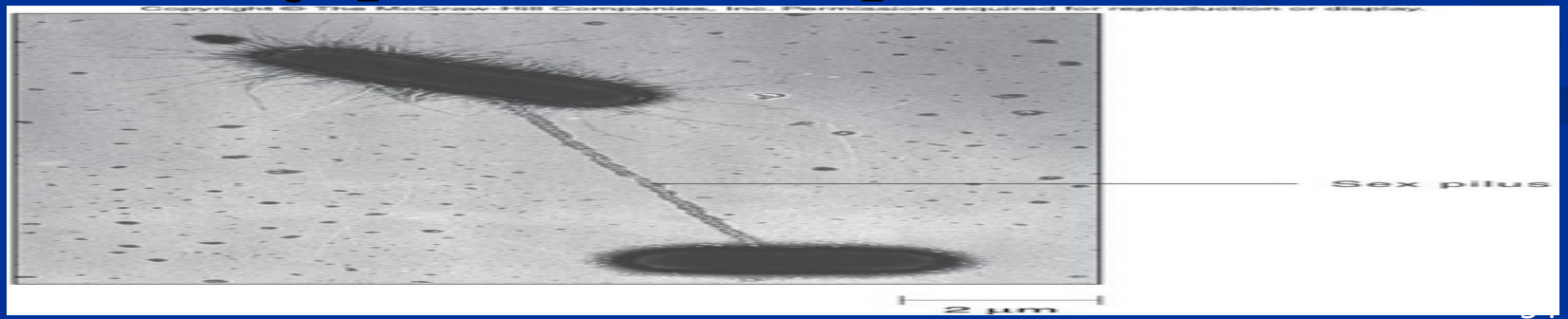
Transduction

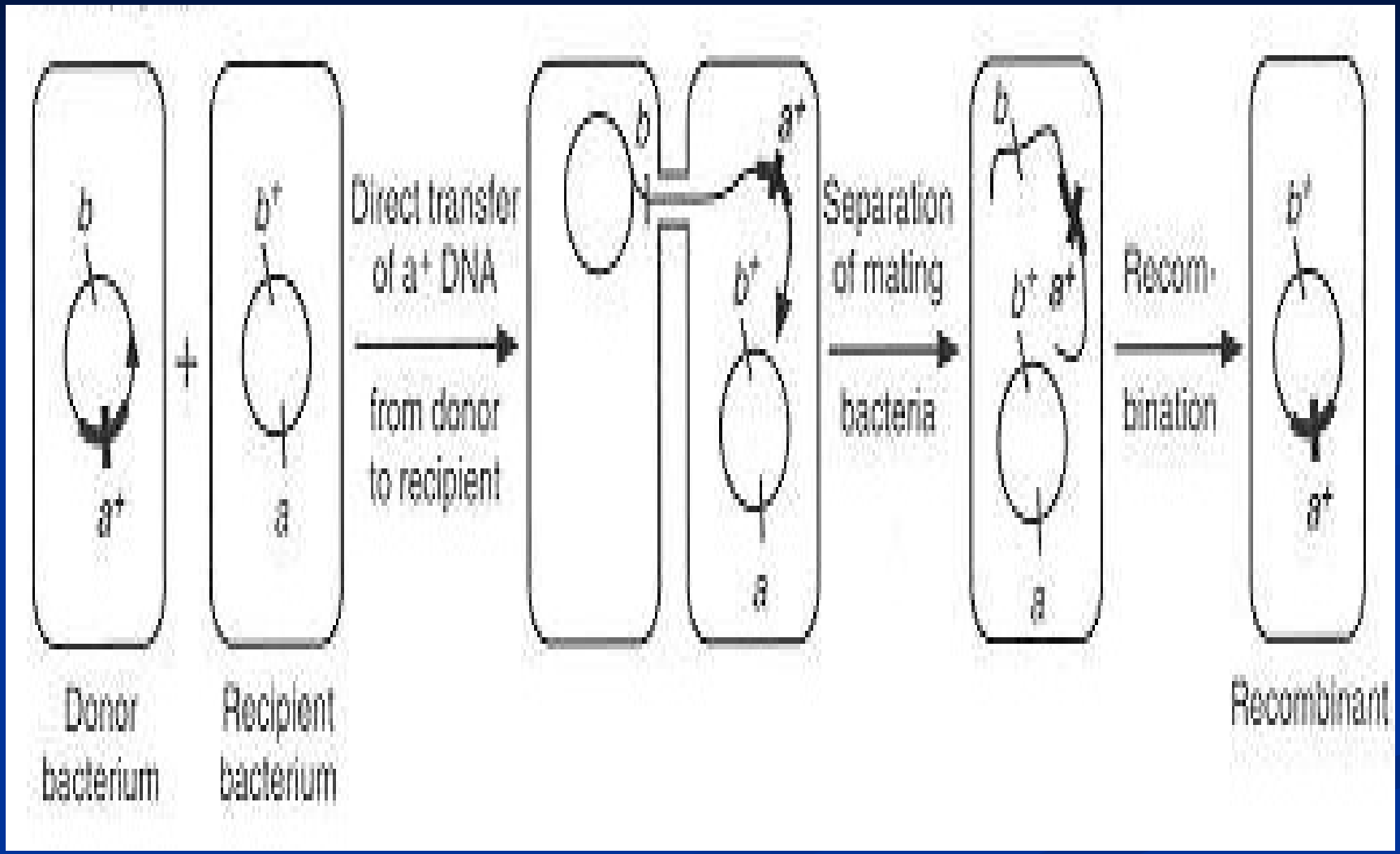
- Infection of donor
- Phage replication and degradation of host DNA
- Assembly of phages particles
- Release of phage
- Infection of recipient



Conjugation

- is the transfer of part or all of genetic information from donor to recipient bacterial cell in a process that requires intimate cell contact
- Donor ability is determined by specific conjugative plasmids called fertility plasmids (F plasmid) or sex




b
a+
b+
a
Direct transfer of a+ DNA from donor to recipient
b
a+
b+
a
Separation of mating bacteria
b
b+
a+
a
Recom-bination
b+
a+
Donor bacterium
Recipient bacterium
Recombinant

Basic Bacteriological Techniques

Specimen collection, storage and transportation

- **Types of specimen**
- Specimen includes:
  - **Cerebrospinal fluid (CSF)**
  - **Feces**
  - **Sputum**
  - **Swabs of Various Fluids**
  - **Blood**
  - **Urine, etc**

Smear preparation and stains used in Bacteriology

- Staining help:-
  - To make bacteria more visible by dark staining in contrast with the bright back ground.
  - It make cellular and group morphology of bacteria more visible (shape and arrangement)
  - It makes structural parts of bacteria more visible such as flagella ,capsule, spore etc
- A stain or dye is a molecule, that can bind to a cellular structure and give it color

Commonly used staining methods

A) Simple staining

- All the cells stain uniformly which is useful to show the size shape and arrangement of bacterial cells
- The common dyes used for simple staining are:
  - Methylene blue (0.3%)for a minute
  - Carbon fuchisin (0.3%) for 15 to 30 seconds
  - Crystal Violet (2.0%) for 50 to 60 seconds

B) Special stains:- these are specialized staining methods to demonstrate certain bacterial components e.g. spore, capsule, etc

C) Differential Staining

- Involves the application of more than one dye to the air dried and heat fixed smears
- Brings difference between bacterial cells e.g. Gram staining & Acid fast staining

The gram's stain

- ✓ Divide the bacteria into two (gram +ve and gram – ve)
- ✓ Four stains used – CV, KI ,Alcohol, Safranin

Acid fast stain

- Developed by France Ziehl and Friedrich Neelsen
- Acid fastness is a property of the *Mycobacterium tuberculosis* and related organisms (Cryptosporidium)
- Carbolfuchisin is a primary dye and Methylene blue is a counter stain
- Acid-fast bacteria take stains poorly but once stained retain it strongly
- They are not easily decolourised by acid-alcohol
- The acid fastness is due to the high lipid content (mycolic acid) in the cell wall
- Acid fast stain organism appear – red and non acid fast stain organism appear- blue

Procedure for gram staining

1) Place the air dried and fixed smear on a staining rack and apply crystal (gentian) violet. Allow to act for one minute and wash with clean water.
2) Apply Gram `s iodine (lugol`s iodine) solution. Allow it for 30-60 seconds and wash with clean water(tape water can be used)
3) Apply decolorized (95% ethanol). Allow to act for 15 to 30 seconds. Then wash with clean water. Time of decolonization could be prolonged up to one minute for thick smears to decolorize it effectively.
4) Apply safranin (counter stain). Allow to act for 30-60 seconds and wash with clean water. Air dried the slides or using blotting papers. The slide is now ready for examination. Use the oil immersion objective and examine all the elements of the smear changing your field until you exhaust the whole area of the smear.

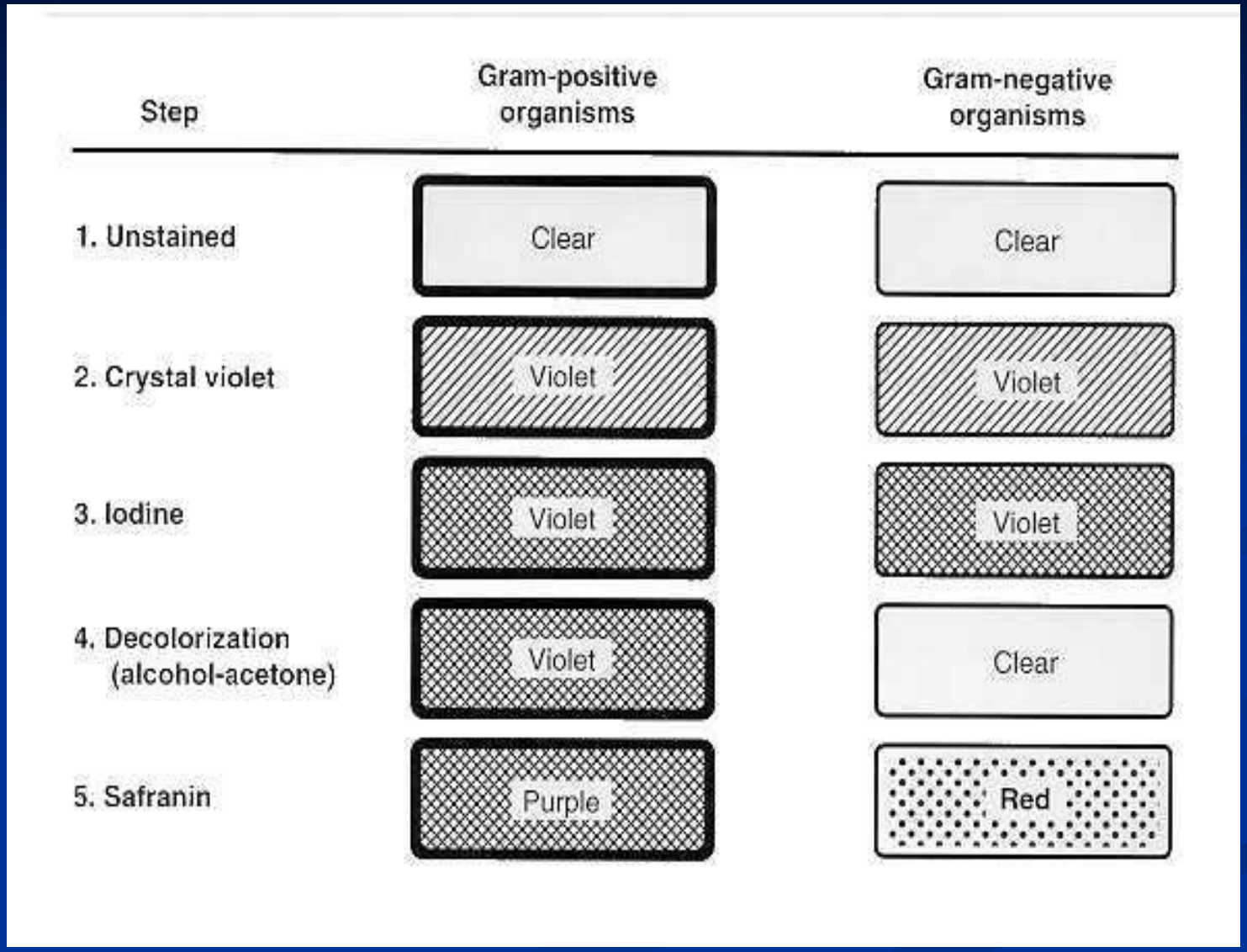
Report of gram stained smear includes

- The number of bacteria present (many ,moderate few, scanty)
- The gram reaction of bacteria (Gram positive or gram negative)
- The morphology of bacteria (cocci ,diplococcic, streptococci, rods or coccobacilli) and other
- Location of the bacteria (as extracellular or intracellular)
- The presence and number of pus cells
- The presence of yeast cells and epithelial cells

Note:* During gram staining the following elements are gram negative in their reaction(red or pink color)

  - Gram negative bacteria
  - Nuclei of pus cells
  - Epithelial cells

- Yeast cells and gram positive bacteria are gram positive during gram staining(blue color)


Step
Gram-positive organisms
Gram-negative organisms
1. Unstained
Clear
Clear
2. Crystal violet
Violet
Violet
3. Iodine
Violet
Violet
4. Decolorization (alcohol-acetone)
Violet
Clear
5. Safranin
Purple
Red

Procedure for acid fast staining

1) Place the air dried and fixed sputum smear on a staining rack and cover the slide with basic fuchsin
2) Flame cotton soaked in alcohol and heats the slide from below till you see a steam coming out of the slides use low and intermittent heat to maintain steaming for 5 minutes
3) Cool and wash with distilled water until a clean water runs off
4) Decolorize by applying 3% Hcl in 95% ethanol for 3 minutes if 3% Hcl is not available use 20% H_2SO_4 as follows. Apply the 20% H_2SO_4 for one minute and wash with water repeating this for ten minutes. Wash the slide well and apply 95% ethanol for 2 minutes and wash with water
5) Counter stain by applying methylene blue or malachite green (0.2%) for one minute. Wash with tape water for one minute continuously and drain off the water. Leave the slide to air dry. The slide is now ready for examination of using oil immersion objective

Culture Media

Media support the growth of microorganisms or cells in the laboratory

- culture media can be classified based on
  - **physical states**,
  - **functional properties**,
  - **chemical composition** etc

A. **Physical state**

I . Liquid media

✓ Do not contain any solidifying agent

e..g. nutrient broth, citrate broth, glucose broth, litmus milk etc

II. Solid media

✓ Contain solidifying agent such as agar, silica gel or gelatin

e. g. Nutrient agar

B. chemical compositions

I . Synthetic media

- Type of media in which all constituents are chemically defined
  e. g. Nutrient agar
- Used to study specific nutritional requirements of micro organisms

e. g. E. coli can grow on minimal media since it can synthesize the remaining nutrients

I I . Complex media

- type of media in which there is one or more nutrients that is chemically undefined
- Usually extracted from biological materials e. g. Beef extract, peptone (casein) extract, yeast extract
- Satisfy the growth of most microorganisms

I I I . **Natural media**

- Naturally available and used as growth media with out modification
  e .g. milk

C. Functional property

I . Simple media

- Allow the growth of all micro organisms e. g. nutrient agar

I I . **Differential media**

- Used to differentiate a specific micro organisms from a given mixed population

  e .g. Blood agar, Eosin methyline blue agar differentiate lactose fermenters from non lactose fermenters

III. Selective media

- Allow the growth of some bacteria and inhibit others

  e .g. Bismuth sulfite agar, bile salt etc

IV . **Enrichment medium**

Is used to enrich the required microbe

e.g. Thioglycholate, Kaufman broth

V . Transport medium

- Used to transport samples (urine, swab, sputum, blood) to laboratory
- It helps the organism to survive until used for diagnosis especially the pathogens

e. g. -Glycerol saline , Bile peptone , Cary- Blair

Storage Media

- Used for maintaining or keeping bacteria for long period of time
- Storage of bacteria can be maintained for long period by:
  - **Serial cultivation (continuous batch):-** continuous transferring of bacteria to new media
  - **Lyopholization**:- rapidly freezing and drying
  - **Deep freezing:-**cooling the media at about –70^0c

Biochemical or Enzymatic Tests

- Many bacteria can not be identified on the basis of microscopic or cultural characteristics alone
- Important biochemical reactions involve oxidation, fermentation, starch hydrolysis, hydrogen sulfide production, urea hydrolysis and tryptophan hydrolysis.
- In each biochemical change may be indicated by the formation of gas or by the formation of color

ANTIMICROBLAL AGENTS

- **Antimicrobial agents** refers to substances produced by a **microorganism** or to a similar substance produced fully or partially by **chemical synthesis**, which in therapeutic concentrations is **lethal** or **inhibits** the growth of other **microorganism**

E.g. sulfonamides

E.g. Penicillin,

Desirable properties of chemotherapeutic agents

1) **Selective toxicity**: refers to the inhibition of the pathogen without injury to the host cells
2) **Bactericidal** rather than bacteriostatic
3) The ideal chemotherapeutic agent is one to which susceptible organisms do not become genetically or phenotypically resistant
4) Effective against a large group of microorganisms commonly encountered in clinical practice
5) Should not be allergenic and combined administration of large doses should not cause adverse side effects
6) Water soluble, stable, and bacterial level in the body should be rapidly reached and maintained for long period

Mode of Action of Antibiotics

- Mechanism of action includes:

1) **Inhibition of synthesis of cell wall/ peptidoglycan/**: E.g. β-lactam antibiotics (penicillins, cephalosporines) and vancomycin.

Bacterial species lacking cell wall such as mycoplasma are not inhibited by these agents

2) **Damage to the permeability of the cytoplasmic membrane**: E.g. polymyxin B, nystatin and other antifungal agents.

3) **Inhibition of proteins synthesis**
   - **Inhibition of transcription**: e.g actionomycin, rifampicin
   - **Inhibition of translation:** e.g aminoglycosides, tetracyclines

4) **Inhibition of nucleic acid synthesis**

e.g. Rifampin

The Action of

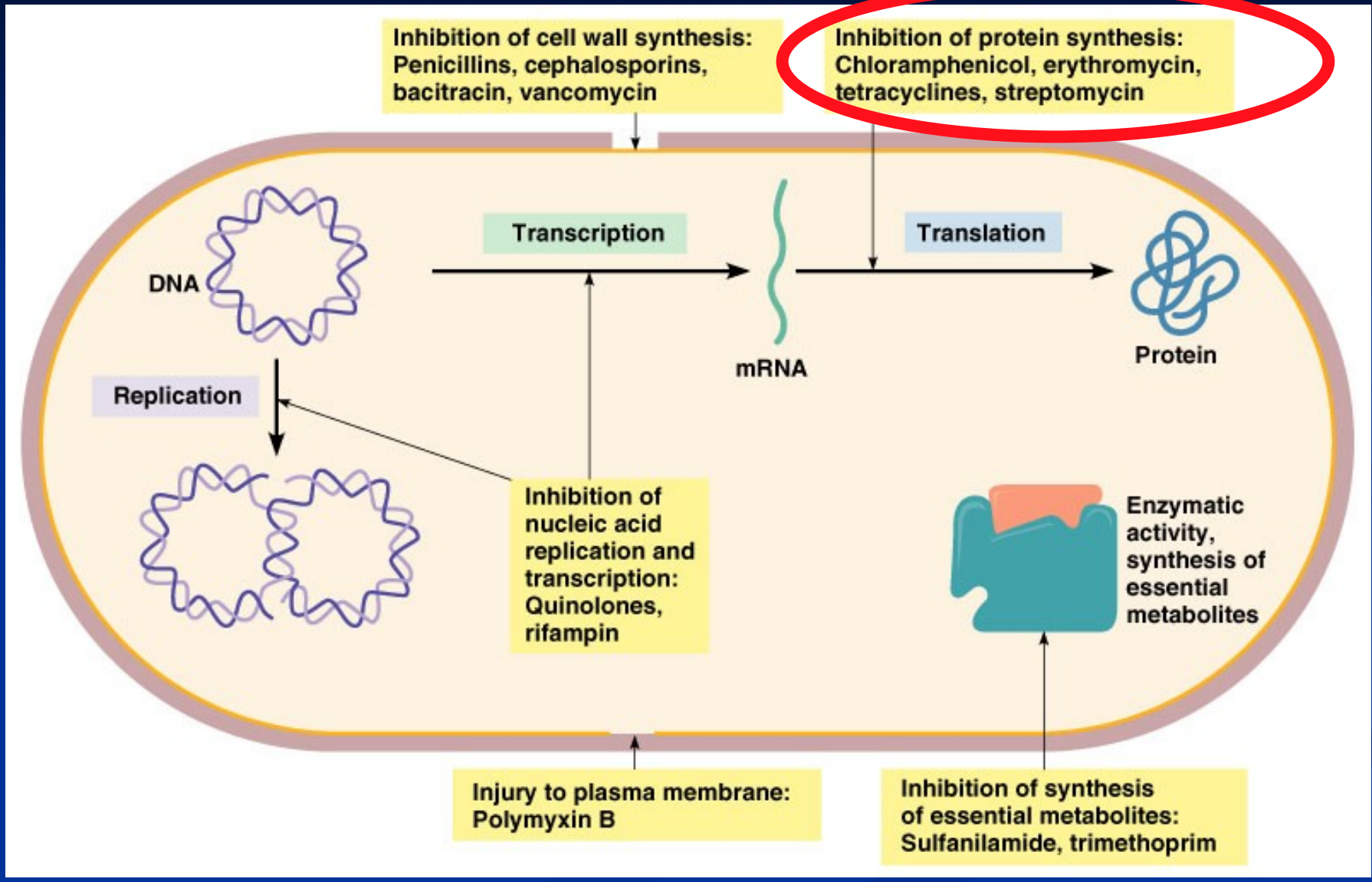
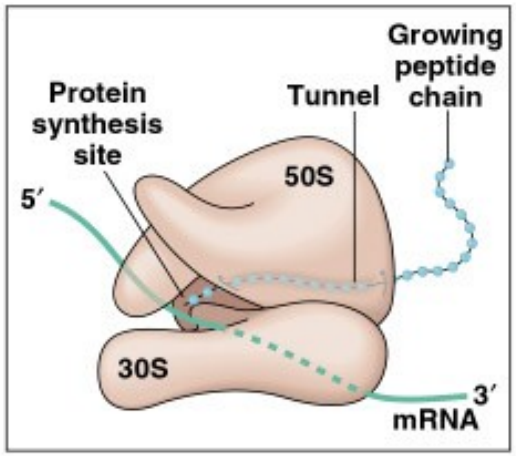
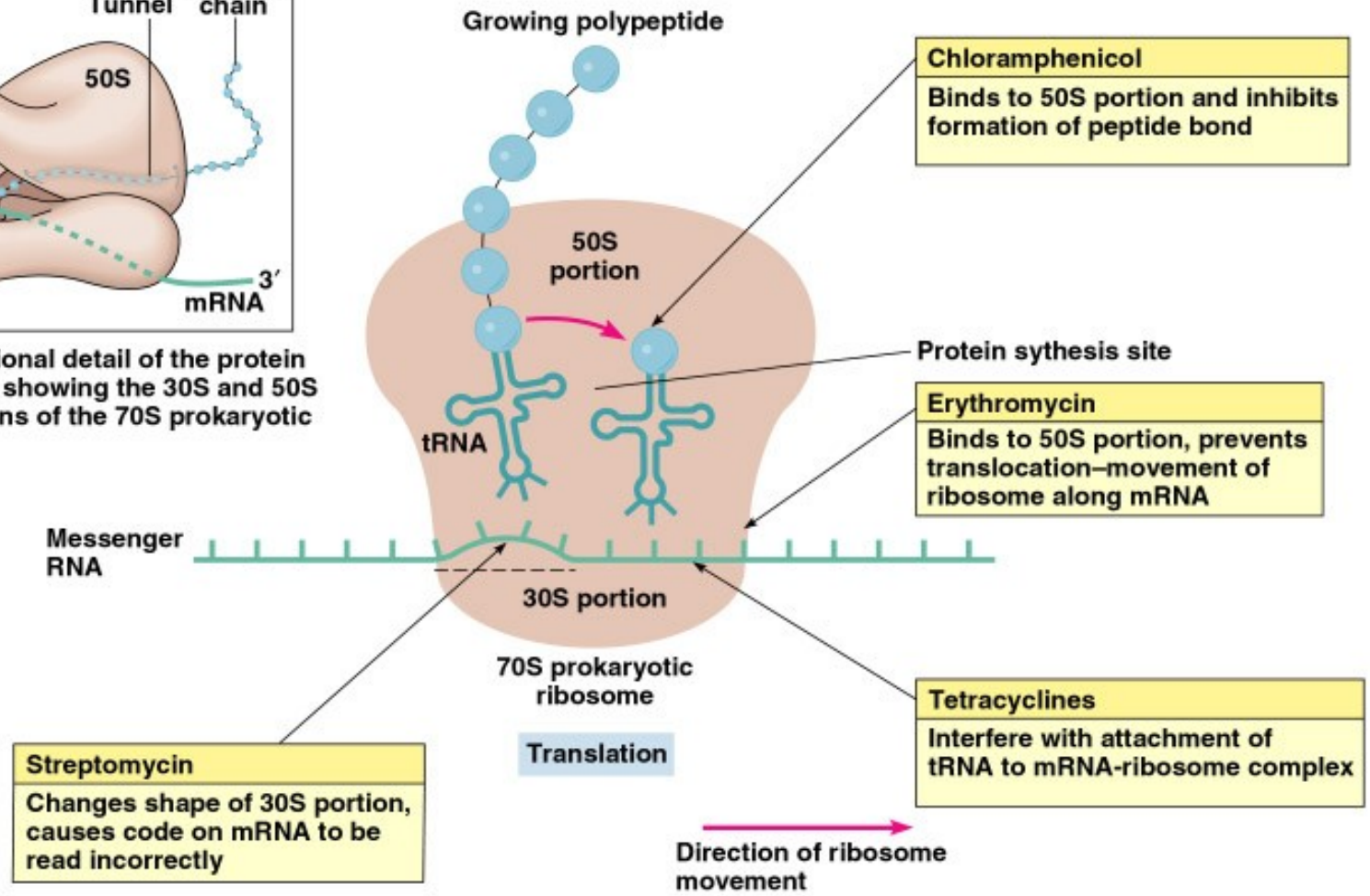


Figure 20.2

Protein Synthesis



(a) Three-dimensional detail of the protein synthesis site showing the 30S and 50S subunit portions of the 70S prokaryotic ribosome.



(b) In the diagram the black arrows indicate the different points at which chloramphenicol, erythromycin, the tetracyclines, and streptomycin exert their activities.

Figure 20.4

BACTERIAL DRUG RESISTANCE

- During treatment microorganisms may acquire resistance to antimicrobial.
- Two major mechanisms:

1) Mutation- bacteria contain drug resistance mutants arising spontaneously once in 10^7- 10^{10} of divisions

- There are two types mutations:
  - **Step wise mutation** in which series of small step mutations result in high levels of resistance, e.g. penicillin, chloramphenicol, tetracycline, etc. however, using adequate dosage of drugs can prevent this type of resistance.
  - **One-step mutation:** in this case resistance develops suddenly even with first exposure of drug, e.g. tubercle bacilli developing resistance to streptomycin

2) Resistance mediated by genetic exchange

- Bacterial drug resistance gene occurs both in the bacterial chromosome and in plasmids (extrachromosomal DNA materials).

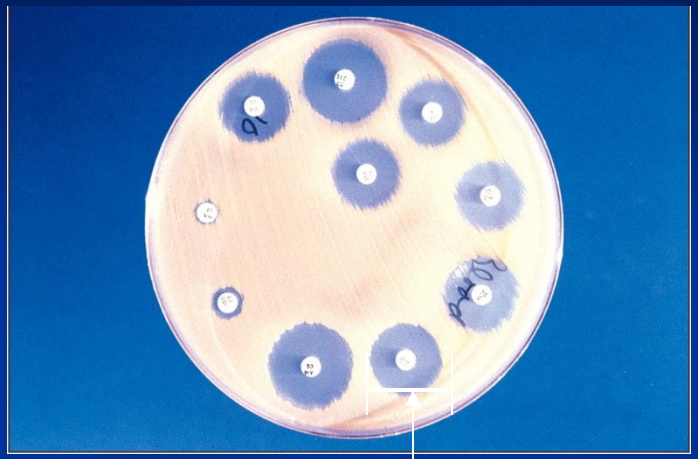
- The resistance trait may be transmitted by the transfer of genetic material from a resistant cell to a sensitive one by:
  - **Transformation**: involves the uptake and assimilation of necked DNA that occurs naturally with only certain bacterial species.
  - **Transduction**: a process whereby a bacterial virus serves as a vector for gene transmission, carrying bacterial genes from one cell to another. Drug resistance by transduction is commonly found in staphylococci
  - **Conjugation**:- one cell directly transmits part or all of its genetic information of suitable recipient plasmids that are associated with the transference of drug resistance markers by conjugation are referred to as ***R- plasmids***

Biochemical mechanisms of drug resistance

- Bacteria become resistant to antimicrobial agent by a number of ways, the common being:
  - Decreased permeability of the organism to the drug (reduce up take)
  - Inactivation of the antimicrobial agents by enzymes produced by resistant organism
  - Modification of the properties of the drug receptor site (target).
  - Development of metabolic pathway by bacteria which enable the site of antimicrobial action to be by pass

SUSCEPTIBILITY OF BACTERIAL TO ANTIMICROBIAL DRUG

- Conventional disc diffusion method
  - Kirby-Bauer disc diffusion routinely used to qualitatively determine susceptibility
  - Standard concentration of strain uniformly spread on standard media
  - Discs impregnated with specific concentration of antibiotic placed on plate and incubated



- Clear zone of inhibition around disc reflects susceptibility
  - Based on size of zone organism can be described as susceptible or resistant

- the minimal inhibitory concentration (MIC) -the lowest concentration of antimicrobial agent that inhibits the growth of the microorganism
- The MIC and the zone diameter of inhibition are inversely correlated
  - the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition
  - the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition

Disinfection and Sterilization

Definitions

- **Sterilization-** Killing or removing ***all forms of microbial life*** (including **endospores**) in a material or an object

- **Disinfection-** Reducing the number of pathogenic microorganisms to the point where they no longer cause diseases

  e.g. vegetative cell

  - **Disinfectant**: Applied to inanimate objects.
  - **Antiseptic**: Applied to living tissue (***antisepsis***).

- **Degerming** -Mechanical removal of most microbes in a limited area. Example: Alcohol swab on skin.
- **Sanitization**- Use of chemical agent on food-handling equipment e.g: Hot soap & water

- **Sepsis-** Comes from Greek for decay or putrid.
  Indicates bacterial contamination.
- **Asepsis**- Absence of significant contamination

- **Bacteriostatic agent-** an agent that ***inhibits*** the growth of bacteria, but does not necessarily kill them
- **Germicide**- an agent that kills certain microorganisms

Several factors influence the effectiveness of sterilization

1. Number of Microbes- The more microbes present, the more time it takes to eliminate population

2. Type of Microbes- Endospores are very difficult to destroy

- Vegetative pathogens vary widely in susceptibility to different methods of microbial control

3. Environmental influences- Presence of organic material (blood, feces, saliva) tends to inhibit antimicrobials, pH etc

4. Time of Exposure- Chemical antimicrobials and radiation treatments are more effective at longer times. In heat treatments, longer exposure compensates for lower temperatures.

- There are two different types of sterilization methods

physical and chemical methods

I. PHYSICAL METHODS

1. Heat- Kills microorganisms **by denaturing their enzymes and other proteins**

a. **Dry Heat:** Kills by **oxidation** effects

- Direct Flaming, Incineration, Hot Air Sterilization

b. **Moist Heat**

- **Boiling:** Heat to 100°C and Kills vegetative forms of bacterial pathogens, viruses, and fungi and their spores within 10 minutes or less
- **Autoclave**: Chamber which is filled with hot steam under pressure.
  - Temperature of steam reaches **121°C** at twice atmospheric pressure

  All organisms and endospores are killed within 15 minutes
- **Pasteurization-**Used to reduce microbes responsible for spoilage of beer, milk, wine, juices, etc.
  - Milk was exposed to 65°C for 30 minutes.

2. Radiation

- **Ionizing Radiation:** Gamma rays, X rays, electron beams, or higher energy rays
  - Used to sterilize pharmaceuticals and disposable medical supplies
- **Ultraviolet light (Nonionizing Radiation)**
  - Used to disinfect operating rooms, nurseries, cafeterias
- **Microwave Radiation**
    - May kill vegetative cells in moist foods

II. Chemical Methods of Microbial Control

1. Phenols and Phenolics

- Used in some throat sprays and lozenges.
- Acts as local anesthetic
- Stable, persist for long times after applied

2. Halogens: Effective alone or in compounds

- Iodine, Chlorine:

3. Alcohols:

- Kill bacteria, fungi, but not endospores or naked viruses
- Not good for open wounds, because cause proteins to coagulate

4. Heavy Metals:

- Selenium – kill fungi and their spore
- mercury – to disinfect skin wounds
- Silver- to protect infants against gonorrheal eye infections until recently.
- Zinc – used in mouth wash and as antfungal

5. Quaternary Ammonium Compounds (Quats)

- Effective against gram positive bacteria
- Also destroy fungi, amoebas, and enveloped viruses

6. Aldehydes- Inactivate proteins by forming covalent crosslink with several functional groups

7. Ethylene Oxide Kills all microbes and endospores

8. Hydrogen Peroxide:

- Used as an antiseptic.
- Not good for open wounds because quickly broken down by catalase present in human cells

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

Normal Microbial flora & Host-parasite interaction

- Symbiosis means “to live together”
- Describes the relationship between microorganisms and their host
- Three types
  - Mutualism-- is associations in which both are metabolically dependent upon each other

    e.g. *E. coli* obtains nutrition and shelter from man and in turn produces Vitamin K, which is essential for Humans
  - Commenalism-an association in which the parasite takes the benefit without causing injury to its host
  - Parasitism-is associations where the parasite is benefited and the host gets nothing in return but always suffers some injury

The Three Types of Symbiotic Relationships

Table 14.1 The Three Types of Symbiotic Relationships

	Organism 1	Organism 2	Example
Mutualism	Benefits	Benefits	Bacteria in human colon
Commensalism	Benefits	Neither benefits nor is harmed	*Staphylococcus* on skin
Parasitism	Benefits	Is harmed	Tuberculosis bacteria in human lung

Table 14.1

Normal flora (indigenous microbiota)

- Refers to the organisms that colonize the body's surfaces without normally causing disease
- Two types
  - Resident microbiota
    - are strains that have an established niche at one of the many body site
    - Most are commensal
  - Transient Microbiota
    - Remain in the body for only hours to months before disappearing
    - Found in the same regions as resident microbiota
    - Cannot persist in the body

Factors Determining the nature of the normal flora

- Physiologic conditions such as local pH ,temperature, moisture etc
  - differing from site to site, and sometimes vary with age
- the amounts and types of nutrients available
- resistance to local antibacterial substances such as bile and lysozyme
- Various microbial interactions
  - competition for nutrients,
  - inhibition by the metabolic products of other organisms eg. hydrogen peroxide
  - production of antibiotics and bacteriocins

Acquisition of Normal Microbiota

- Development in the womb is generally free of microorganisms
- Microbiota begins to develop during the birthing process
- Much of ones resident microbiota established during the first months of life

NORMAL FLORA AT DIFFERENT SITES

Blood, Body Fluids, and Tissues

- Tissues and body fluids such as blood are sterile in health
- Transient bacteremia can result from trauma or during childbirth
- **Skin-**
  - they are most numerous in the ducts of hair follicles and of the sebaceous glands
  - Propionibacteria and staphylococci are dominant bacteria
  - Skin flora is not easily removed
  - Conjunctiva resembles skin

Con…

Digestive Tract

- the **mouth** and **pharynx** contain large numbers of facultative and strict anaerobes (streptococci and *Neisseria*
- Stomach and small bowel have few residents-because of hydrochloric acid and peptic enzymes
- Small intestinal flora is scanty but increases toward lower ileum
- The colon carries the most prolific flora in the body
- Adult colonic flora is abundant and predominantly anaerobic

Con…

Respiratory Tract

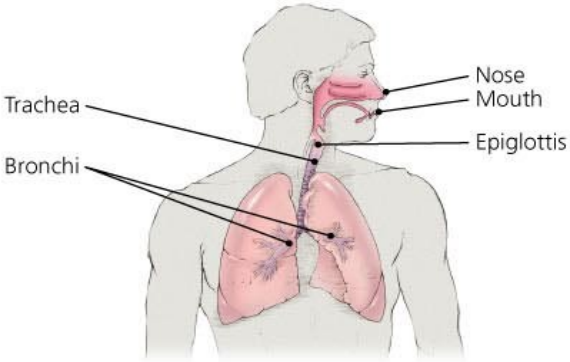
- Nares is the primary site of carriage of a pathogen, *Staphylococcus aureus*
- Nasopharynx is often a site of carriage of potential pathogens such as pneumococci, meningococci, and *Haemophilus* species
- Lower tract is protected by mucociliary action

Genitourinary Tract

- Bladder and upper urinary tract are sterile
- Before puberty and after menopause, vagina contains organisms derived from the flora of the skin and colon
- During the childbearing years, it is composed predominantly of the genus *Lactobacillus*

Resident Microbiota

Table 14.2 Some Resident Microbiota[a]



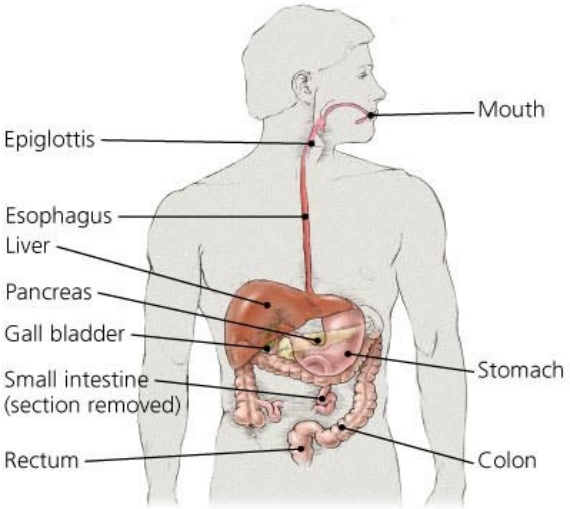
Upper Respiratory Tract

Genera

Fusobacterium, Haemophilus, Lactobacillus, Moraxella, Staphylococcus, Streptococcus, Veillonella, Candida (fungus)

Notes

The nose is cooler than the rest of the respiratory system and has some unique microbiota. The trachea and bronchi have a sparse microbiota compared to the nose and mouth. The alveoli of the lungs, which are too small to see at this magnification, have no natural microbiota.



Upper Digestive Tract

Genera

Actinomyces, Bacteroides, Corynebacterium, Haemophilus, Lactobacillus, Neisseria, Staphylococcus, Treponema, Entamoeba (protozoan), *Trichomonas* (protozoan)

Notes

Microbes colonize surfaces of teeth, gingiva, lining of cheeks, and pharynx, and are found in saliva in large numbers. Dozens of species have never been identified.

Lower Digestive Tract

Genera

Bacteroides, Bifidobacterium, Clostridium, Enterococcus, Escherichia, Fusobacterium, Lactobacillus, Proteus, Shigella, Candida (fungus), *Entamoeba* (protozoan), *Trichomonas* (protozoan)

Notes

Bacteria are mostly strict anaerobes, though some facultative anaerobes are also resident.

[a]Genera are bacteria unless noted.

Table 14.2.1

Epidemiology

- Study of where and when diseases occur and how they are transmitted within populations
- Track occurrence of diseases using two measures
  - **Incidence** – number of *new* cases of a disease in a given area during a given period of time
    - It tells how rapidly the outbreak is growing on
  - **Prevalence** – number of *total* cases of a disease in a given area during a given period of time
    - It is an indication of how widespread the infection is

Occurrence of Disease

- **Endemic** - implies that the disease is present at a low but fairly constant level
- **Epidemic**- an outbreak of an infectious disease within a given geographical region for a limited period of time (sudden localized outbreak)
- **pandemic** -Communicable infections that are worldwide, and have high attack
- **Sporadic**- a disease that appears in scattered or isolated instances . It is not an outbreak

Con…

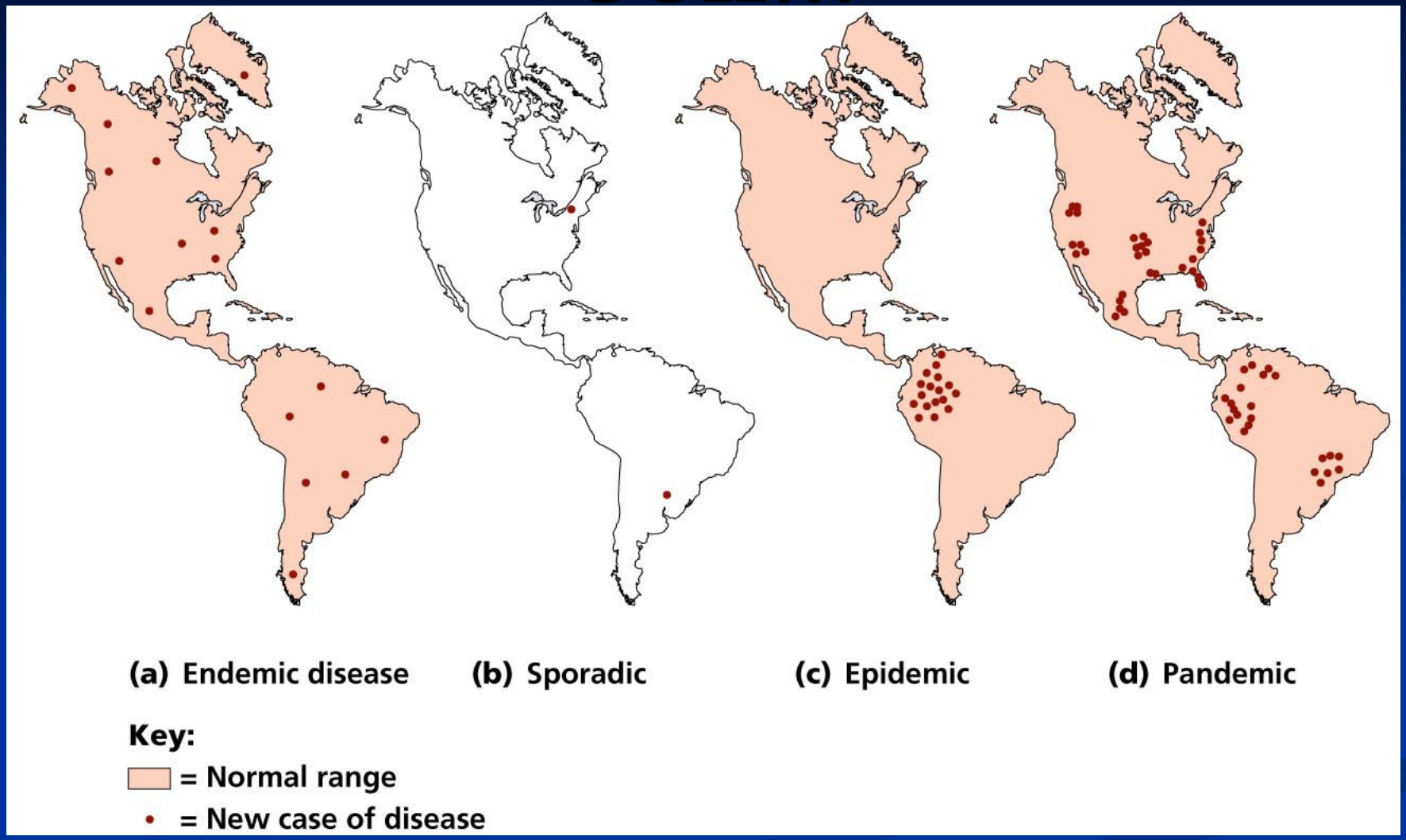


Figure 14.15a-d

Infection vs. Disease

- **Infection** is the invasion of the host by a pathogen
- **Disease** results only if the invading pathogen alters the normal functions of the body
- **Pathogenicity** – ability of a microorganism to cause disease

Manifestations of Disease

- **Symptoms** – subjective characteristics of disease felt only by the patient
- **Signs** – objective manifestations of disease that can be observed or measured by others
- **Syndrome** – group of symptoms and signs that characterize a disease or abnormal condition
- **Asymptomatic**, or **subclinical**, infections lack symptoms but may still have signs of infection

The Stages of Infectious Disease

- Following infection, sequence of events called the disease process occurs
- Many infectious diseases have five stages following infection
  - Incubation period
  - Prodromal period
  - Illness
  - Decline
  - Convalescence

The Stages of Infectious Disease

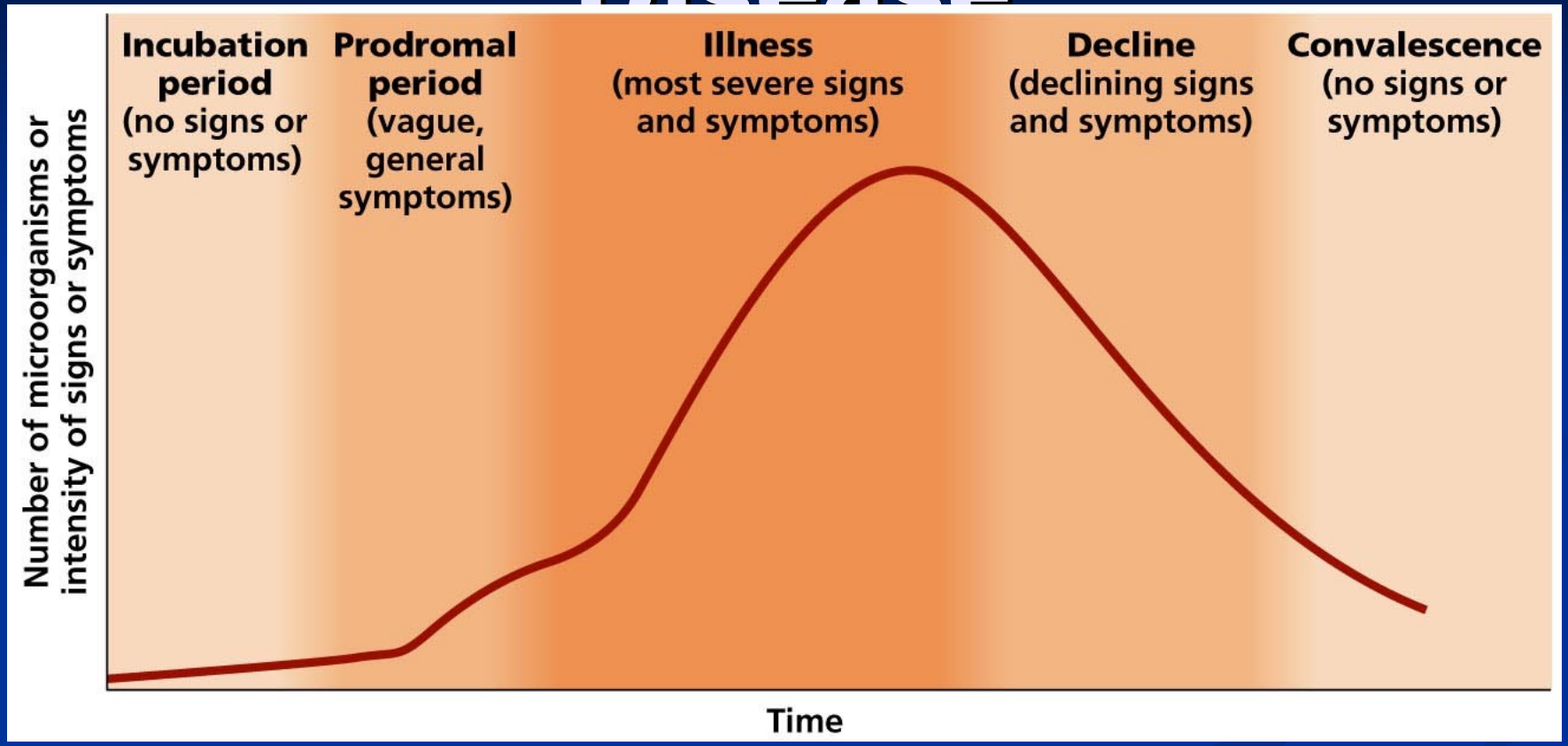


Figure 14.10

Reservoirs of Infection

- Most pathogens cannot survive long outside of their host
- The **reservoir** is the primary habitat of the organism
- The **source** of infection is the actual site from where the infection is acquired
- The **source** is not necessarily the same as the reservoir
- Three types of reservoirs
  - Animal reservoir
  - Human carriers
  - Nonliving reservoir

Animal Reservoirs

- **Zoonoses** – diseases that are naturally spread from their usual animal host to humans
- Acquire zoonoses through various routes
  - Direct contact with animal or its waste
  - Eating animals
  - Bloodsucking arthropods
- Humans are usually dead end host to zoonotic pathogens

Human Carriers

- **Carriers**: Infected individuals who are asymptomatic but infective to others
- Some individuals will eventually develop illness while others never get sick
- Healthy carriers may have defensive systems that protect them from illness

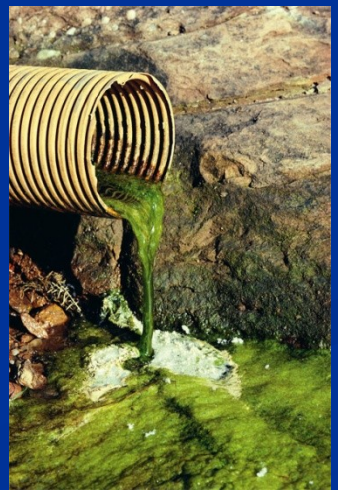
Nonliving Reservoirs

- Soil, water, and food can be reservoirs of infection
  - Presence of microorganisms is often due to contact with feces or urine











Spectral scan of chicken carcass showing areas of fecal contaminants (nasaimages.org)

Modes of Infectious Disease Transmission

- **Transmission** from either a reservoir or portal of exit
  - Horizontal transmission direct or indirect person-to-person
  - Vertical transmission mother to fetus

    e.g. *Treponema pallidum* (syphilis)
- Three groups
  - Contact transmission
  - Vehicle transmission
  - Vector transmission

Modes of Disease Transmission

Table 14.10 Modes of Disease Transmission

Mode of Transmission	Diseases Spread Include:
Contact Transmission	
Direct Contact: e.g., handshaking, kissing, sexual intercourse, bites	Cutaneous anthrax, genital warts, gonorrhea, herpes, rabies, staphylococcal infections, syphilis
Indirect Contact: e.g., drinking glasses, toothbrushes, toys, punctures	Common cold, enterovirus infections, influenza, measles, Q fever, pneumonia, tetanus
Droplet transmission: e.g., droplets from sneezing (within 1 meter)	Whooping cough, streptococcal pharyngitis (strep throat)
Vehicle Transmission	
Airborne: e.g., dust particles	Chickenpox, coccidiomycosis, histoplasmosis, influenza, measles, pulmonary anthrax, tuberculosis
Waterborne: e.g., streams, swimming pools	*Campylobacter* infections, cholera, *Giardia* diarrhea
Foodborne: e.g., poultry, seafood, meat	Food poisoning (botulism, staphylococcal); hepatitis A, listeriosis, tapeworms, toxoplasmosis, typhoid fever
Vector Transmission	
Mechanical: e.g., (on insect bodies) flies, roaches	*E. coli* diarrhea, salmonellosis, trachoma
Biological: e.g., lice, mites, mosquitoes, ticks	Chagas' disease, Lyme disease, malaria, plague, Rocky Mountain spotted fever, typhus fever, yellow fever

Table 14.10