

Microbiology

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Rickettsiaceae:

Rickettsiae are small, pleomorphic, gram negative bacilli. They are fastidious bacteria that are **obligate intracellular parasites**. They require an **arthropod vector** as part of their natural cycle and are transmitted to man by blood sucking arthropods. They possess both DNA and RNA. They possess a cell wall made of peptidoglycan. They are non motile and non capsulated. They reproduce by binary fission and are susceptible to antibacterial agents. However they are **not visible by light microscopy**.

Morphology:

- They are pleomorphic coccobacilli. They possess trilaminar cytoplasmic membrane and cell wall as seen by electron microscopy.
- They are Gram- negative though do not take stain well.
- They stain deep red with Macchiavello and Gimenez while bluish purple with Giemsa and Castaneda stain.

Cultivation:

- They are obligate intracellular parasites. They cannot be grown on cell free media. They generally grow in cytoplasm of infected cell but spotted fever rickettsiae grow in nucleus as well. Optimum temperature for growth is 32-35°
- They can be cultivated in yolk sac of 5-6 days old embryonated egg.
- They can grow well on continuous cell lines.
- Mice and guinea pig can be used for primary isolation of rickettsiae from clinical samples.

Antigenic structure:

Rickettsiae possess 3 types of antigens:

Group specific soluble antigen: It is present on surface of organism and is protein in nature.

Species specific antigen: It is adherent to the cell and act as adhesin for host cell.

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An alkali stable polysaccharide:- Found in some rickettsiae and in some non motile strains of Proteus (OX- 19, OX- 2, OX-K). This sharing of antigens forms the basis for Weil- Felix reaction used in diagnosis of rickettsial infections. In this test agglutinins are detected against these Proteus strains.

Pathogenesis:

Man acquire infection by bite or faeces of an infected arthropod vector. On entry into the human body they become localised chiefly in the vascular epithelium leading to thrombus formation.

1- Typhus fever group

This consist of :

- (a) Epidemic (classical) typhus / transmitted by **louse**
- (b) Brill-Zinsser disease / transmitted by **louse**
- (c) Endemic typhus/ transmitted by **fleas**

2- Spotted fever group

Main vector of spotted fever group are ticks.

3- Rocky Mountain spotted fever: It is the most serious type of infection, transmitted by **tick**.

4- Rickettsial Pox: Mildest form of rickettsial disease, self-limited, non-fatal. Also known as varicelliform rickettsiosis. **Vector is mite.**

Diagnosis is carried out by

- (a) Isolation of rickettsiae in lab animals, fertile hen's egg and cell cultures
- (b) Direct detection of organism and their antigen in clinical samples
- (c) Serology

Isolation of Rickettsiae

Blood clots ground in skimmed milk or BHI broth is inoculated intraperitoneally in guinea pig or mice. Animal will be observed for 3-4 weeks.

Direct detection of organism and their antigen

Aggregates of the organism or their antigen in biopsy specimen from rashes and liver, impression smears from organs of infected animals may be demonstrated by:

- Giemsa staining

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- Macciavello staining
- Gimenez staining
- Direct immunofluorescence
- Indirect immunofluorescence
- PCR

Serology

(a) Non-specific reaction:- Weil- Felix Reaction.

(b) Specific:- using rickettsial antigen.

Treatment: Tetracyclines and chloramphenicol can be given to treat rickettsial infection.

Mycoplasma and L-forms

Mycoplasma species are the smallest free-living organisms. These organisms are unique among prokaryotes in that **they lack a cell wall**, hence lack fixed shape or size and also lack Gram stain reaction and their lack of susceptibility to beta-lactams. Because of their plasticity, they can pass through bacterial filters of 45µm pore size and have often been mistaken for viruses. Mycoplasmal organisms are usually associated with mucosal surfaces of respiratory and urogenital tracts. They rarely penetrate the submucosa, except in the case of immunosuppression or instrumentation, when they may invade the bloodstream and disseminate. Species most commonly associated with infections are **Mycoplasma pneumoniae**, **Mycoplasma hominis**, and **Mycoplasma genitalium**.

Pathophysiology:

M. pneumoniae causes community-acquired **atypical pneumonia**, tracheobronchitis or bronchiolitis. Pneumonia develops in only 5-10% of persons who are infected. Acute pharyngitis may also occur.

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After inhalation of respiratory aerosols, the organism attaches to host epithelial cells in the respiratory tract. It produces adhesions and other accessory proteins which mediate attachment, followed by local inflammation and tissue destruction that may be mediated by liberation of hydrogen peroxide. Recently, *M. pneumoniae* has been shown to produce an exotoxin: **community acquired respiratory disease**. The organism **replicates intracellularly**, which contribute to chronicity of illness and difficult eradication. Spread of infection throughout households is common. The incubation period is 2-3 weeks.

Culture: They can be cultivated on fluid (broth) or solid media (agar) enriched with 20% horse or human serum and yeast extract and addition of antibiotics as selective agents. Colonies appear after incubation for 2-6 days and are about 10-600 μm in size with a typical “fried egg” appearance. Colonies may be seen with a hand lens but are best studied after staining by **Dienes method**.

Antimicrobials :

Oral erythromycin or one of the newer macrolides such as azithromycin or clarithromycin have long been the drug of choice for mycoplasmal respiratory tract infections. Tetracycline and its analogues are also active. As would be predicted by the lack of a cell wall, none of the beta-lactams is effective against *M. pneumoniae*.

L-FORM BACTERIA:

L-form bacteria, also known as L-phase bacteria, and cell wall deficient (CWD) bacteria, are strains of bacteria that lack cell walls.

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Two types of L-forms are distinguished: **unstable L-forms**, spheroplasts that are capable of dividing, but can revert to the original morphology, and **stable L forms**. L-forms that are unable to revert to the original bacteria.

L-forms can be generated in the laboratory from many bacterial species that usually have cell walls, such as *Bacillus subtilis* or *Escherichia coli*. This is done by inhibiting peptidoglycan synthesis with antibiotics or treating the cells with lysozyme, an enzyme that digests cell walls. Some of the species of L-form bacteria that have been implicated in chronic disease include: *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Treponema pallidum*, and *Rickettsia prowazekii*. Although L-forms can develop from Gram-positive as well as from Gram-negative bacteria, in a Gram stain test, the L-forms always colour Gram-negative due to the lack of a cell wall.

Chlamydiae

Chlamydiae are obligate, aerobic, intracellular parasites of eukaryotic cells. They are small Gram-negative coccoid or rod-shaped, non-motile bacteria.

Chlamydiae exhibit characteristics intermediate between bacteria and viruses. They are widespread in the natural world, being parasites of people, animals and birds with tropism for squamous epithelial cells and macrophages of the respiratory and gastrointestinal tract.

They are recognized as bacteria as

- They have both DNA and RNA.
- They have cell wall (that resembles that of GNB) and ribosomes
- Replicate by binary fission
- Susceptible to antibiotics

Cell structure :

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Chlamydiae have a cytoplasmic membrane and an outer membrane similar to Gram-negative bacteria but lack a peptidoglycan cell wall. Chlamydiae cannot synthesize their own ATP and require intracellular abode to remain viable.

Chlamydiae exist in two forms: the elementary body and the reticulate body. Both of them play a pivotal part in the life cycle of chlamydia. Although Gram negative, Chlamydiae stain better with Castaneda, Machiavello or Gimenez stains.

Elementary body (EB):

The elementary body is the dispersal form, which is analogous to a spore. This dispersal form is about 200-300 nm in diameter. It is the extracellular infective form. It induces its own endocytosis upon exposure to target cells.

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Reticulate body (RB):

Reticulate body is the intracellular, multiplicative form. It represents the non-infectious growing form.

Life cycle :

The life cycle of *Chlamydia trachomatis* consists of two stages: elementary body and reticulate body. Upon endocytosis into the host cell EB prevents phagolysosomal fusion enabling intracellular survival of the bacteria. Once inside the endosome, the elementary body transforms into the larger reticulate body (500 – 1000 nm) as a result of the glycogen that is produced.

The reticulate body is the reproductive form. It divides through binary fission. It contains no cell wall and is detected as an inclusion in the cell arranged as a mantle around the nucleus. The inclusion bodies are basophilic. They can also be stained by Lugol's iodine because of the presence of glycogen matrix. After division, the reticulate body transforms back to the elementary form and is released by the cell by exocytosis.

One phagolysosome usually produces 100-1000 elementary bodies. The entire process takes 24 – 48 hours. The EB may infect new cells and the cycle continues.

Antigenic structure :

Chlamydia antigens consist of 3 groups:

- 5- **genus-specific antigen**
- 6- **species specific protein antigen**
- 7- **serotype-specific**

Culture:

Chlamydiae can be isolated by the following methods:

(a) **Animal inoculation:** Mice can be inoculated through intranasal,

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intraperitoneal or intracerebral route. Mice die within 10 days. Smears

made from lung, spleen, brain or peritoneal exudate demonstrate elementary bodies.

(b) **Egg inoculation:** Organisms can be isolated by egg yolk inoculation of the specimen.

(c) **Tissue culture:** Inclusion bodies can be visualized by staining the cell lines.

Diseases produced by *Chlamydia*:

(a) **Ocular infections:** *C. trachomatis* serotype A, B, Ba, C- is the leading cause of preventable blindness (caused by a chlamydia infection called trachoma) in the world. Other diseases produced are inclusion conjunctivitis and ophthalmia neonatorum.

(b) **Genital infections:** *C. trachomatis* is also the leading cause of sexually transmitted disease worldwide. It is associated with non-gonococcal urethritis and lymphogranuloma venereum. *trachomatis* is one of the major causes of pelvic inflammatory disease (PID) and infertility in women.

(c) **Respiratory infections:** *C. pneumoniae* causes pneumonia. , *C. psittaci* causes *psittacosis*.

Laboratory diagnosis :

Specimen collection: Specimen should be collected by scraping the mucosa. Depending on the site of infection. In suspected Psittacosis, blood and sputum are collected for microscopy and culture and serum for serology.

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□ **Light Microscopy:** Inclusion bodies of *C. trachomatis* can be detected by staining with Lugol's iodine. Iodine can be used because inclusion bodies contain a glycogen matrix. Giemsa, Castaneda, Machiavello and Giminez methods are better and can be used to stain ocular, cervical or urethral specimen.

□ **Isolation:** Mice, fertilized hen's egg and tissue cultures can be used for isolation of chlamydia. The clinical specimen can be inoculated into the yolk sac of 6 to 8 day old eggs.

□ **Immunofluorescence:** Direct fluorescent antibody test detects major outer membrane proteins. It is now considered by many the test of choice for diagnosis.

□ **ELISA:** Antigen and antibodies can be detected by ELISA. Antigen detection is more specific than antibody detection.

□ **Molecular tools:** Polymerase chain reaction, can be used for detection of Chlamydia.

Treatment:

Sulphonamides and tetracycline are the drugs of choice. Single dose azithromycin is the drug of choice for non-gonococcal urethritis.

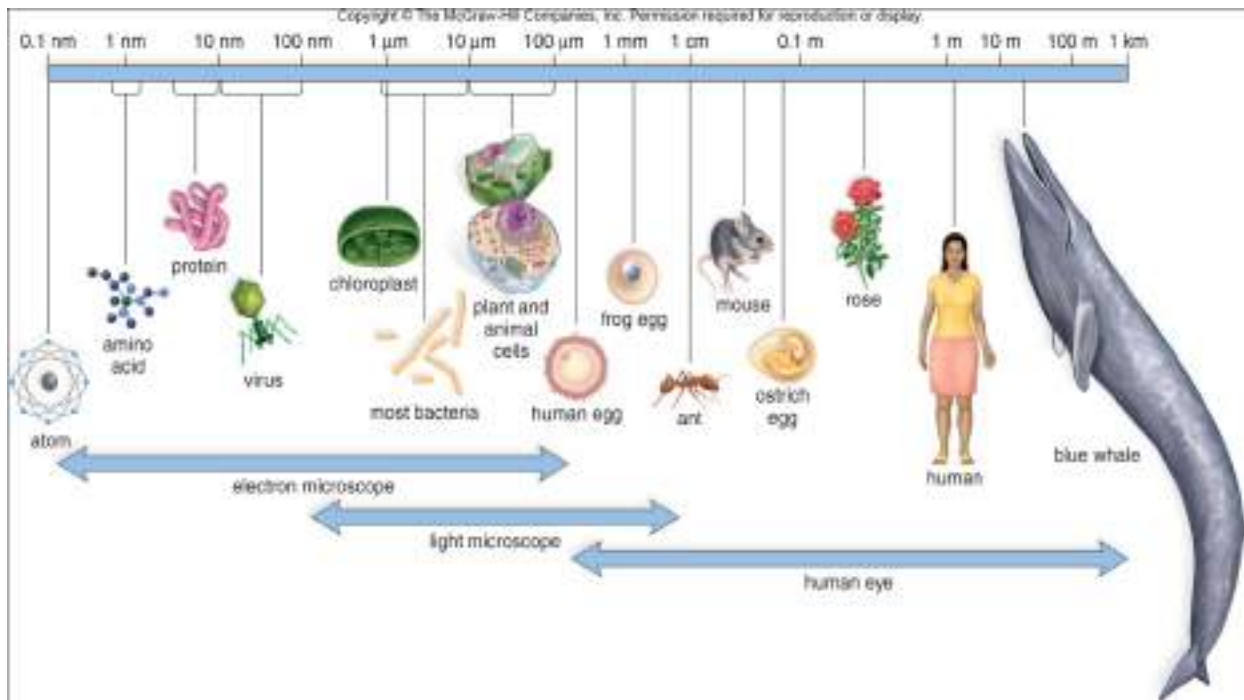
Introduction to microbiology

Microbiology : is the study of microbes.

Microbiology began in the mouth : Antony van Leeuwenhoek developed and used the first microscope to examine material collected from teeth, and described motile ‘ animalcules’.

Microbiology can be divided into the following major groups :

- 1- Bacteria
- 2- Viruses and prions
- 3- Fungi
- 4- protozoa



Prokaryotes and Eukaryotes

all forms of life fall into three domains: **Archaea, Bacteria** and **Eucarya**

Viruses are not included in this classification as they are unique, acellular, metabolically inert organisms and therefore replicate only within living cells. Other differences between viruses and cellular organisms include:

- **Structure.** Cells possess a nucleus or, in the case of bacteria, a nucleoid with DNA. This is surrounded by the cytoplasm where energy is generated and proteins are synthesized. In viruses, the **inner core of genetic material is either DNA or RNA**, but they have **no cytoplasm** and hence depend on the host for their energy and proteins (i.e. they are metabolically inert).

- **Reproduction.** Bacteria reproduce by **binary fission** (a parent cell divides into two similar cells), but **viruses disassemble, produce copies of their nucleic acid and proteins, and then reassemble to produce another generation of viruses.** As viruses are metabolically inert, they must replicate within host cells. Bacteria, however, can replicate extracellularly (except rickettsiae and chlamydiae, which are bacteria that also require living cells for growth).

- There are fundamental differences in eukaryotic and prokaryotic cell structure and gene expression :
- The defining difference is the presence of a nuclear membrane surrounding the genetic material of eukaryotes, but not prokaryotes.

TABLE 4.1			
Comparison of Prokaryotic Cells and Eukaryotic Cells			
	Prokaryotic Cells (1–20 μm in diameter)	Eukaryotic Cells (10–100 μm in diameter)	
		Animal	Plant
Cell wall	Usually (peptidoglycan)	No	Yes (cellulose)
Plasma membrane	Yes	Yes	Yes
Nucleus	No	Yes	Yes
Nucleolus	No	Yes	Yes
Ribosomes	Yes (smaller)	Yes	Yes
Endoplasmic reticulum	No	Yes	Yes
Golgi apparatus	No	Yes	Yes
Lysosomes	No	Yes	No
Mitochondria	No	Yes	Yes
Chloroplasts	No	No	Yes
Peroxisomes	No	Usually	Usually
Cytoskeleton	No	Yes	Yes
Centrioles	No	Yes	No
9 + 2 cilia or flagella	No	Often	No (in flowering plants) Yes (sperm of bryophytes, ferns, and cycads)

The Morphology of Bacteria

- Bacterial cells are between 0.3 and 5 μm in size.

They have three basic forms: **cocci**, **straight rods**, and **curved or spiral rods**.

-The nucleoid consists of a very thin, long, circular DNA molecular double strand that is not surrounded by a membrane.

- Among the nonessential genetic structures are the plasmids.

-The membrane is surrounded by the cell wall, the most important element of which is the supporting murein skeleton. The cell wall of Gram-negative bacteria poses outer membrane contain LPS

-. The cell wall of Gram-positive bacteria does not possess such an outer membrane. Its murein layer is thicker and contains teichoic acids

.-Many bacteria have capsules made of polysaccharides that protect them from phagocytosis.

- Attachment pili or fimbriae facilitate adhesion to host cells.

- Motile bacteria possess flagella

-Some bacteria produce spores, dormant forms that are highly resistant to chemical and physical conditions.

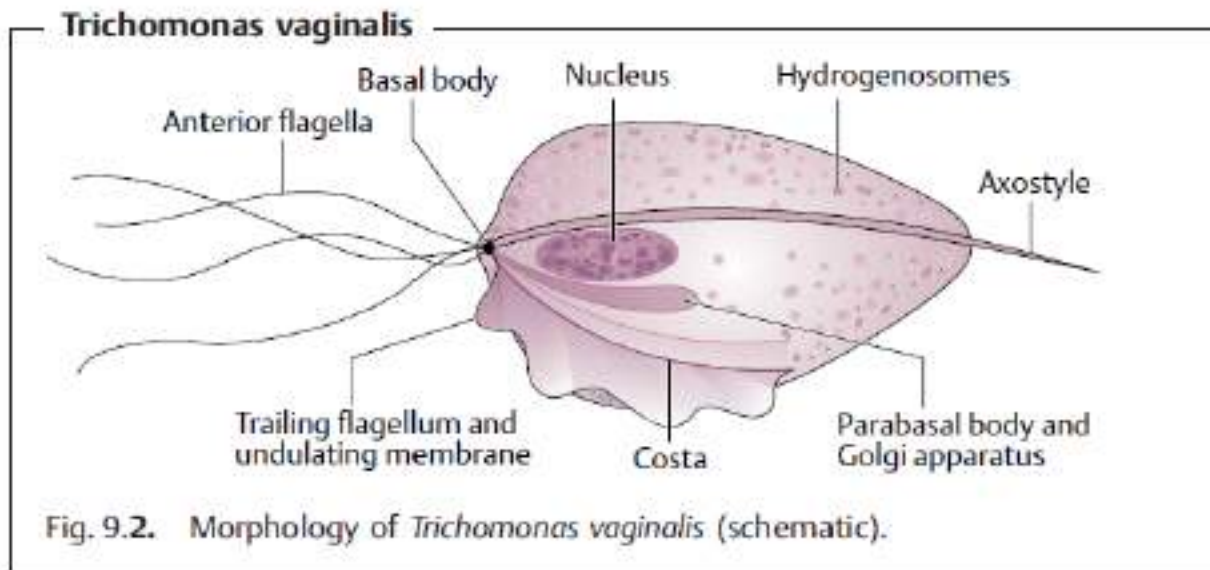
Bacterial Forms

Bacteria differ from other single-cell microorganisms in both their cell structure and size, which varies from 0.3–5 micron. Magnifications of 500–1000!—close to the resolution limits of light microscopy—are required to obtain useful images of bacteria. Techniques like phase contrast and dark field microscopy, both of which allow for live cell observation, are used to overcome this difficulty. Chemical-staining techniques are also used, but the prepared specimens are dead.

Protozoa

- Protozoa are a diverse group of eukaryotic organisms, usually unicellular, exhibiting a great variety of structures and life styles.
- They range in size from 1 mm to several millimetres.

- Most are free living (found in soil and water)., and most are aerobic. However, some can grow anaerobically or microaerophilically.




- In the mouth a few species have been isolated (eg *Entamoeba gingivalis*, *Trichomonas tenax*, *Hamblia spp*)
- But there true prevalence and importance in the oral cavity is unclear.


Viruses : are obligate intracellular parasites

Human DNA viruses

- Parvovirus 
- Papovavirus 
- Adenovirus 
- Herpesvirus 
- Poxvirus 


Bacteriophage MS2






Bacteriophage M13


Tobacco mosaic virus


Bacteriophage T2

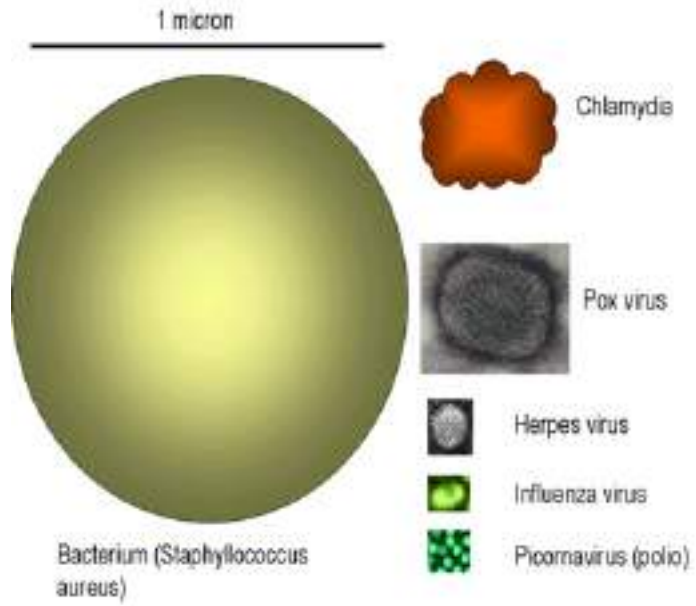

Chlamydia

Human RNA viruses

- Picornavirus 
- Reovirus 
- Togavirus 
- Coronavirus 
- Orthomyxovirus 
- Rhabdovirus 
- Paramyxovirus 



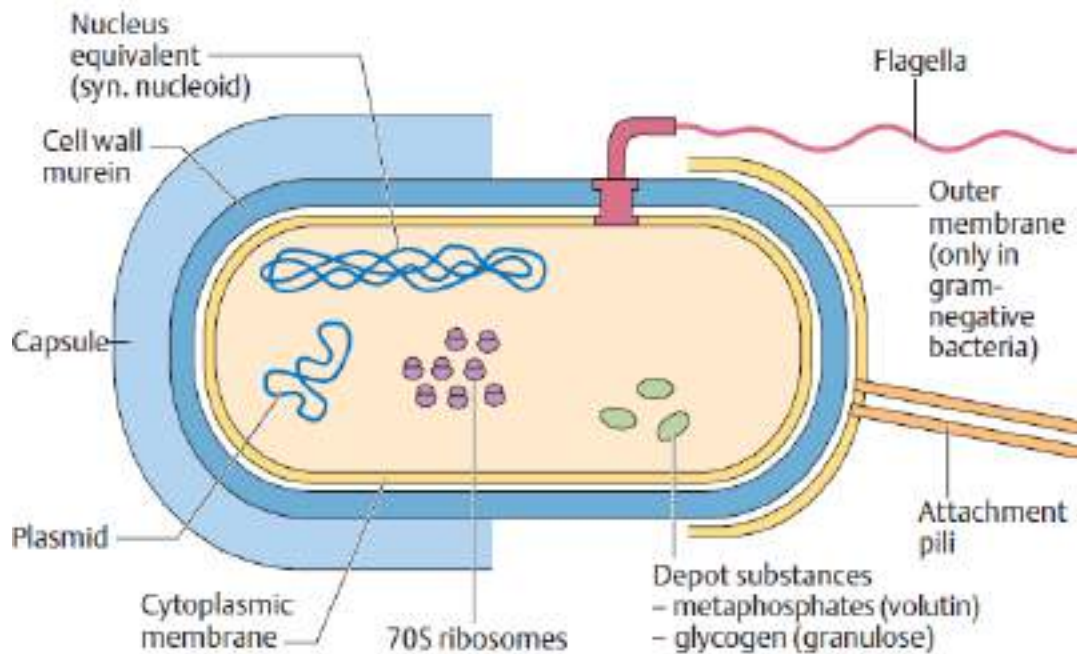
Escherichia coli (6 μ m long)



Bacterial Structure

Essential and Particular bacterial structures

Basic Bacterial Cell Structure



Cell wall

- Situation: outmost portion That covers the entire bacteria. 15-30nm in thickness, 10%-25% of dry weight.
- **Cell Wall function**
 - 1- The tasks of the complex bacterial cell wall are to protect the protoplasts from external environment
 - 2- to withstand and maintain the osmotic pressure gradient between the cell interior and the extracellular environment
 - 3- to give the cell its outer form
 - 4- to facilitate communication with its surroundings.

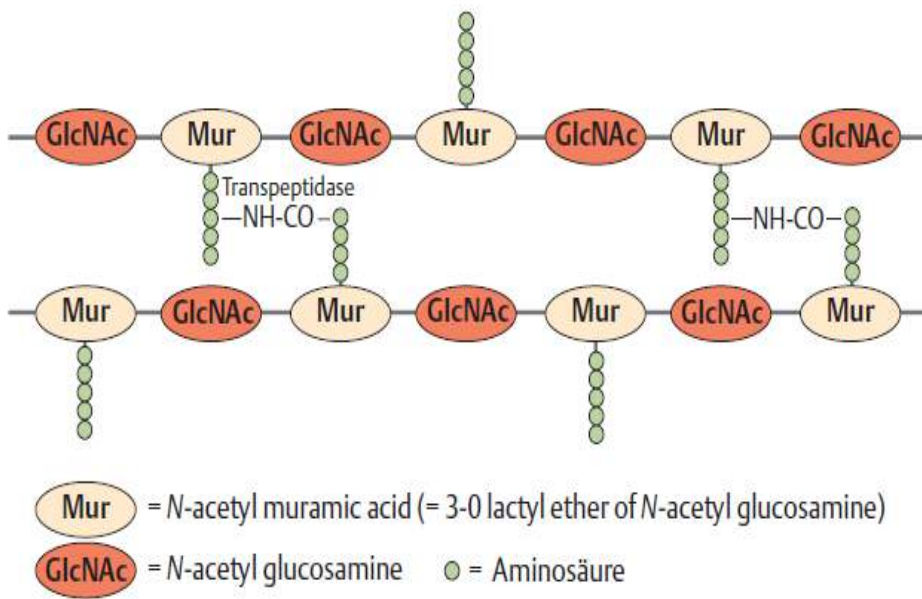
peptidoglycan

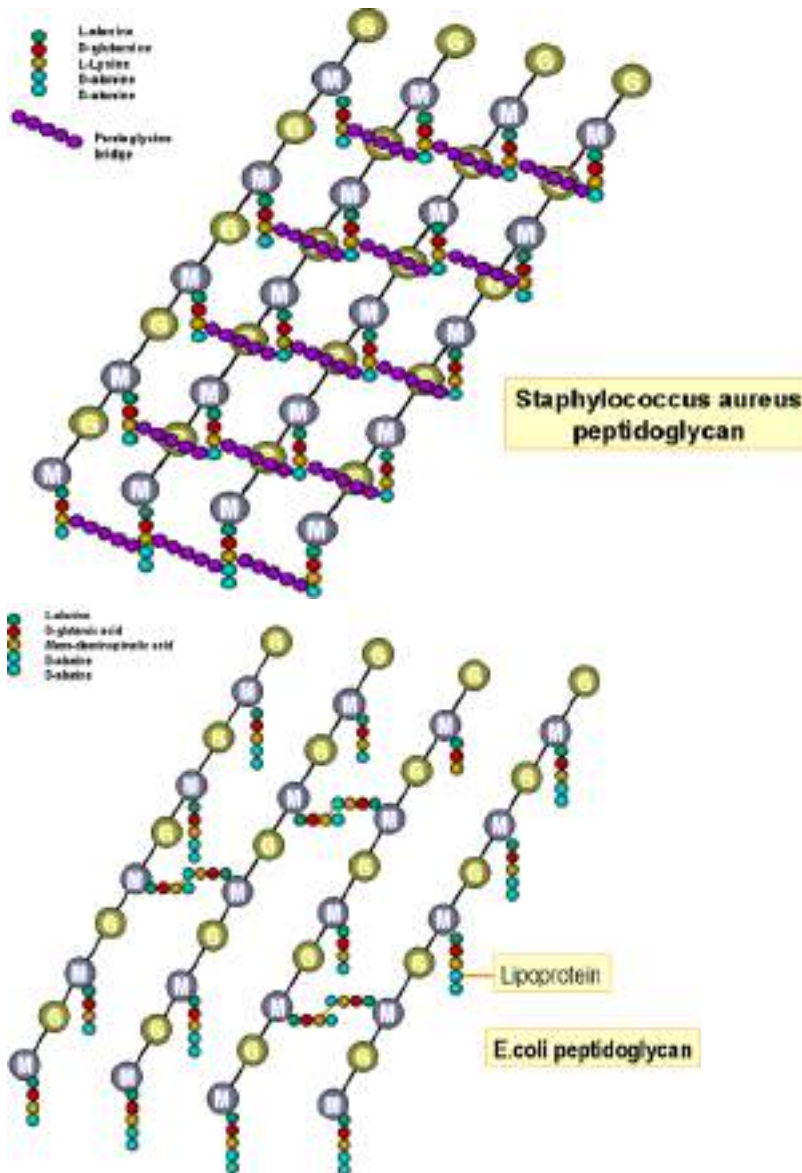
Murein (syn. peptidoglycan). The most important structural element of the wall is murein, a netlike polymer material surrounding the entire cell . It is made up of polysaccharide chains crosslinked by peptides

Common peptidoglycan layer

- 1- A backbone of N-acetyl glucosamine and N-acetylmuramic acid: Both discovered in Gram positive and Gram negative bacteria.
- 2- A set of identical tetrapeptide side chain attached to N-acetylmuramic acid: different components and binding modes in Gram positive and Gram negative bacteria.
- 3- A set of identical peptide cross bridges: only in Gram positive bacteria

The Structure of Murein

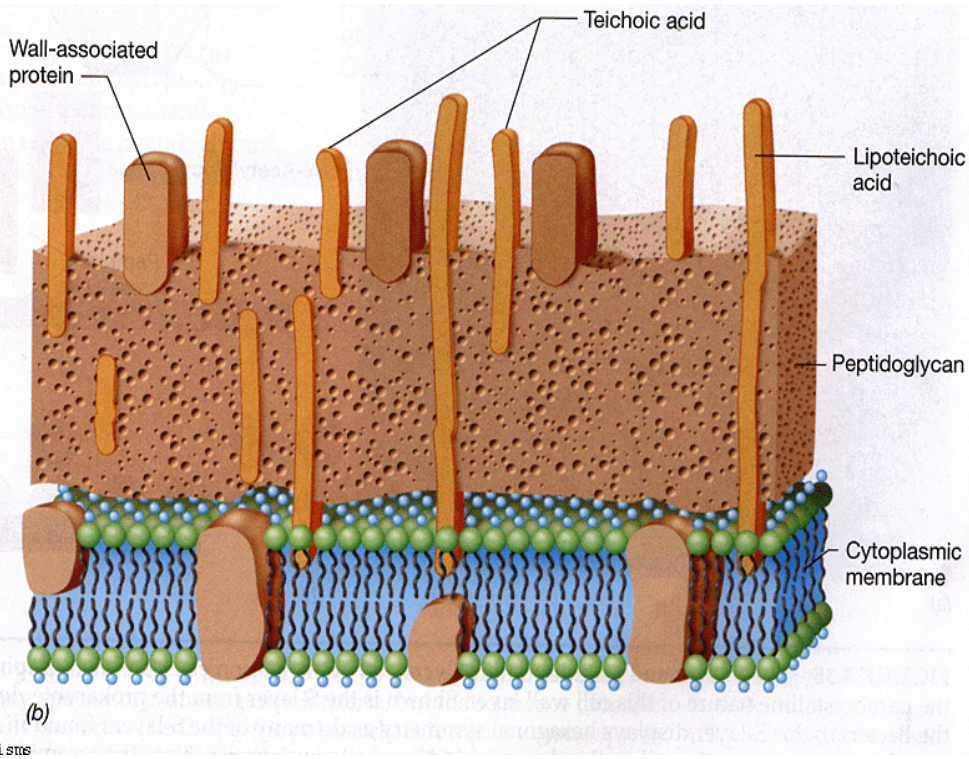




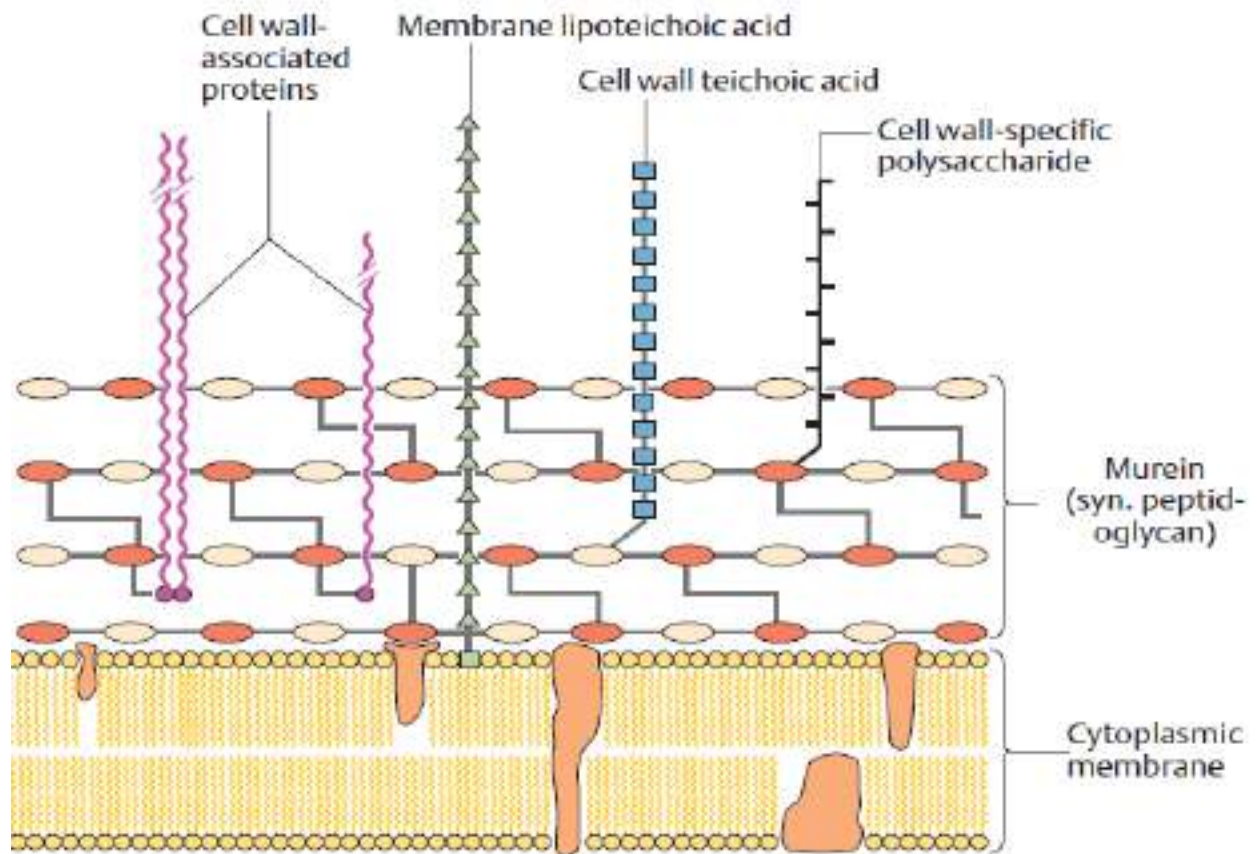
Special components of Gram positive cell wall

Teichoic acid

- The membrane lipoteichoic acids are anchored in the cytoplasmic membrane
- whereas the cell wall teichoic acids are covalently coupled to the murein

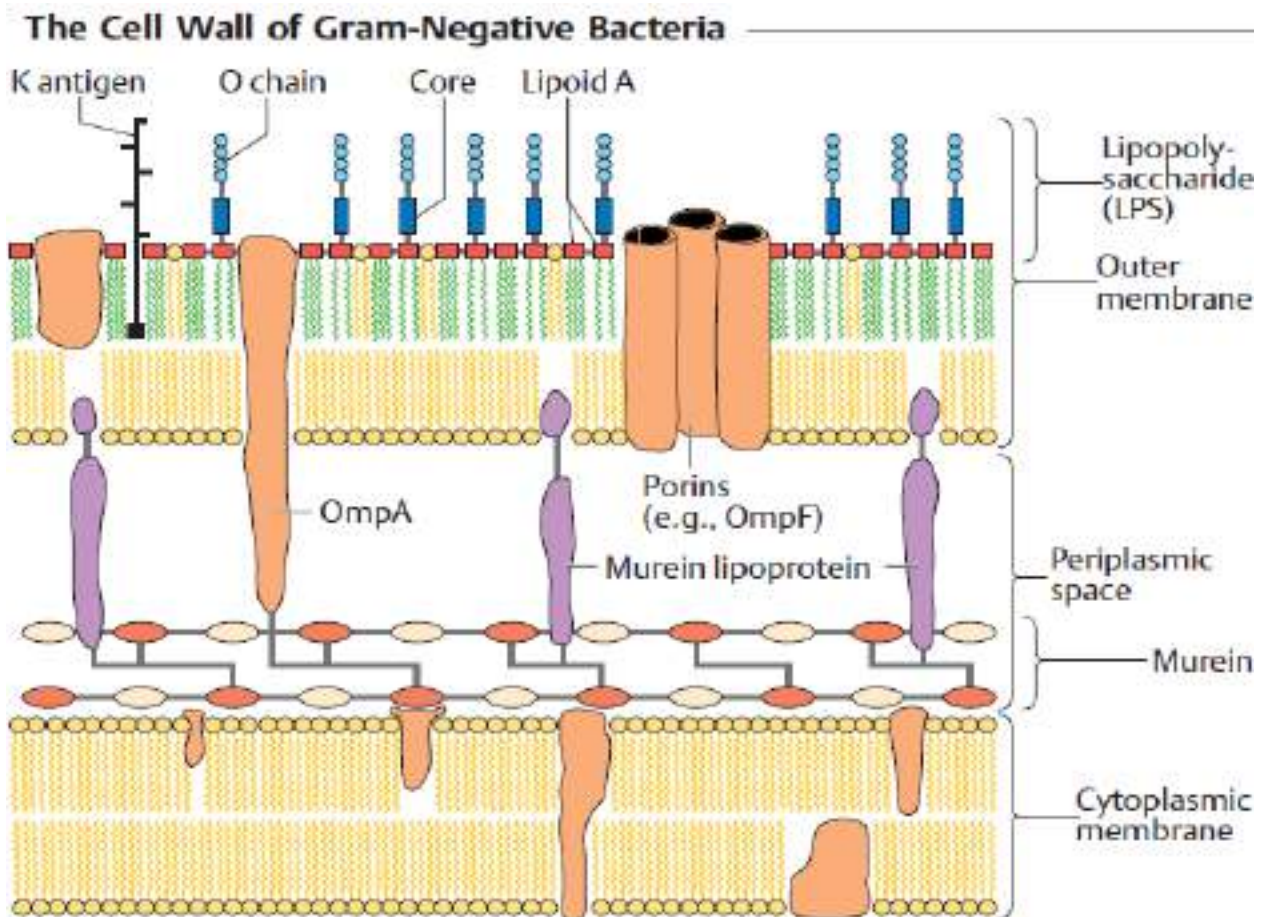


The Cell Wall of Gram-Positive Bacteria



cellwall of Gram-negative bacteria

- the murein is only about 2 nm thick and contributes up to 10% of the dry cell wall mass
- With outer membrane
- It contains numerous proteins
- as well as the medically critical lipopolysaccharide. (LPS)



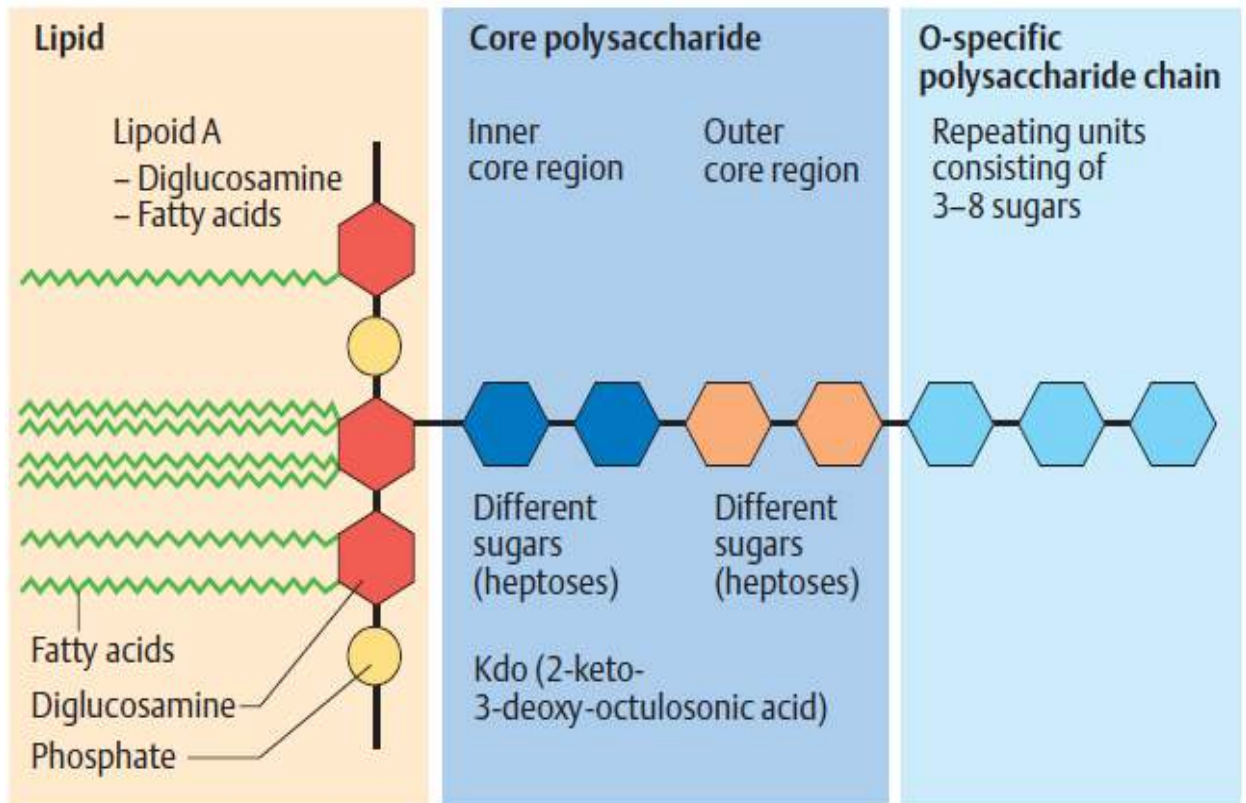
Special components of Gram negative cell wall

- Lipopolysaccharide (LPS).
- This molecular complex, also known as endotoxin, is comprised of the
 - 1- lipid A
 - 2- the core polysaccharide

3- and the O-specific polysaccharide chain

Lipid A is responsible for toxic effect

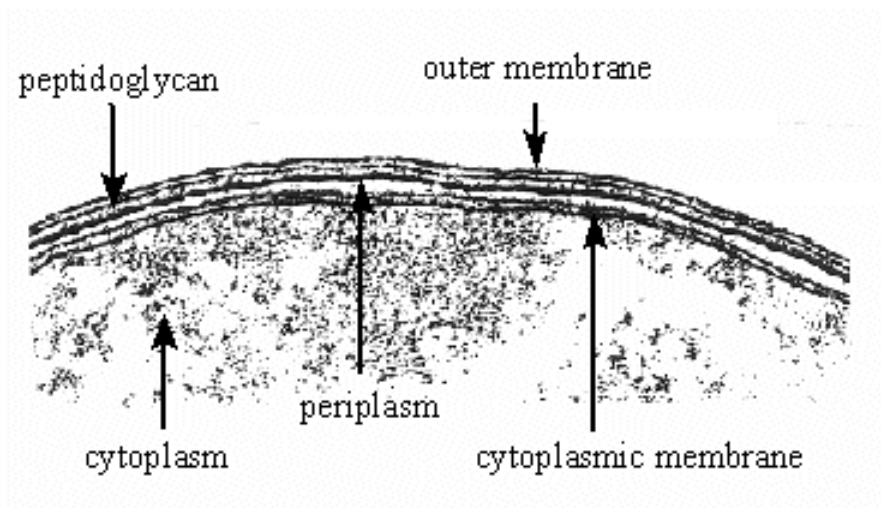
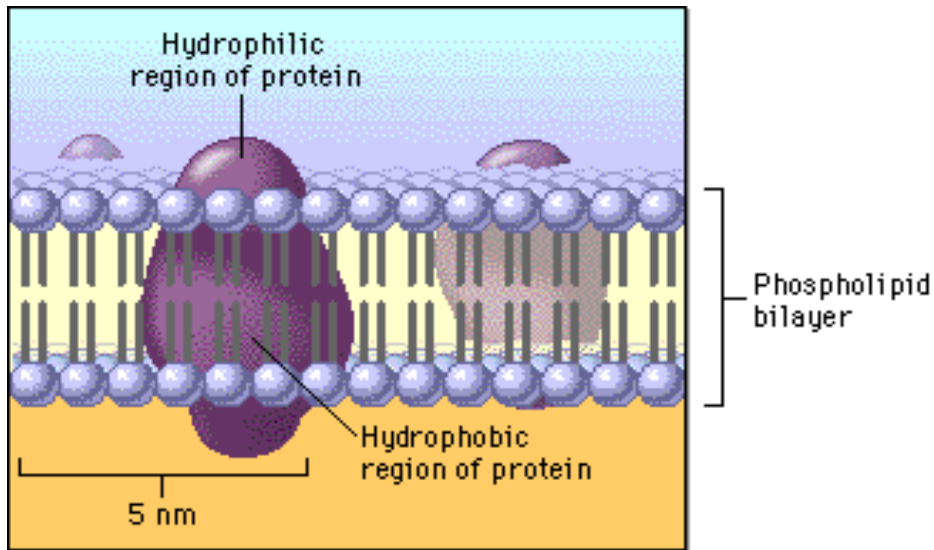
The Lipopolysaccharide Complex



Cell membrane

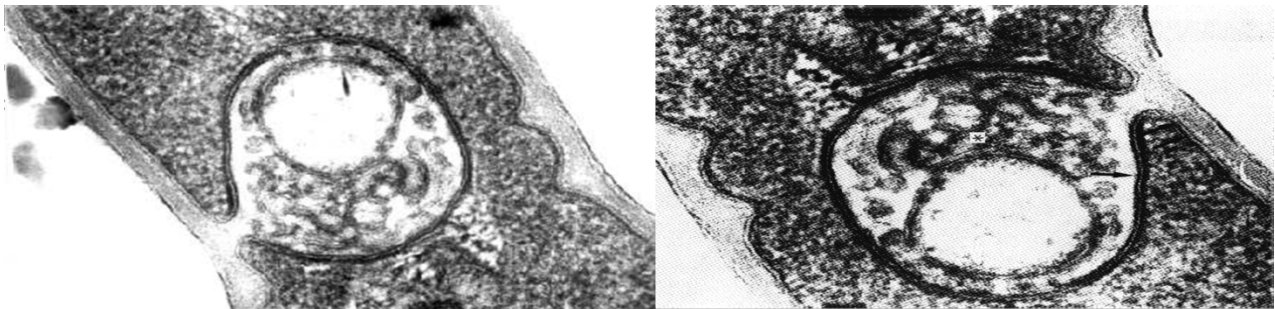
Is a phospholipid bilayer located beneath the cell wall. The functions are:

- Site of biosynthesis of DNA, cell wall polymers and membrane lipids.
- Site of selective permeability and transport of solutes into cells
- Electron transport and oxidative phosphorylation
- Excretion of hydrolytic exoenzymes



Mesosomes

- Mesosomes are specialized structures formed by convoluted invaginations of cytoplasmic membrane, and divided into septal and lateral mesosome.

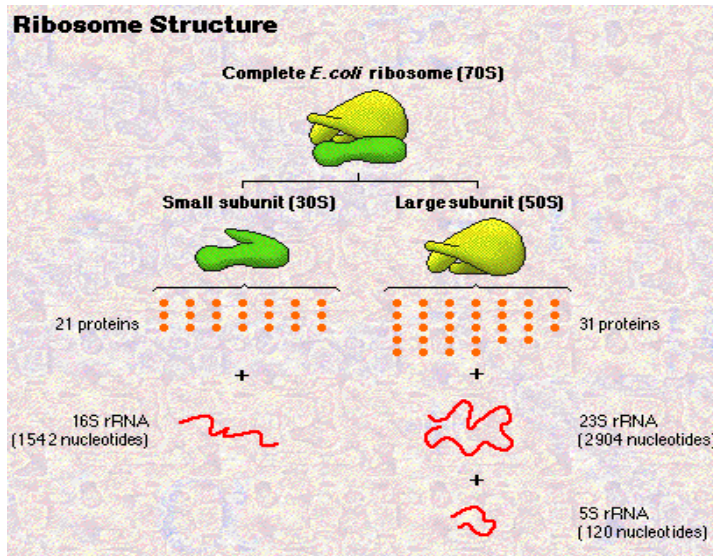


Cytoplasm

- It is the fluid phase of bacterial cell composed largely of water, together with proteins, nucleic acid, lipids and small amount of sugars and salts

Contains :

- Ribosomes: numerous, 15-20nm in diameter with 70S; distributed throughout the cytoplasm; sensitive to streptomycin and erythromycin site of protein synthesis
- Plasmid: extrachromosomal genetic elements
- Inclusions: sources of stored energy



Nucleus

- Lacking nuclear membrane, absence of nucleoli, hence known as nucleic material or nucleoid, one to several per bacterium.

Flagella

Some bacterial species are mobile and possess locomotory organelles - flagella. Flagella is a long hair like protrusions consist of flagellin protein

Function:

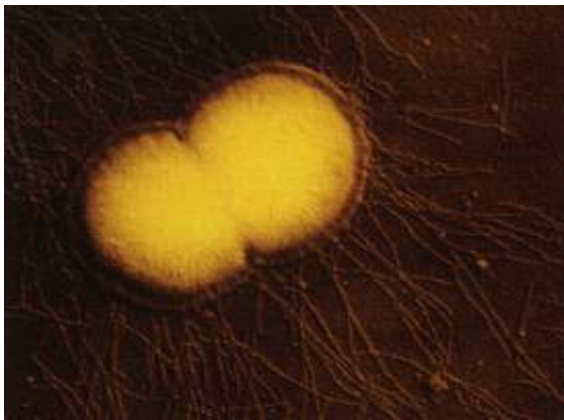
- Motility of bacteria
- Identification of Bacteria
- Pathogenesis

Pili

- Pili are hair-like projections of the cell, They are known to be receptors for certain bacterial viruses. Chemical nature is pilin

Classification and Function

- a. Common pili or fimbriae: fine, rigid numerous, related to bacterial adhesion
- b. Sex pili: longer and coarser, only 1-4, related to bacterial conjugation



Capsule

- Many pathogenic bacteria make use of extracellular enzymes to synthesize a polymer that forms a layer around the cell called **capsule**. structures surrounding the outside of the cell envelope
- The capsule protects bacterial cells from phagocytosis.
- The capsule of most bacteria consists of a polysaccharide in certain bacilli they are composed of a polypeptide (polyglutamic acid)

- The bacteria of a single species can be classified in different capsular serovars (or serotypes) based on the fine chemical structure of this polysaccharide.

Endospores (spores) are spherical to oval dormant life form characterized by a thick spore wall and a high level of resistance to chemical and physical factors.

Characteristics :

- Dormant cell
- Resistant to adverse conditions
 - high temperatures
 - organic solvents
- Produced when starved
- Contain calcium dipicolinate DPA, Dipicolinic acid
- *Spore forming bacteria : Bacillus and Clostridium*

NOTE:

In prokaryotes, the bacterial genome, or chromosome, is a single, circular molecule of double-stranded DNA, lacking a nuclear membrane (smaller, single or multiple circular DNA molecules called plasmids may also be present in bacteria), whereas the eukaryotic cell has a true nucleus with multiple chromosomes surrounded by a nuclear membrane

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Bacterial growth and metabolism

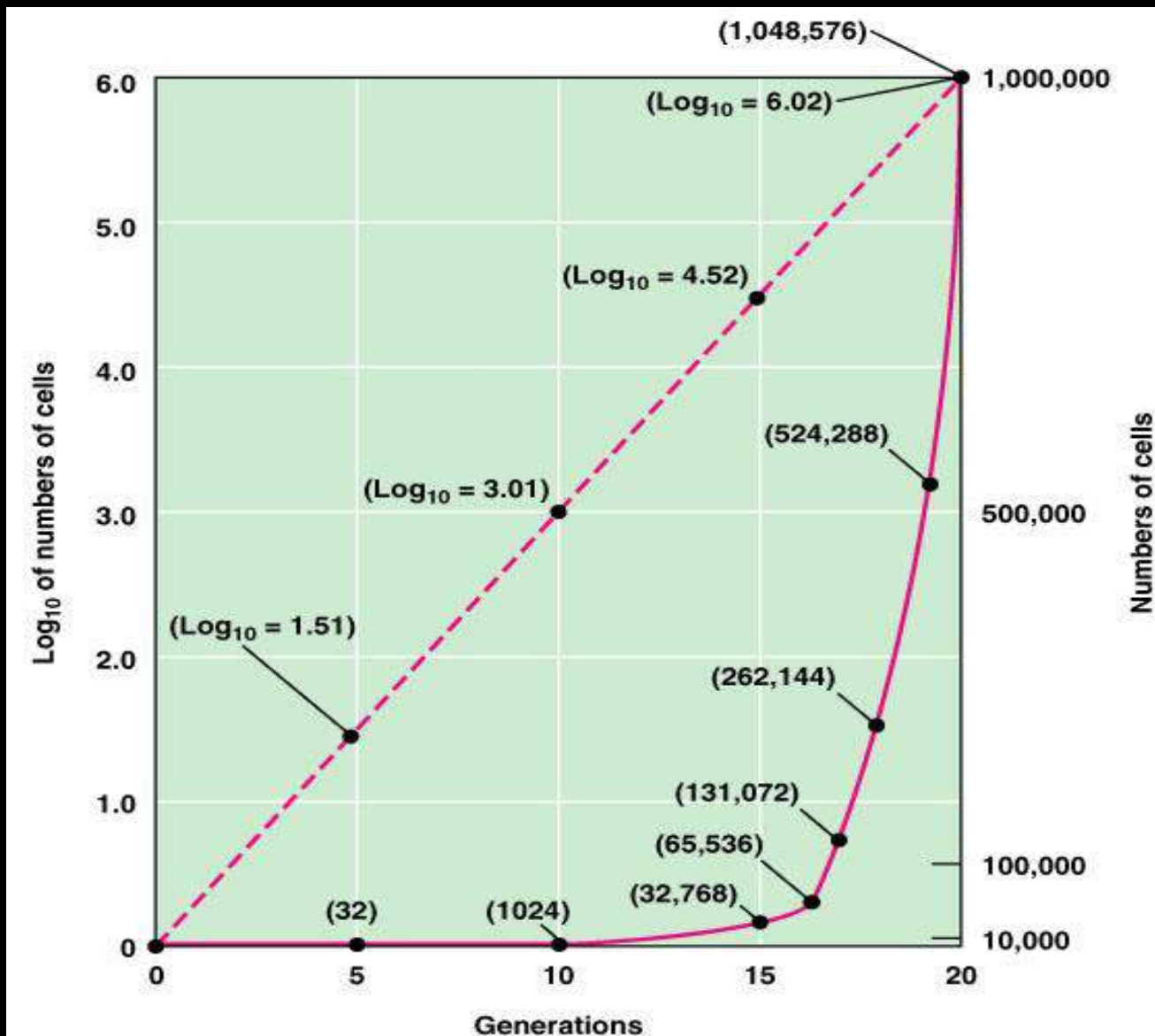
Growth of Microbes

Microbial growth: Microbes grow via binary fission, resulting in exponential increases in numbers

Bacterial “growth” means an increase in the number of cells, not an increase in cell size

One cell becomes colony of millions of cells

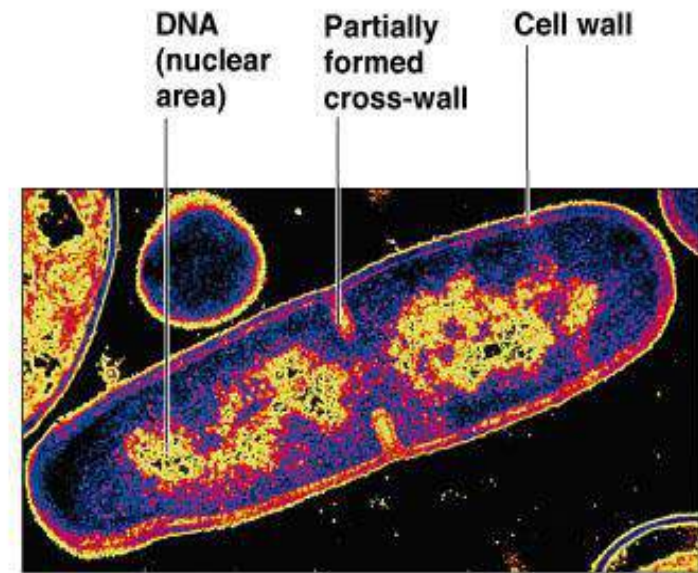
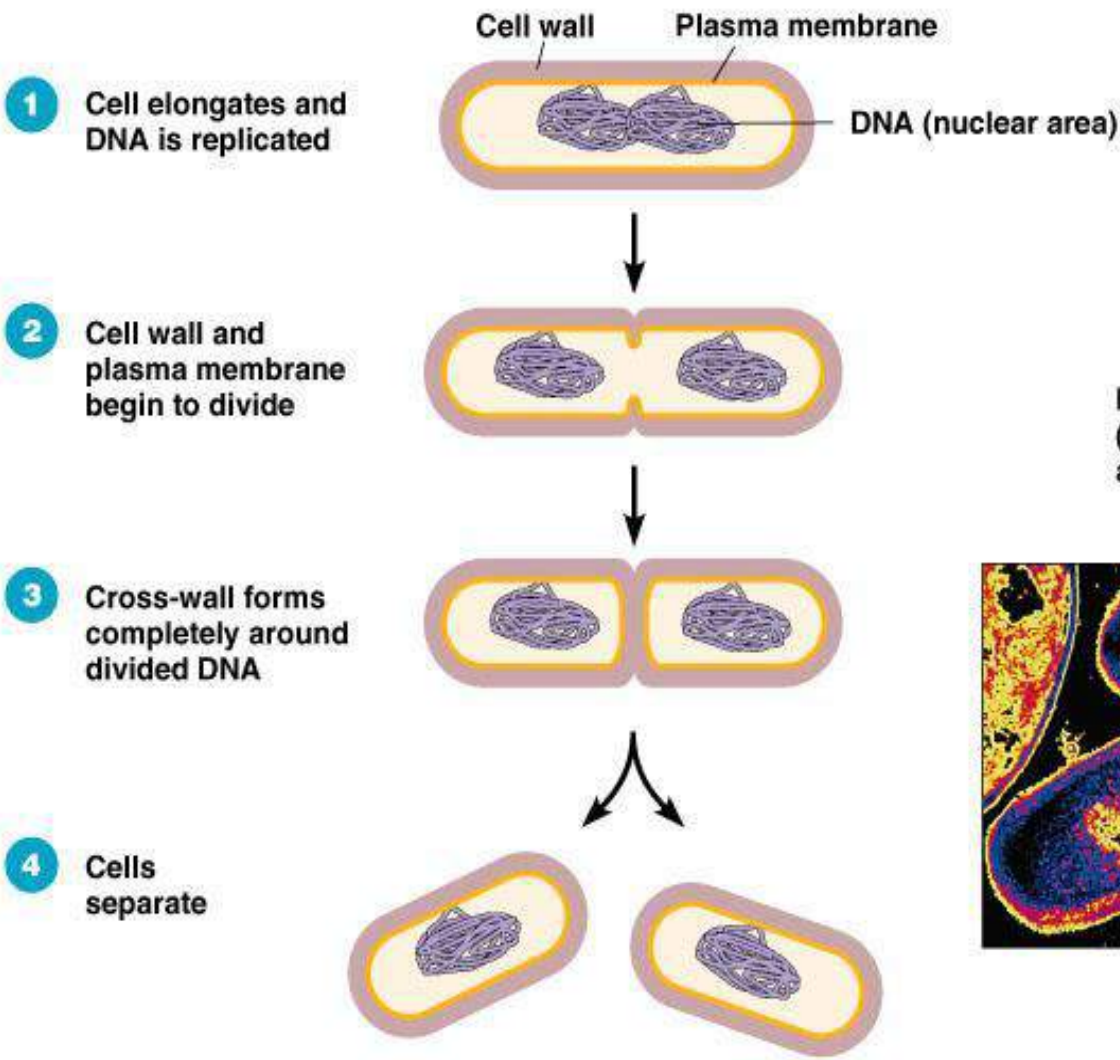




- ◆ Bacteria grow by binary fission to produce identical offspring, which cannot be distinguished as a parent or offspring



Binary Fission



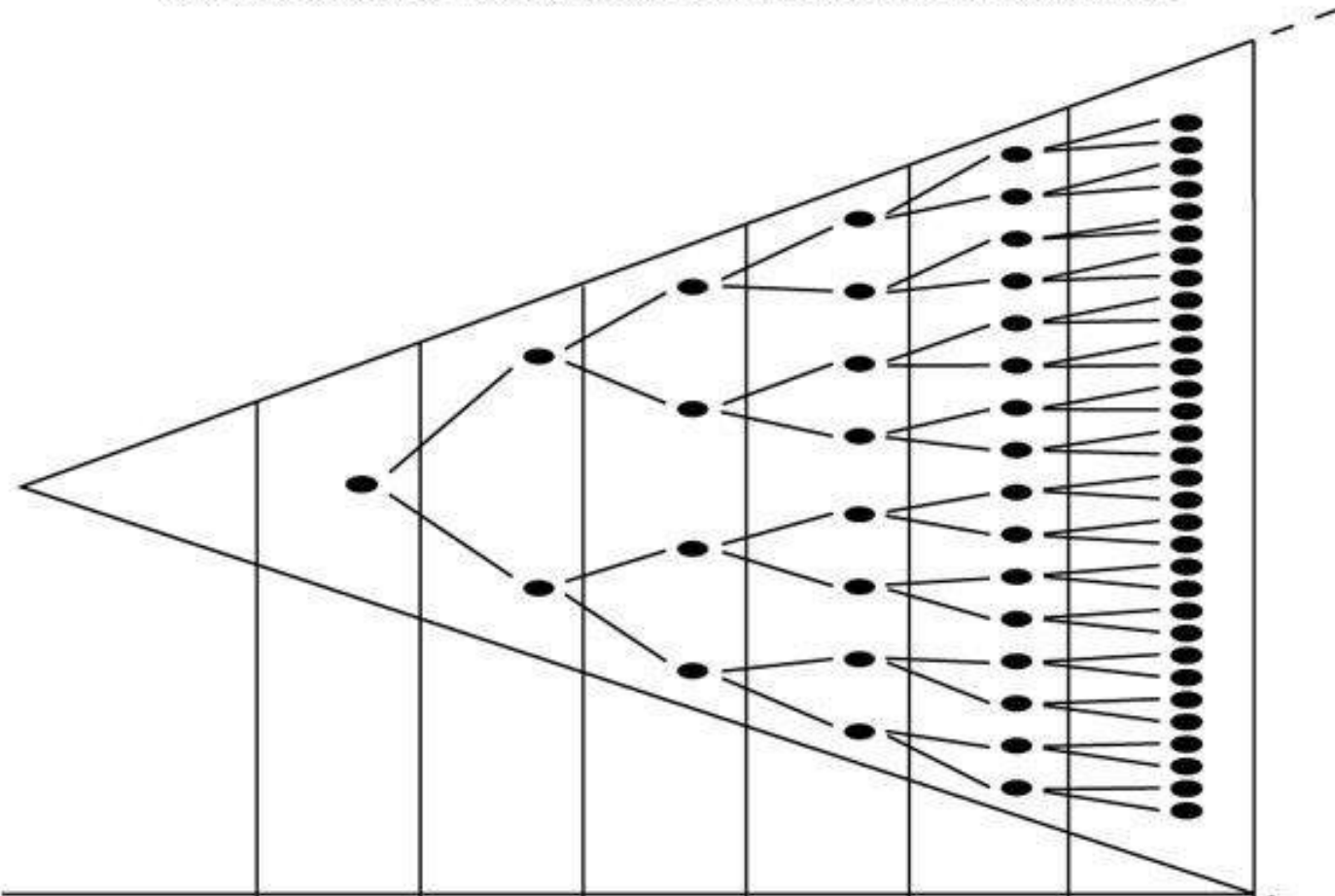
(a) A diagram of the sequence of cell division.

(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.



Generation Time

- ◆ Generation time; is the time it takes for a single cell to grow and divide
- ◆ Average for bacteria is 1-3 hours
- ◆ ***Escherichia coli***: 20 minutes.....20 generations (7 hours), 1 cell becomes 1 million cells!
- ◆ ***Mycobacterium*** much slower: (12-24h)



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)

(a)



Phases of Growth

Four main growth phases

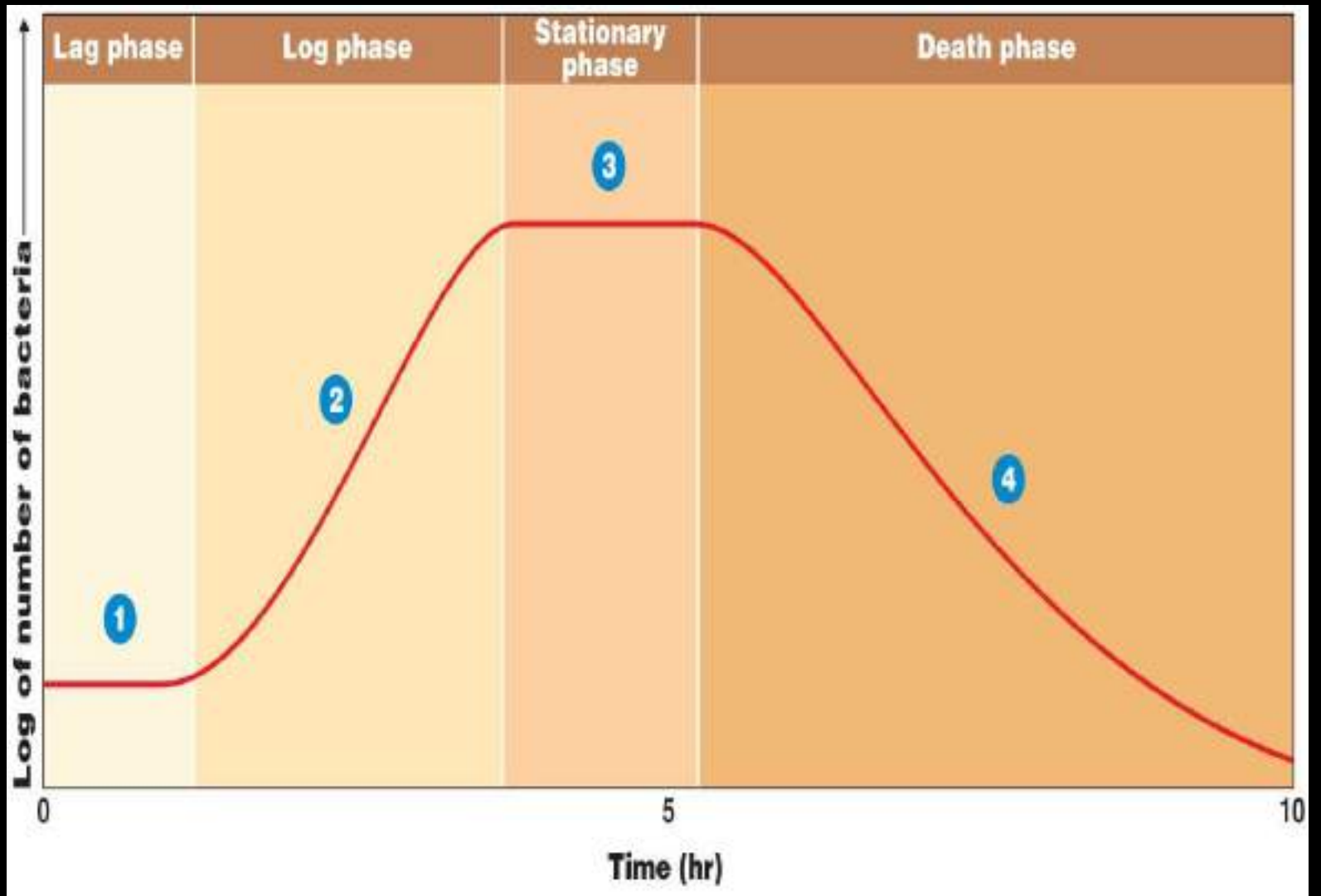
- Lag phase
- Exponential (Log) phase
- Stationary phase (Post-exponential)
- Decline phase




In **lag phase**, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide.

During the lag phase synthesis of RNA, enzymes and other molecules occurs.


In this phase cells change very little because the cells do not immediately reproduce in a new medium. During this phase cells are not dormant.






The **log phase** (sometimes called the logarithmic phase or the *exponential phase*) is a period characterized by cell doubling.

The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period.




Exponential growth cannot continue indefinitely,
because the medium is soon depleted of nutrients
and enriched with wastes.



The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid.

Stationary phase results from a situation in which growth rate and death rate are equal.



Mutations can occur during stationary phase. **DNA damage** is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria.

Endogenously generated **reactive oxygen species** appear to be a major source of such damages.



- ◆ At **death phase** (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.



- **Media for bacterial growth**

- For identification of bacteria, a culture is obtained by growing the organisms on artificial culture media.

- **Types of culture media**

- Simple media
- Enriched media
- Selective media
- Differential media



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a. Simple media

- Contain basic nutrients for bacterial growth like **broth** with peptone, e. g. **Nutrient broth**

b. Enriched media

- Enriched by some substances like: Blood & Serum, e.g. Blood **agar**, Chocolate **agar**

c. Selective media

- Contain substances such as bile salts or antibiotics that inhibit the growth of some organisms but have little or no effect on the required organism.

- 
- e. g. Salmonella Shigella agar.

d. Differential media

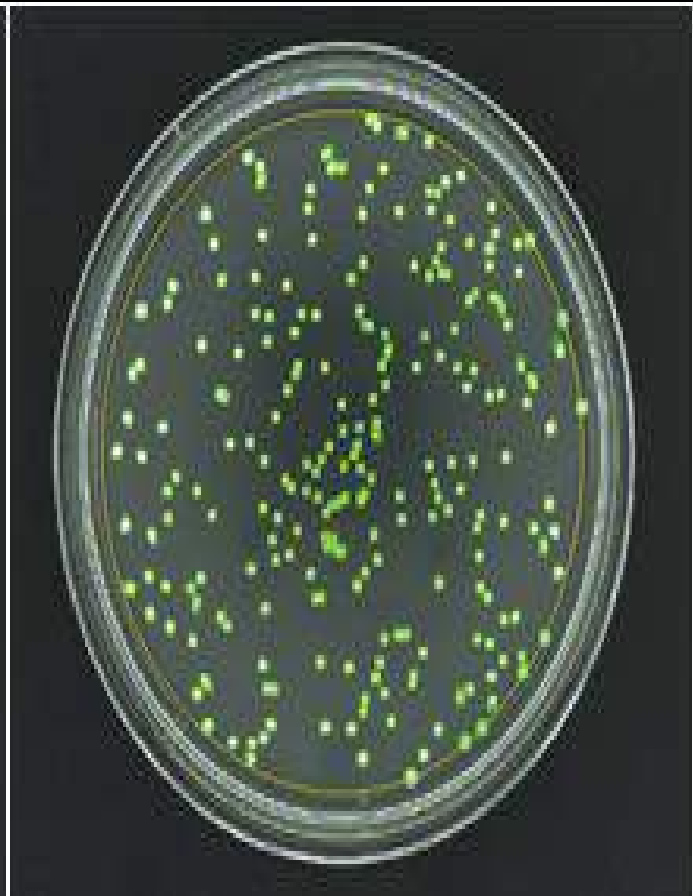
- Differential shows up as visible changes, variations in colony size or in media color, or in the formation of gas bubbles or precipitates, e.g. MacConkey agar





Methods used to measure microbial growth

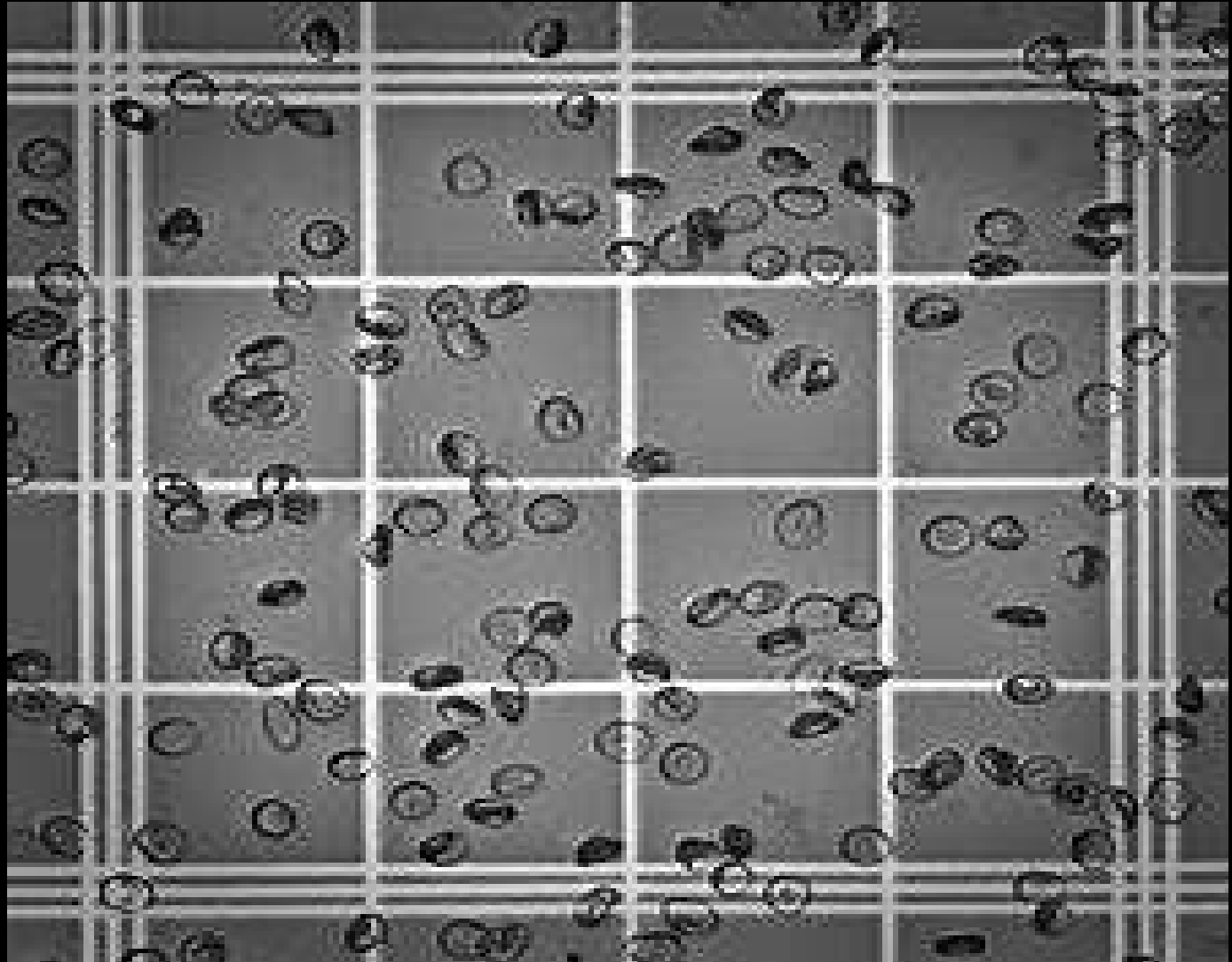
- Count colonies on plate (counts live cells)
- Microscopic counts
- Flow cytometry
- Turbidity



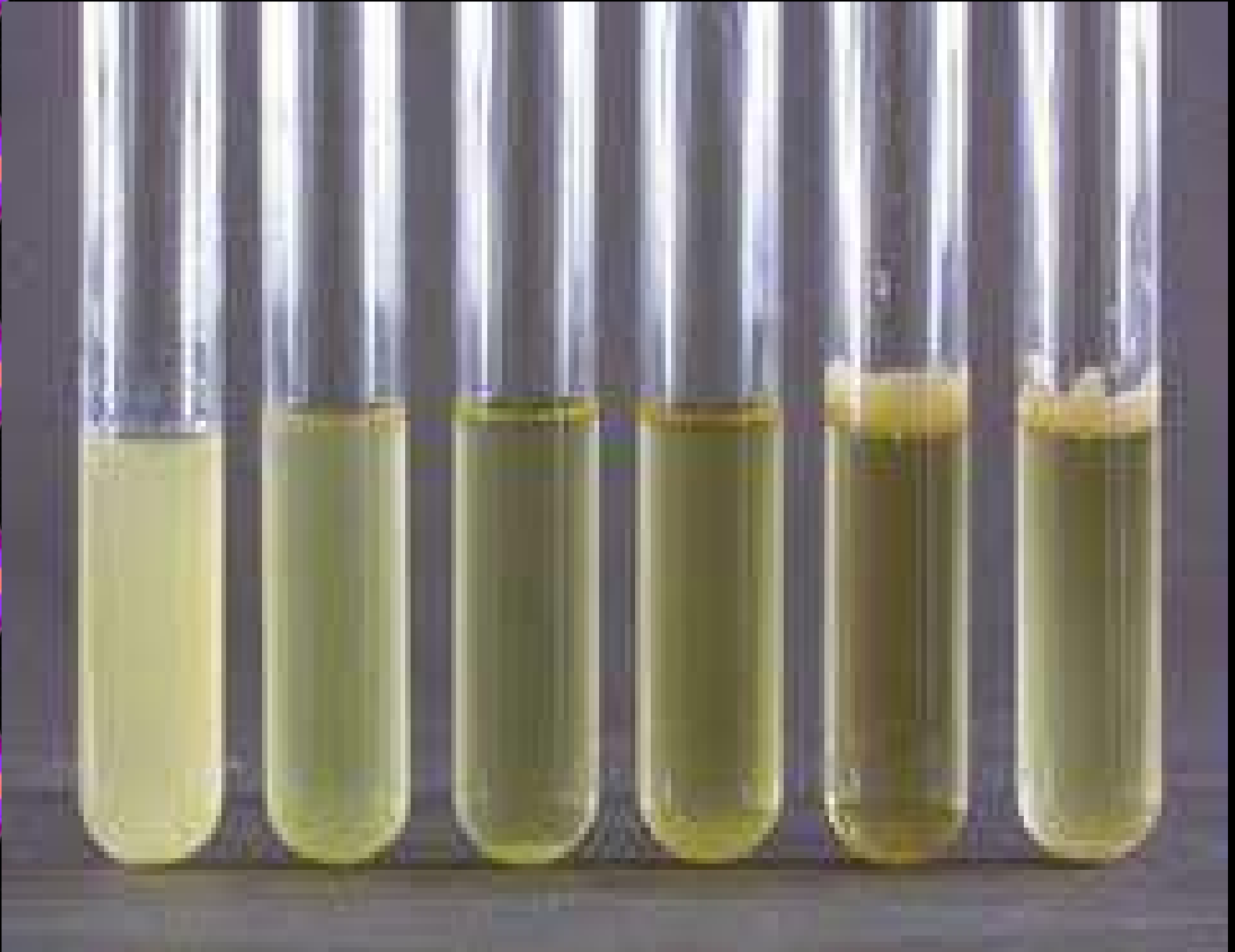
- Count colonies on plate (counts live cells)



- Microscopic counts



- Turbidity





Requirements for Growth

- Bacteria must obtain or synthesize **Amino acids**, **Carbohydrates & Lipids** => build up the cell.

Requirement of growth included:

1. Nutrients
 2. Temperature
 3. Oxygen
 4. pH (**potential of hydrogen**)
 5. Osmotic pressure
- Growth requirements & metabolic by-products
=> **Classify different bacteria.**



1. Nutrient


- Carbon sources
- Nitrogen sources
- Inorganic salts and trace elements
- Growth factors
- Water



Nutritional types of bacteria

A. Depend on how the organism obtains **carbon** for synthesizing cell mass divided into:

- ♦ autotrophic – carbon is obtained from carbon dioxide (CO₂)
- ♦ heterotrophic – carbon is obtained from organic compounds
- ♦ mixotrophic – carbon is obtained from both organic compounds and CO₂



B. Depend on how the organism obtains **reducing equivalents** used either in energy conservation or in biosynthetic reactions:


- ♦ **lithotrophic** – are obtained from **inorganic compounds**

- ♦ **organotrophic** – rare obtained from organic compounds

C. Depend on how the organism obtains **energy** for living and growing:

- ♦ **chemotrophic** – energy is obtained from **chemical compounds**

- ♦ **phototrophic** – energy is obtained from light



◆ **chemolithoautotrophs** obtain energy from chemical compounds, red. eq. from inorganic compounds and carbon from CO₂ . e.g.: Knallgas-bacteria

◆ **photolithoautotrophs** obtain energy from light, reducing equivalents from inorganic compounds and carbon from CO₂. e.g.: Cyanobacteria

◆ **chemolithoheterotrophs** obtain energy chemical compounds and red. eq from inorganic compounds, carbon by organic compounds . e.g.: Nitrobacter spp

◆ **chemoorganoheterotrophs** obtain energy, carbon, and reducing equivalents from organic compounds. e.g.: most bacteria, e. g. Escherichia coli

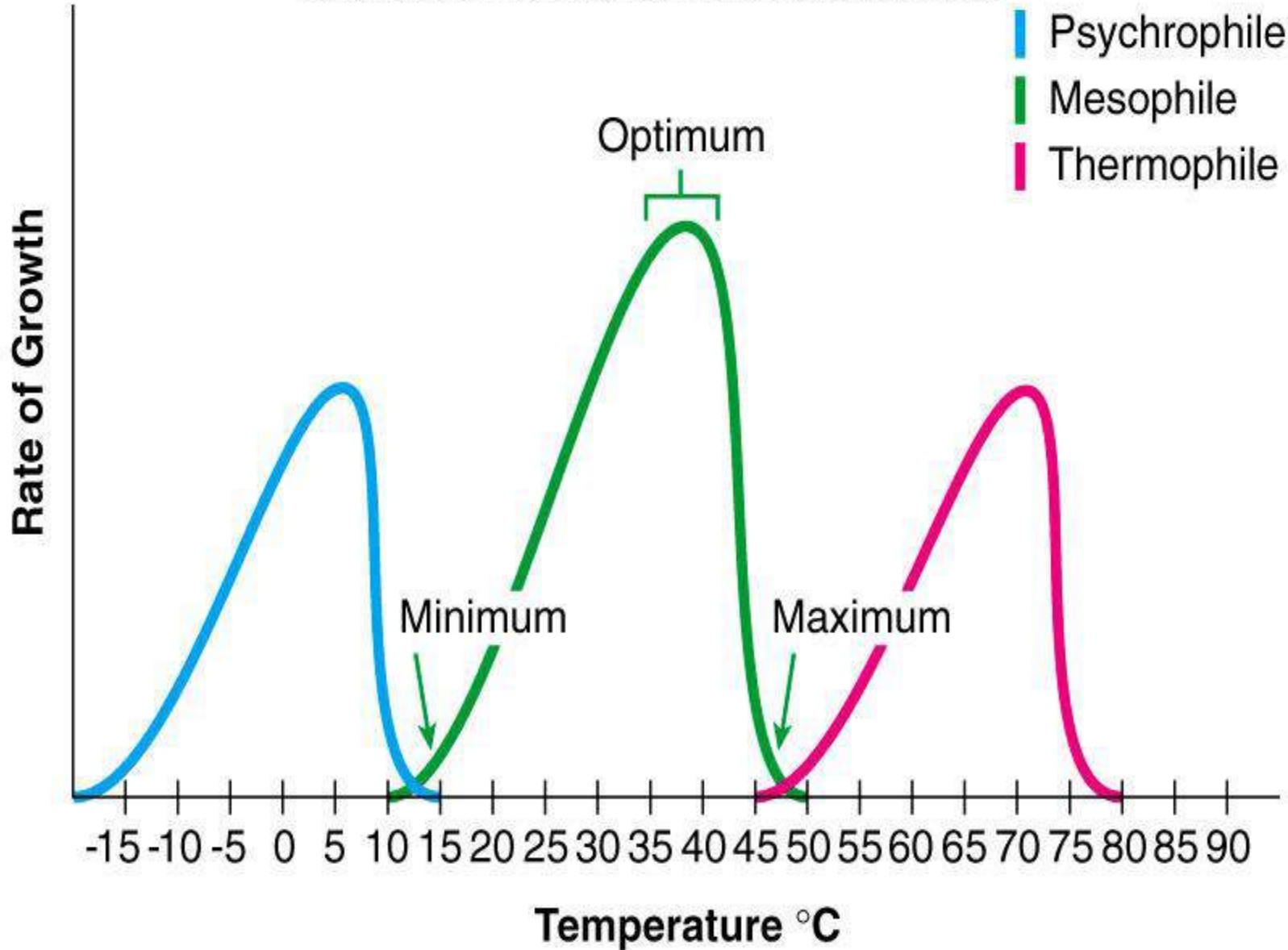


2. Temperature

- ◆ Psychrophiles: cold-loving, can grow at 0 C.
- ◆ Mesophiles: moderate temperature-loving
(Most bacteria)
 - Include most pathogens.
 - Best growth between 25 to 40 C.
 - Optimum temperature commonly 37C.
 - Many have adapted to live in the bodies of human.
- ◆ Thermophiles: heat-loving



- Optimum growth between 50 to 80 C.
- Many cannot grow below 45 C.
- Adapted to live in sunlit soil and hot springs.



- Psychrophile
- Mesophile
- Thermophile

Rate of Growth

Temperature °C

Optimum

Minimum

Maximum



3. Oxygen

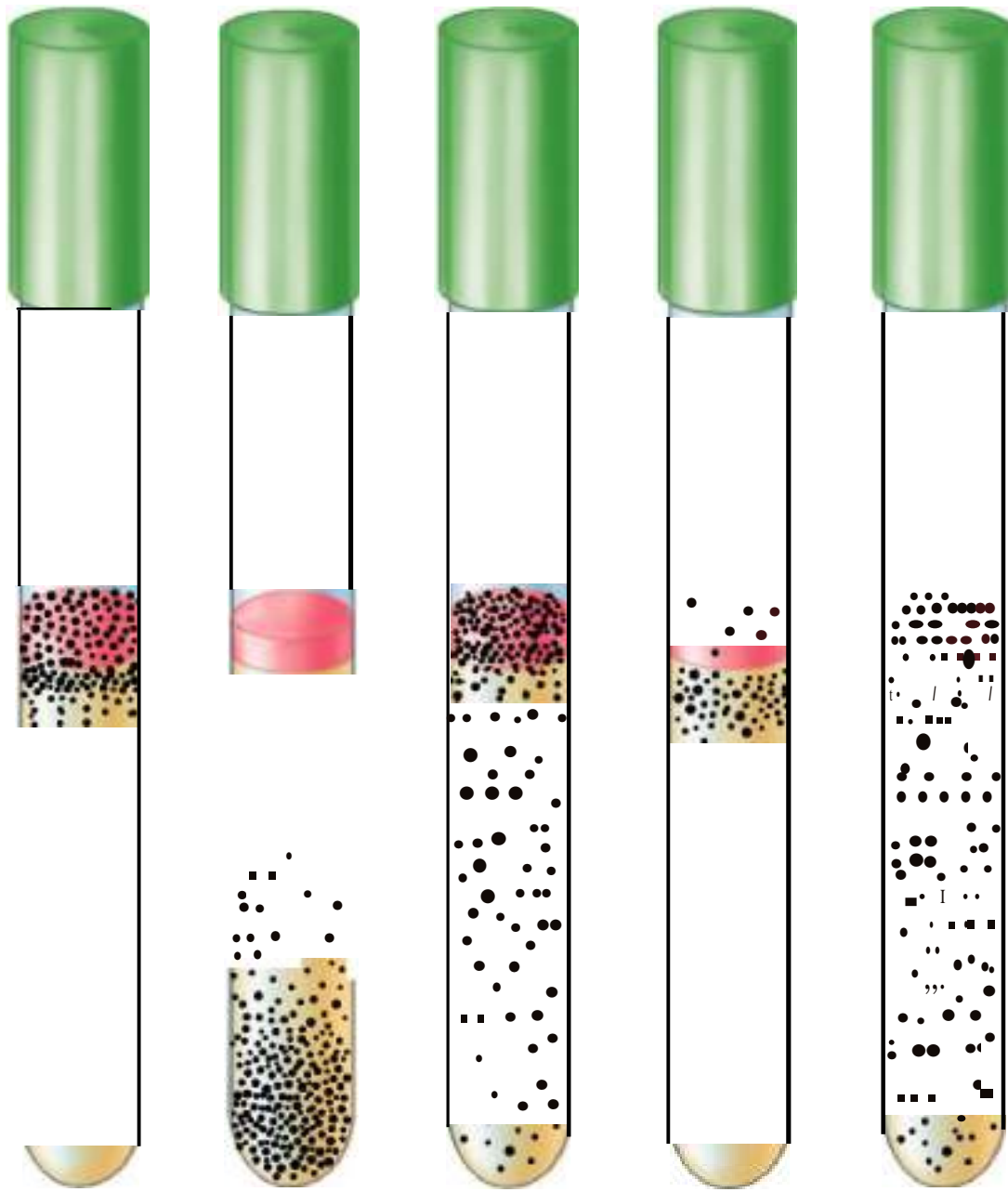
(a) Obligate aerobes – require O_2

(b) Obligate anaerobes – die in the presence of O_2

(c) Facultative anaerobes – can use O_2 but also grow without it

(d) Microaerophilic -requires lower oxygen to survive.

(e) Aerotolerant anaerobe: tolerate the presence of oxygen but does not require it for its growth



(a)

(b)

(c)

(d)

(e)



4. pH

Organisms can be classified as:

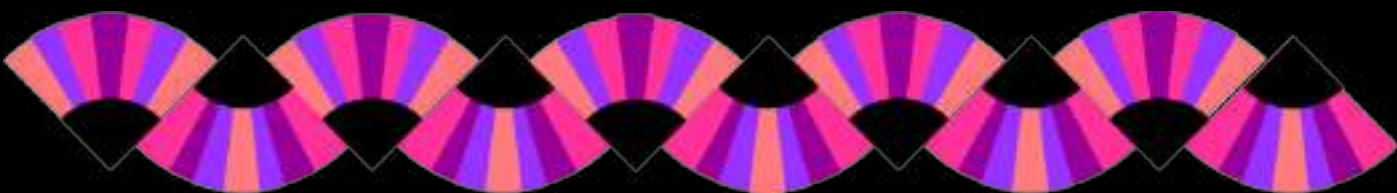
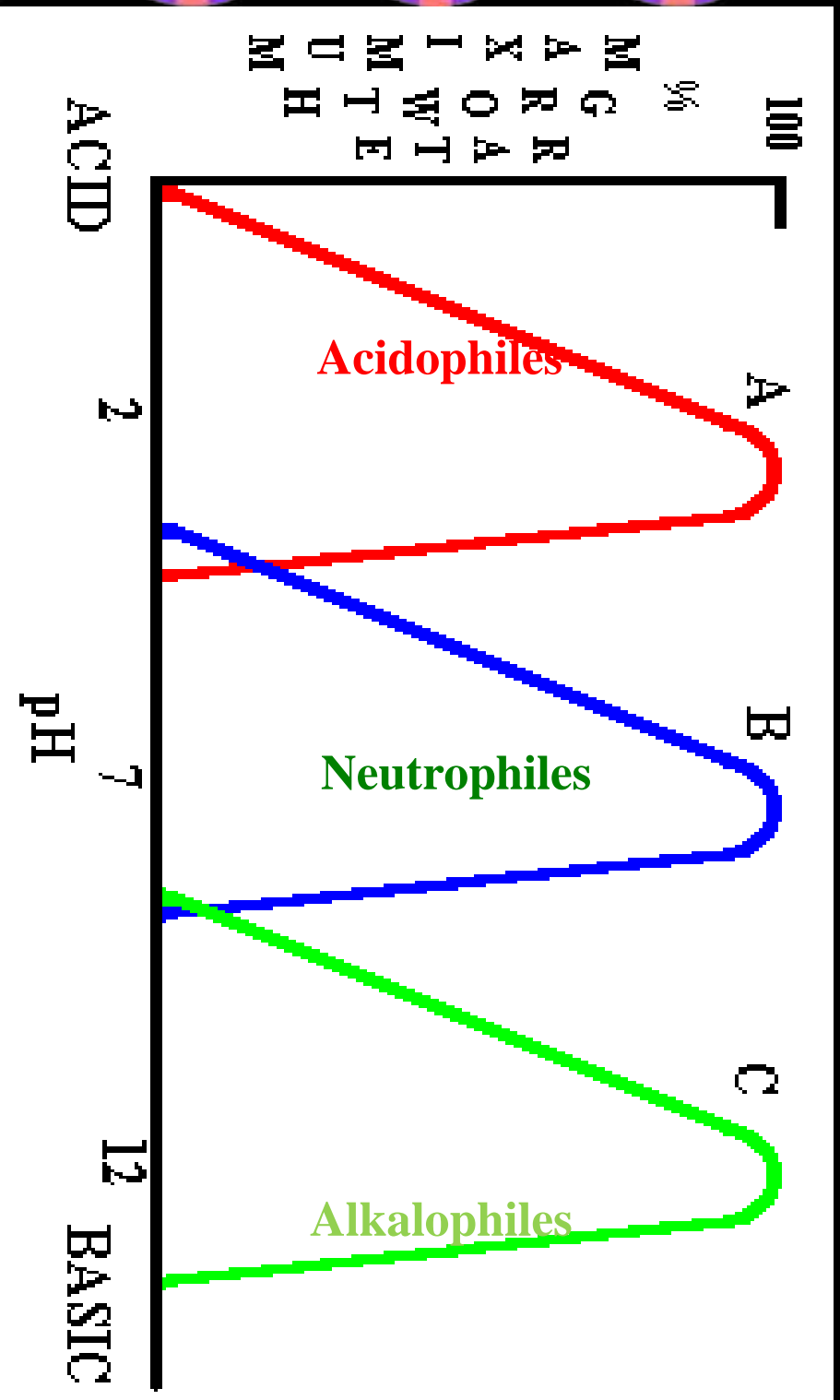
- ◆ **Acidophiles**: “Acid loving”.
- ◆ Grow at very low pH (0.1 to 5.4)
(many fungi).

- ◆ **Neutrophiles**:
 - Grow at pH 5.4 to 8.5.
 - Includes most human pathogens.



- ◆ Alkaliphiles: “Alkali loving”.
 - Grow at alkaline or high pH (7 to 12 or higher)
 - *Vibrio cholerae* -optimal pH 9.
 - Soil bacterium *Agrobacterium* grows at pH 12.


Most bacteria grow between pH 6.5 and 7.5



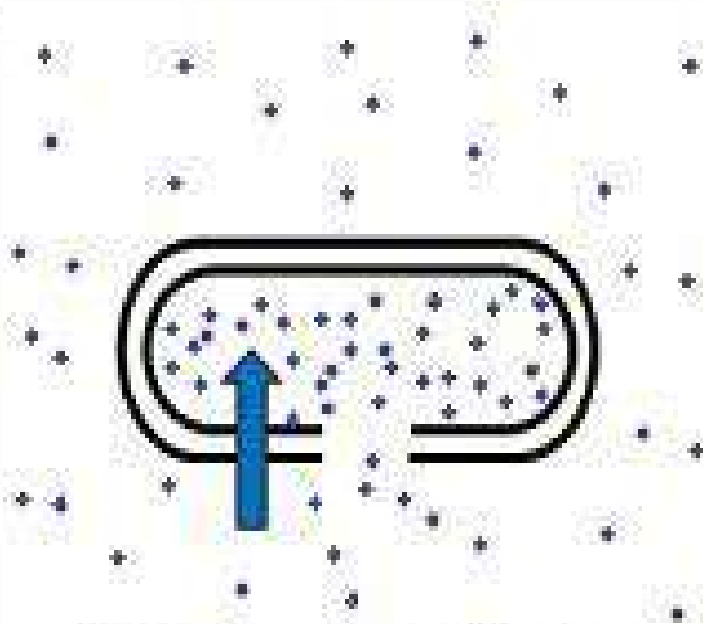


5. Osmotic Pressure

- ◆ Microbes require minerals or nutrients for their growth, which can be obtained from the surrounding water.
- ◆ Osmotic pressure and salt concentration of the solution can influence bacterial growth. The bacterial cell wall gives a mechanical strength that allows the bacteria to withstand alternations in the osmotic pressure.
- ◆

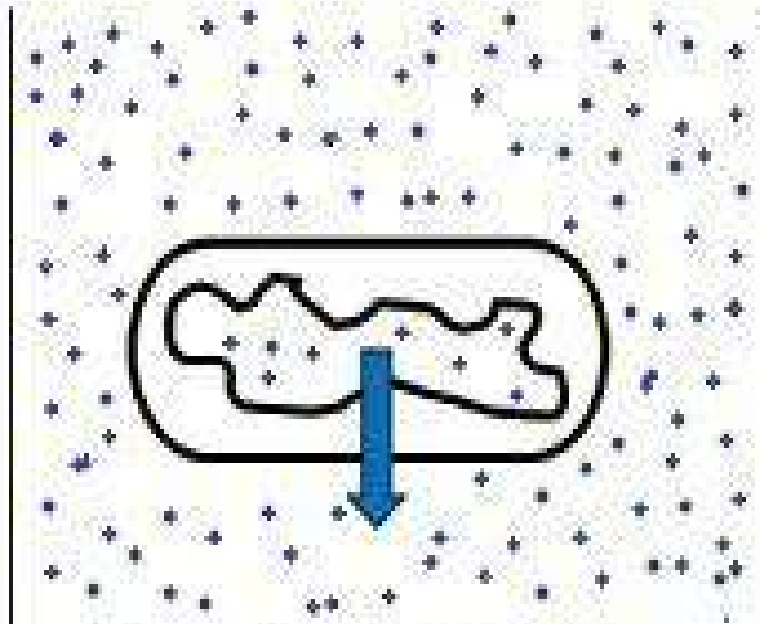
- 
- ◆ **Osmophilic bacteria** requires high osmotic pressure. When the bacterial cell is subjected to the **hypertonic solution**, it may cause osmotic removal of water, resulting in **plasmolysis** or osmotic shrinkage of the protoplasm.
 - ◆ In contrast, when the bacterial cell is subjected to the hypotonic solution, it may cause excessive imbibition of water resulting in **plasmolysis** or cell bursting.
 - ◆

EFFECT OF OSMOTIC PRESSURE TO BACTERIAL CELL



Hypotonic solution

Causes osmotic cell lysis



Hypertonic solution

Causes osmotic
plasmolysis

BIOLOGY READER

Metabolism

– Sum up all the chemical processes that occur within a cell

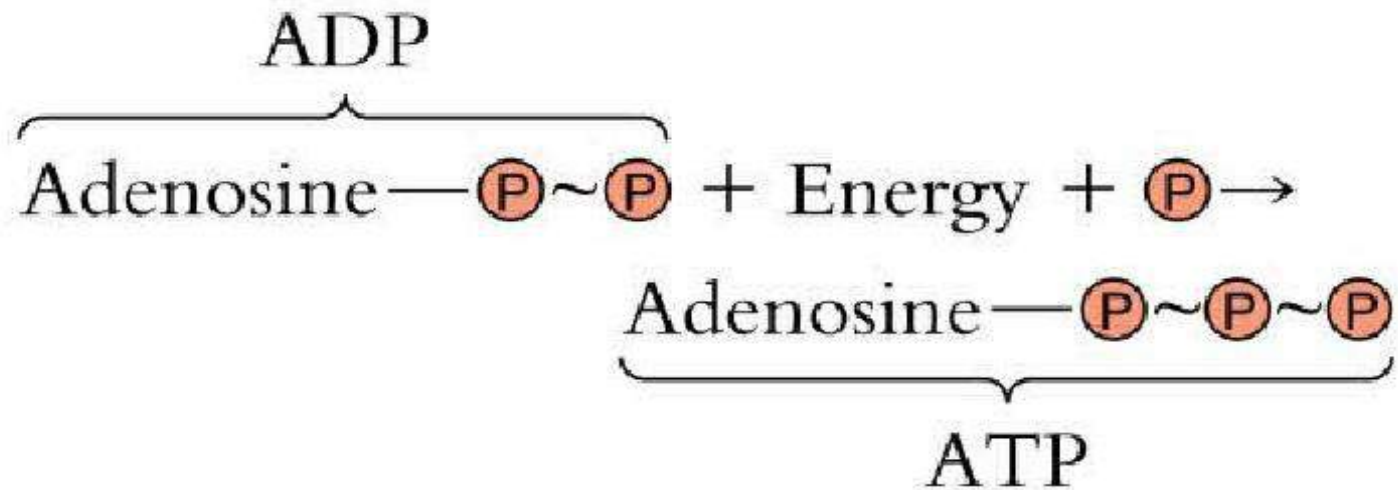
1. Anabolism: Synthesis of more complex compounds and use of energy

2. Catabolism: Break down a substrate and capture energy for growth and maintenance.

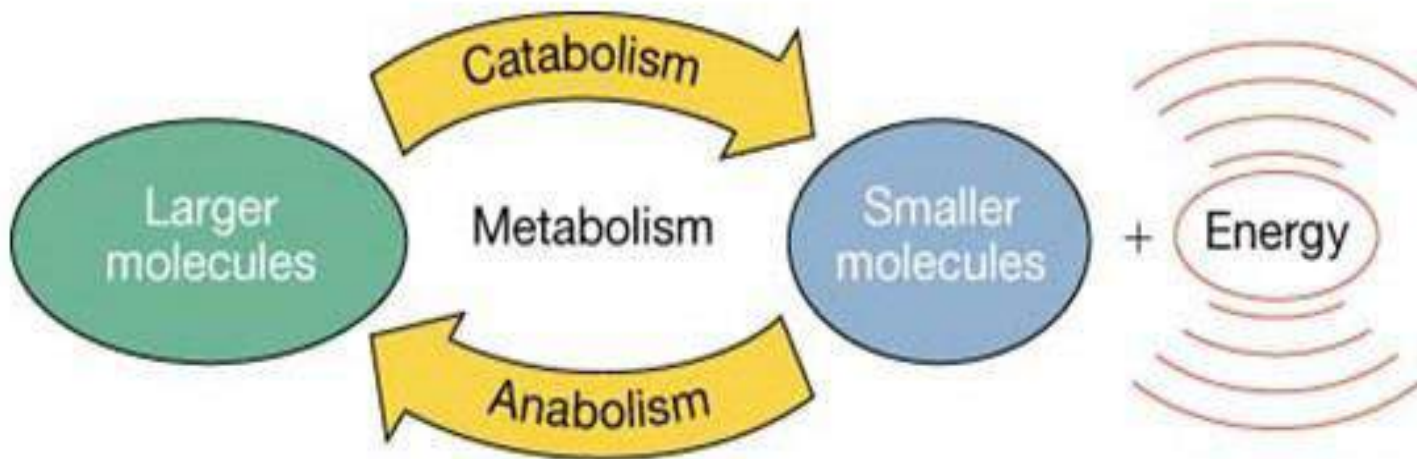
– All cells require the energy supply to survive. The common energy form => **ATP (Adenosine Tri-Phosphate)**

ATP

is generated by the phosphorylation
of ADP



Metabolism Relationships





Microbial metabolism


-Is the means by which a microbe obtains the energy and nutrients, it needs to living and reproduce.

-Microbes use many different types of metabolic strategies, and microbes species can often be differentiated from each other based on metabolic characteristics.



Metabolism of Glucose

- Bacteria can metabolism of glucose, proteins or lipids.
- Bacteria can produce energy from glucose. Glucose breakdown (Glycolysis) can be aerobic (using oxygen) or anaerobic (without oxygen).
- Anaerobic metabolism** of glucose is also known as anaerobic glycolysis or **fermentation**.
- Aerobic metabolism** of glucose is known as aerobic glycolysis and **respiration**.



Catabolism/Aerobic Respiration of Glucose

**The breakdown of carbohydrates to release
energy**

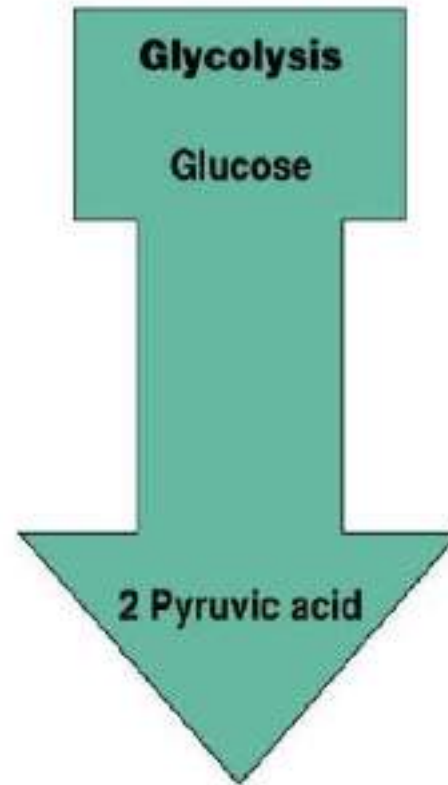
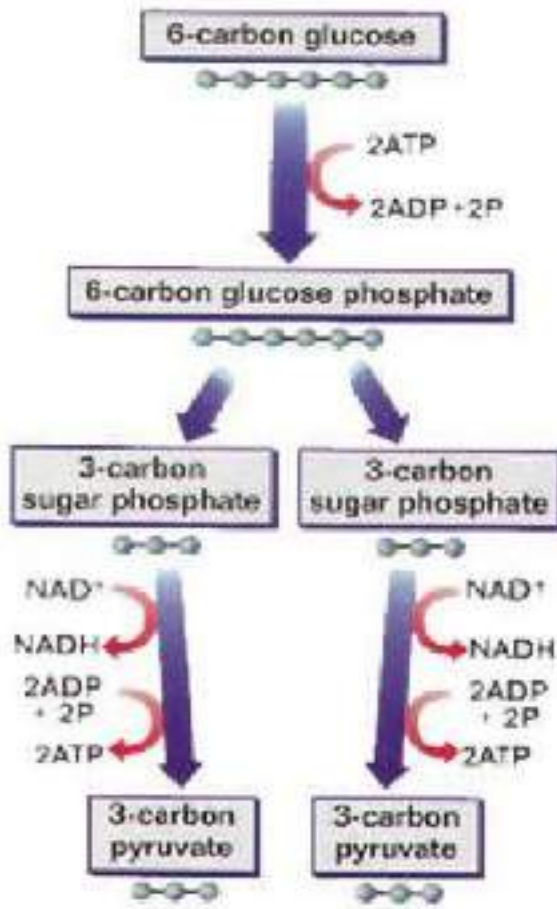
- Glycolysis
- Krebs cycle
- Electron transport chain



Glycolysis

- Glycolytic pathway, the Embden-Meyerhof-Parnas pathway.
- A nine-step biochemical reactions, each of which requires specific enzymes. Six-carbon molecule of glucose is broken down into three-carbon molecules of pyruvic acid
- Can take place with or without oxygen
- Produces very little energy—only 2 ATP

- Takes place in the Cytoplasm of both prokaryotic and eukaryotic cells.



Metabolism of Glucose



-i) the splitting of glucose to 2 pyruvate (pyruvic acid)


-(i) 2 ATP are used

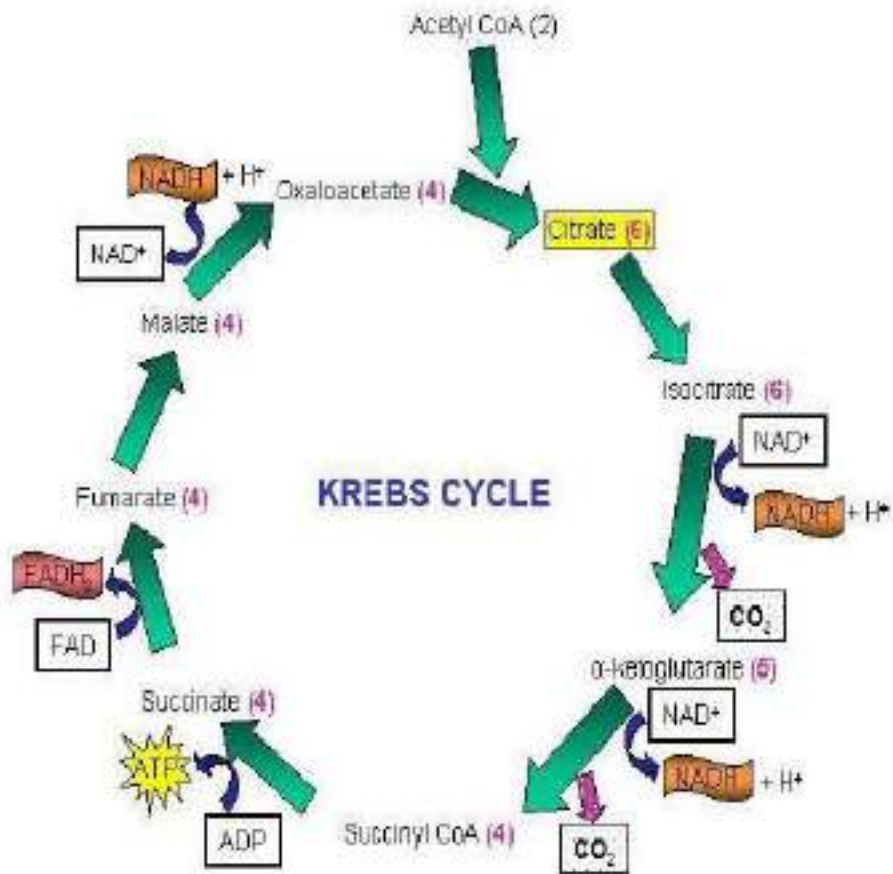
-(ii) 4 ATP are produced (a net gain of 2)

-(iii) 2 NADH (nicotinamide adenine dinucleotide phosphate hydrogen) are produced





Krebs Cycle

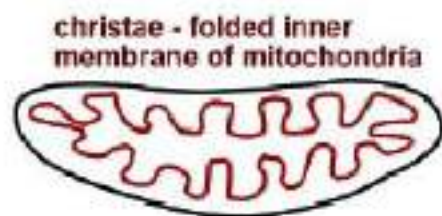
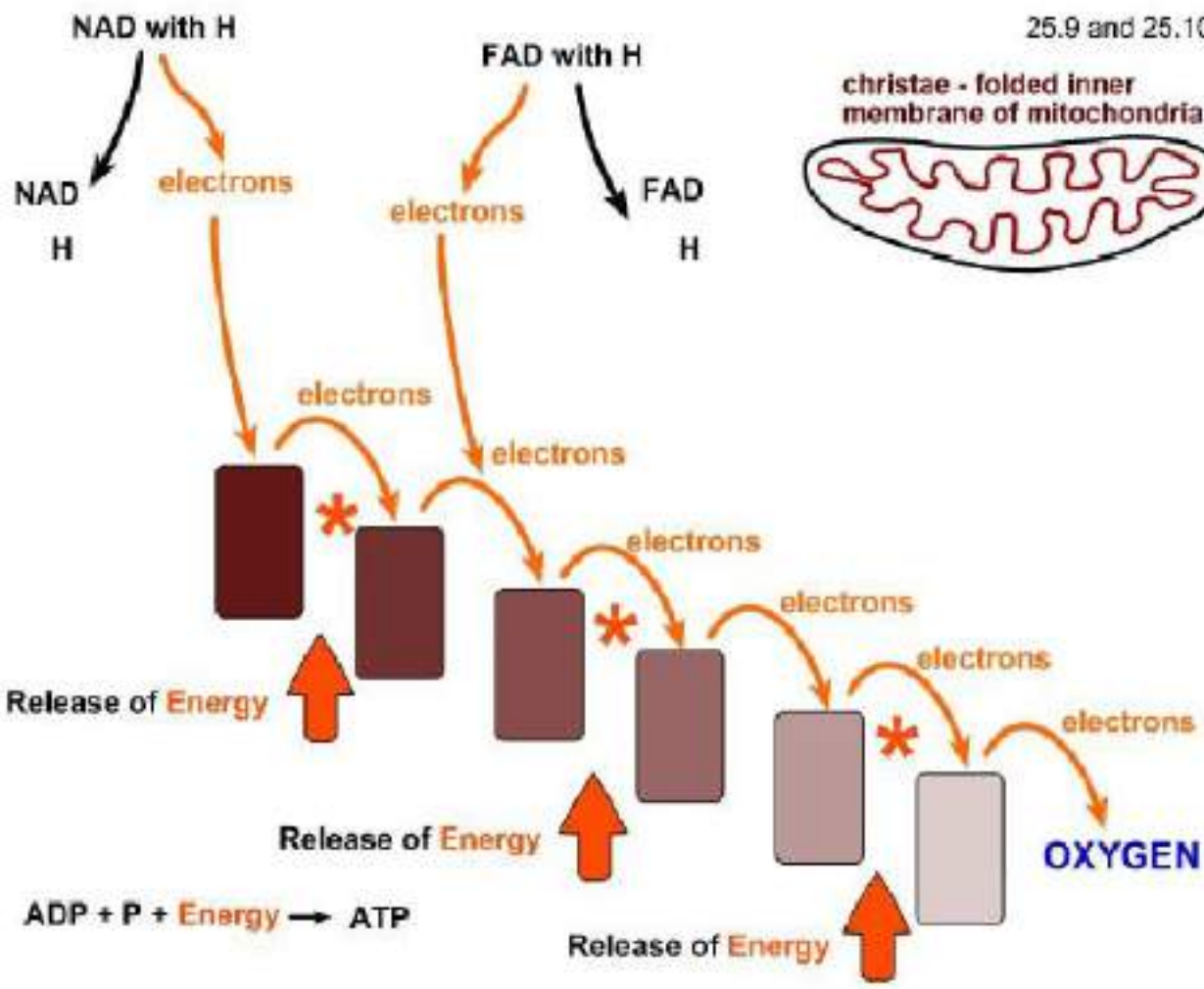
- 
- The pyruvic acid produced during glycolysis are converted into acetyl-CoA.
 - The Krebs Cycle is consists of eight reactions.
 - Acetyl-CoA combine with oxalate to produce citric acid (tricarboxylic acid).
 - Only 2 ATP produced, but a number of products like NADH, FADH₂ and H ions
- Mitochondria (eukaryotes); cell membrane (prokaryotes).




Electron Transport Chain

- 
- Certain of the products produced during the Krebs cycle enter the **electron transport chain**
 - Consist of a series of **oxidation-reduction reactions**, whereby energy is released as electrons are transferred from one compound to another.
 - **Oxygen** is the end of the chain; referred to as the final or terminal electron acceptor.

- 
- **Cytochrome oxidase** enzyme responsible for transferring electrons to oxygen.
 - Produces **32 ATP** in prokaryotic cells, and **34ATP** in eukaryotic cells.





Number of ATP Produced From One Molecule of Glucose by Aerobic Respiration

Biochemical pathways	Prokaryotic	Eukaryotic
Glycolysis	2	2
Krebs cycle	2	2
ETC	32	34
Total ATP	36	38

Bacterial genetics

-Genetics : is the study of inheritance and variation .All inherited characteristics are encoded in DNA except RNA viruses .

-The bacterial chromosome :

Contains the genetic information that defines all characteristics of organism.it is single continuous strand of DNA with closed circular structure attached to the cell membrane of the organism.

-Replication:

*Chromosomal replication is an accurate process that ensures that the progeny cells receive identical copies from mother cell.

*DNA replication is the synthesis of new strands of DNA using the original DNA strands as templates .

*DNA replicates by process called semiconservative replication, DNA dependent DNA polymerase is the main enzyme that mediate replication ,while restriction enzymes of bacteria delete foreign nucleotides from their genome .These enzymes are therefore extremely useful in molecular biological technique.

-Gene variation :

Occur by :1- mutation 2- gene transfer

***Mutation** :a change in the base sequence of DNA can be due to either base substitution frame shifts or insertion of additional places of DNA .

***Gene transfer**: may occur by :

1-conjugation : is the mating two bacteria which DNA is transfer from donor to the recipient cell

2- transduction: is the process of DNA transfer by means of bacterial virus(bacteriophage) during the replication of phage ,a piece of bacterial DNA is incorporated accidentally into the phage particle and carried into the recipient cell at the time of infection.

3-transformation :this is transfer of exogenous bacterial DNA from one cell to another it occur in nature when dying bacteria release their DNA ,which is then taken by recipient cell DNA. This process appears to play an insignificant role in disease

4- transposition :this occur when transposable elements (transposons)move from one DNA site to another within the genome of the same organism(e.g. E.coli).

-Plasmids:

are extra chromosomal ,double strand circular DNA molecule capable of independent replication within the bacterial host .

The clinical relevance of plasmids that they code antibiotic resistance ,resistance to heavy metals ,exotoxin production and pili formation .

-Gene :

the genetic code of bacteria is contained a series of units called genes. As the normal bacterial chromosome has only one copy of each gene ,bacteria are called haploid , while contain two copies of gene called diploid .

*A gene is a chain of purine and pyrimidine nucleotides.

***Gene cloning** :is the introduction of foreign DNA into another cell where it can replicate and express itself.

***Gene probes** used in diagnostic microbiology are labeled with chemical radioactively piece of --DNA that can be used to detect specific sequence of DNA of pathogen (in the clinical sample) by pairing complementary bases.

-- Polymerase Chain Reaction (PCR):

Gene cloning technique revolutionized the molecular advance in 1970s , IS a widely used technique that enables multiple copies of DNA molecule to be generated by enzymatic amplification of the target DNA sequence .

*Materials:

The following materials are required:

- 1-the region of the DNA molecule to be amplified
- 2-Taq polymerase (a heat stable enzyme from *T. aquaticus*, a bacteria that live in hot springs)
- 3-deoxyribonucleoside 5- triphosphate dNTP: adenine , guanine ,cytosine ,thymine
- 4-primers(with a known DNA sequence)

* Methods :

- 1- Choose a region of DNA molecule where the nucleotide sequence of borders are known (because two short oligonucleotide must hybridize ,one to each strand of the double helix DNA molecule for the PCR to begin)
- 2- The double strand of DNA molecule is first split into single strand by heating at 94°C (denaturation step)
- 3- The oligonucleotides now act as primers for DNA synthesis and stick (or hybridize)to the region adjacent to target DNA sequence ,thus delimiting the region that is copied and amplified (hybridization step around 55°C)
- 4- The DNA polymerase enzyme (Taq polymerase and the nucleotide are added to the primed template DNA and incubated at 72°C for synthesis of new complementary strands or amplicons (synthesis step)
- 5- The mixture is again heated to 94°C to detach the newly synthesized strands (amplicon)from the template.

- 6- The solution is cooled ,enabling more primers hybridize at their respective positions,including positions on the newly synthesized strand.
- 7- A second round of DNA synthesis occurs (this time on four strands)with the help of Taq polymerase.
- 8- This three step PCR cycle of denaturation ,hybridization, synthesis can be repeated usually 25-30 times (in a thermo cycler)resulting in exponential accumulation of several million copies of amplified fragment (amplicon).
- 9- Finally a sample of the reaction mixture is run through agarose gel electrophoresis system in order to visualize the product,which manifest as a discrete band after staining with ethidium bromide.
- 10- The latter step is obviated in newer variations of PCR such as real time PCR where the amplicon can be identified using labeled probes and labeled flourophores.

***There are several variations of PCR :**

1-Nested PCR 2- Multiplex PCR 3- Real time PCR

*Real time PCR (quantitative)is a valuable tool for identification of bacteria.

*PCR widely used in :study of molecule quantitative of DNA ,rapid clinical diagnostic procedures , amplification of RNA ,and comparison of different genome.

اد.هديل مزهر يونس ا.م.د.جتين عز الدين علي

Host-parasite Relationship

The relation of bacteria to disease

When microorganism first associated with a host, the host is said to be "contaminated". If the microorganisms establish themselves and grow and multiply for period time, the host is said to be "infected". If infection causes damage, the host is said to have an "infectious disease".

Ecological Interactions between Organisms in a Community:

Dynamic interrelationships based on nutrition and shared habitat

SYMBIOSIS: neutral, antagonistic or synergistic relationship between two dissimilar organisms living in close association with each other.

MUTUALISM: mutually beneficial relationship between two species. e.g. 1- certain indigenous enteric microorganisms produce large amount of the B & K vitamins which absorbed through the intestine wall of the human body and used in metabolism. In the same time the intestine provides the microorganisms with favorable Temp., moisture and nutrients for growth. 2- growth of *Lactobacillus arabinosus* and *Strept. faecalis*. *Lactobacillus* produce folic acid and the *Streptococcus* produce phenylalanine, each organism produce a sufficient amount of the factor require by other organism.

COMMENSALISM: relationship between two species in which one is benefited and the other is not affected, neither negatively or positively. e.g. *Veillonella* in the dental plaque require lactate for growth which provided by other dental plaque bacteria but fermenting glucose to produce lactic acid (such as *lactobacilli* & *Streptococi*) the lactic acid used for growth of *Veillonella* while *lactobacilli* & *Streptococci* still unaffected.

(syntrophism – metabolic products of one are useful nutrients for another)

PARASITISM: relationship between two species in which one benefits (parasite) from the other (host); usually involves detriment to the host.

Amphibiosis (opportunistic pathogens): Commensal microorganism of the human body that possess the potential for causing infection disease when conditions becomes favor for their invasion of tissue.

Antibiosis: is a relationship of antagonism. The antagonism among microorganisms is important to the host because it helps control the microbial population and thus helps prevent the over growth of certain microorganism.(e.g. some bacteria produce lethal substances called colicins or bacteriocins which inhibit the growth of other bacteria, also production of antibiotics is an example of antagonism relationship

Synergism: two usually independent organisms cooperate to break down a nutrient neither one could have metabolized alone (This is relationship in which different organisms produce a reaction that none can produce by individual growth.). (e.g. the relationship of *Proteus vulgaris* and *Staph.aureus* when growing separately both organisms ferment glucose resulting in the production acid only. When the species are grown together they produce acid and gas).

Entry of a Microbe

- Need to adhere, penetrate, and then cause damage
- Gain access via portal of entry and may a have preferred portal of entry - *Streptococcus pneumoniae* via GI tract?

Portals of Entry

1-Mucous membranes :First of Entry Portal :

Mucous Membranes: Respiratory (microbes inhaled into mouth or nose in droplets of moisture or dust particles. Easiest and most frequently traveled portal of entry)

- Common cold, Flu, Tuberculosis, Whooping cough, Pneumonia, Measles, Strep Throat, Diphtheria

• **Mucous membranes: G.I. Tract :**

- Salmonellosis (Salmonella sp.)
- Shigellosis (Shigella sp.)
- Cholera(Vibrio cholerae)
- Ulcers(Helicobacter pylori)
- Botulism(Clostridium botulinum)
- Fecal - Oral Diseases
- These pathogens enter the G.I. Tract at one end and exit at the other end.
- Spread by contaminated hands & fingers or contaminated food & water
- Poor personal hygiene.

• **Mucous Membranes of the Genitourinary System:**

- Gonorrhoea(Neisseria gonorrhoeae), Syphilis(Treponema pallidum), Chlamydia(Chlamydia trachomatis), HIV, Herpes Simplex II

• **Mucous Membranes: Conjunctiva**

- Trachoma(Chlamydia trachomatis)

2-Skin: 2nd. Portal of Entry:

Skin - the largest organ of the body. When unbroken is an effective barrier for most microorganisms.

Some microbes can gain entrance thru openings in the skin: hair follicles and sweat glands.

3-Parenteral :3rd. Portal of Entry: Microorganisms are deposited into the tissues below the skin or mucous membranes

Punctures, injections, bites, scratches, surgery, splitting of skin due to swelling or dryness

Preferred Portal of Entry:

- Just because a pathogen enters your body it does not mean it's going to cause disease.

- **pathogens - preferred portal of entry**

- Streptococcus pneumoniae (if inhaled can cause pneumonia, if enters the G.I. Tract, no disease)

- Salmonella typhi (if enters the G.I. Tract can cause Typhoid Fever, if on skin, no disease)

In general the source of infection includes:

1- Exogenous infection:

- Infections due to some microbial species are acquired from ill persons with active or manifest infection (e.g. T.B, leprosy. Whooping cough)

- Healthy carrier:

Convalescent carrier: are persons limits localized infection continues for a period of week or months after clinical recovering from manifest infection.

Contact carrier: those of them who acquire the pathogen from patient.

Paradoxical carrier: those of them who acquired the pathogen from other carriers.

- Infected animals: some pathogens that are primarily parasites of different animal species spread from the infected animal to man and cause human disease such infection are called zoonosis (e.g. anthrax, Brucellosis)

- Soil: a few infection disease of man are caused by microbes derived from soil (e.g. tetanus, gas-gangrene).

2- Endogenous infections: the source of endogenous infection are microorganisms grow as a commensal in the certain site of patient's body and under abnormal condition, these microorganisms cause disease in the other site of the body, e.g. E.coli have a commensalisms relationship and

grow in the intestine as a normal flora but can cause urinary tract infection when invade the urinary tract.

KOCH'S POSTULATES:

Four criteria that were established by Robert Koch to identify the causative agent of a particular disease, these include:

1. The microorganism (pathogen) must be present in all cases of the disease
2. The pathogen can be isolated from the diseased host and grown in pure culture
3. The pathogen from the pure culture must cause the same disease when inoculated into a healthy, susceptible laboratory animal
4. The pathogen must be reisolated from the new host and shown to be the same as the originally inoculated pathogen.

Currently, these four postulates are complemented by another:

5. The antibody to organism should be detected in the patient's serum

Types of bacterial pathogens:

1-Opportunistic pathogens: these rarely cause disease in individual with intact immunological and anatomical defenses. Only when such defenses are impaired or compromised, as a result of congenital or acquired disease or by the use of immune-suppressive therapy or surgical techniques, are these bacteria able to cause disease. Many opportunistic pathogens (e.g. coagulase-negative staphylococci & E.coli) are part of the normal human flora and are carried on the skin or mucosal surface where they cause no harm and may actually have a beneficial effect by preventing colonization by other potential pathogens. However, introduction of these organisms into anatomical sites in which they are not normally found, or removal of competing bacteria by the use of broad-spectrum antibiotics, may allow their localized multiplication and subsequent development of disease.

2 –primary pathogens: these are capable of establishing infection and causing disease in previously healthy individuals with intact immunological defenses.

Microbial Pathogenicity:

The structural and biochemical mechanisms where by microorganisms cause disease.

Numbers of Invading Microbes:

Virulence: The degree of the pathogenicity (measure of pathogenicity).can be measured by:

- ID50: Infectious dose for 50% of the test population
- LD50: Lethal dose (of a toxin) for 50% of the test population
- ID50 and LD50 : are the quantity of organism that will infect or kill 50% of inoculated animals.
- Example: ID50 for Vibrio cholerae 10⁸ cells (100,000,000 cells)
- ID50 for Inhalation Anthrax - 5,000 to 10,000 spores ????

Mechanisms of Bacterial pathogenicity:

- Colonization of surface(adherence)
- Invasion of tissue(invassiveness)
- Production of toxin(Toxigenicity)

Colonization (Adherence Factors):

Adherence alone does not mean that an organism is pathogenic, so the pathogenicity of most microorganisms is related to the sequence of their ability to (adhere, penetrate& multiplication, bring about pathogenic changes that resulting disease)

Once bacteria enter the body of the host, they must adhere to cells of a tissue surface. If they did not adhere, they would be swept away by mucus and other fluids that bathe the tissue surface. Adherence, which is only one step in the infectious process, is followed by development of microcolonies and subsequent steps in the pathogenesis of infection.

The interactions between bacteria and tissue cell surfaces in the adhesion process are complex. Several factors play important roles: surface hydrophobicity and net surface charge, binding molecules on bacteria (ligands), and host cell receptor interactions. Bacteria and host cells commonly have net negative surface charges and, therefore, repulsive electrostatic forces. These forces are overcome by hydrophobic and other more specific interactions between bacteria and host cells. In general, the more hydrophobic the bacterial cell surface, the greater the adherence to the host cell. Different strains of bacteria within a species may vary widely in their hydrophobic surface properties and ability to adhere to host cells.

Bacteria also have specific surface molecules that interact with host cells. Many bacteria have pili, hair-like appendages that extend from the bacterial cell surface and help mediate adherence of the bacteria to host cell surfaces. For example, some E coli strains have type 1 pili, which adhere to epithelial cell receptors containing D-mannose; adherence can be blocked in vitro by addition of D-mannose to the medium. The E coli that cause diarrheal diseases have pilus-mediated adherence to intestinal epithelial cells.

Other specific ligand-receptor mechanisms have evolved to promote bacterial adherence to host cells, illustrating the diverse mechanisms employed by bacteria. Group A streptococci (*Streptococcus pyogenes*) also have hair-like appendages, termed fimbriae that extend from the cell surface. Lipoteichoic acid, protein F, and M protein are found on the fimbriae. The lipoteichoic acid and protein F cause adherence of the streptococci to buccal epithelial cells; this adherence is mediated by fibronectin, which acts as the host cell receptor molecule. M protein acts as an antiphagocytic molecule.

Antibodies that act against the specific bacterial ligands that promote adherence (eg, pili and lipoteichoic acid) can block adherence to host cells and protect the host from infection.

Invasion of tissue (invasiveness)

The ability of organisms to penetrate tissues. The invasion of a host by a pathogen may be aided by the production of bacterial extracellular substance which acts against the host by breaking down primary or secondary defenses of the body.

Examples:

- Hyaluronidase(spreading factor)..... produce by Staph., Strept.,,, Clostridium tetani.
- Collagenase..... produce by Clostridium, Bacteroides
- Lecithinase.... produce by Clostridium
- Catalase.... Produce by T.B, Brucella
- Hemolysins.... Produce by Staph., Strept.

TOXIGENICITY:

The ability of a microorganism to cause disease as determined by the toxin.

1. ENDOTOXIN: a complex bacterial toxin that is composed of protein, lipid, and

polysaccharide (LPS) which is released only upon lysis of the cell. Endotoxins - part of the Gram (-) Bacterial cell wall. Lipid A - Toxin portion of the LPS.

2. EXOTOXINS: a potent toxic substance formed and secreted by species of certain bacteria. Mostly seen in Gram (+) Bacteria. Most genes that code for exotoxins are located on plasmids or phages (LDLD5050 Small Small -- Very potent Very potent 1 mg of 1 mg of Clostridium botulinum Clostridium botulinum toxin can kill 1 million guinea toxin can kill 1 million guinea pigs).

Exotoxins - three types:

- 1-Cytotoxins (kill cells)
2. Neurotoxins (interfere with normal nerve impulses)
3. Enterotoxins(effect cells lining the G.I. Tract)

Many toxins have A-B subunit toxins or type III toxins

A - Active Causes change in host

B – Binding

Superantigens or type I toxins

- Cause an intense immune response due to release of cytokines from host cells
- Fever, nausea, vomiting, diarrhea, shock, death

Membrane-disrupting toxins or type II toxins

Lyses host's cells by:

- Making protein channels in the plasma membrane (e.g., leukocidins, hemolysins)
- Disrupting phospholipid bilayer
- Streptococcus pyogenes Membrane--disrupting. Type II disrupting. Type II Erythrogenic.

Other factors that enhance the pathogenicity of bacteria are

AVOIDING THE HOST DEFENSE

Capsules

Allow some organisms to avoid phagocytosis and digestion

Changing the antigenic determinants

Some organisms can avoid the immune system

Similar proteins

Others avoid the host defense by coating themselves with proteins similar to that coating red blood cells

Special proteins

M protein or protein A of some organisms prevent opsonization

IRON

Most bacteria require iron for certain enzymes to function

In humans

Iron forms a complex with iron-binding proteins that are bacteriostatic

transferrin in blood

lactoferrin in milk and saliva

This bacteriostatic effect is lost when these molecules are saturated with iron some bacteria secrete Siderophores remove iron from the host for their growth and enhance their virulence Examples of siderophores are:

Aerobactin

Enterobactin

The properties which are essential for pathogenicity are:

- Transmissibility
- Infectivity
- Virulence

The pathogens can transmit by:

- Direct transmission of the disease(e.g. syphilis, gonorrhoea)
- From carrier(e.g. Salmonella typhi)
- Transmission by droplets(e.g.T.B, whooping cough)

- By toxin(Food born infection)(e.g. neurotoxin of Clostridium botulinum, Enterotoxin of Staph. aureus)
- By vector insect (arthropod-blood infection)(e.g. mosquito/ malaria, yellow fever, flea/ plague, louse/typhus fever, tick/ Rocky- mountain spotted fever)
- Water born infection(e.g.typoid, cholera)

Wound infection:

Surgical wound infection :it is account approximately a quarter of hospital (nosocomial)infections .it is significant cause of morbidity prolonging the hospital stay of surgical patients and frequently results in death.

A etiology :

Staphylococcus aureus , *E.Coli* ,*pseudomonas aeruginosa* ,*klebsilla spp*,*clostridium spp*, and *bacteroids spp*.

Clinical features:

- 1-reddened wound edge ,with or without pus formation.
- 2-wound abscess may noticed in deeper layer and discharge thru suture line.
- 3-patient may or not be pyrexial depending on the degree of infection .
- 4-may cause septicemia or breakdown of wound necessitating re-suture.
- 5-tetanus or gas gangrene may occur by clostridium spp.

Pathogenesis:

The infection could be :endogenous or exogenous .

The source of exogenous are: person in a joining bed ,or carrier ,member of staff.

The reservoir include :human skin, environmental dust ,air-born ,fomites, bed linen.

Factors affecting the incidence of wound infection:

1-types of wound(clean, contaminated, or infected)

2-length of stay in hospital

3-length of operation

4-foreign body and drain

5-general health of patient.

Burn infection :

Major burns create large ,moist ,exposed surfaces that are ideal for bacterial growth because the protective skin cover has been lost.

Etiology :

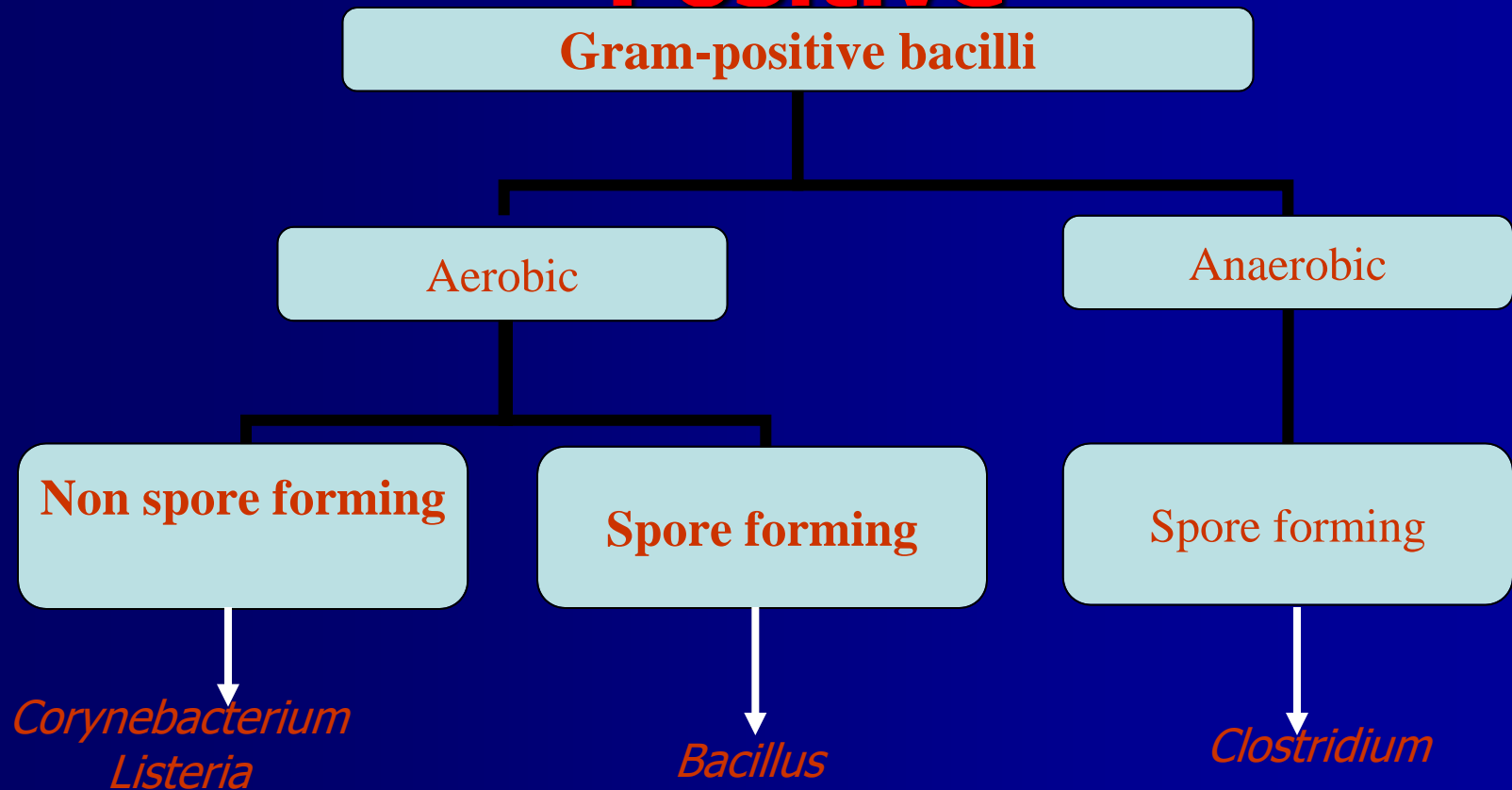
Streptococcus pyogenes ,pseudomonas aeruginosa .staphylococcus aureus

Pathogenesis:

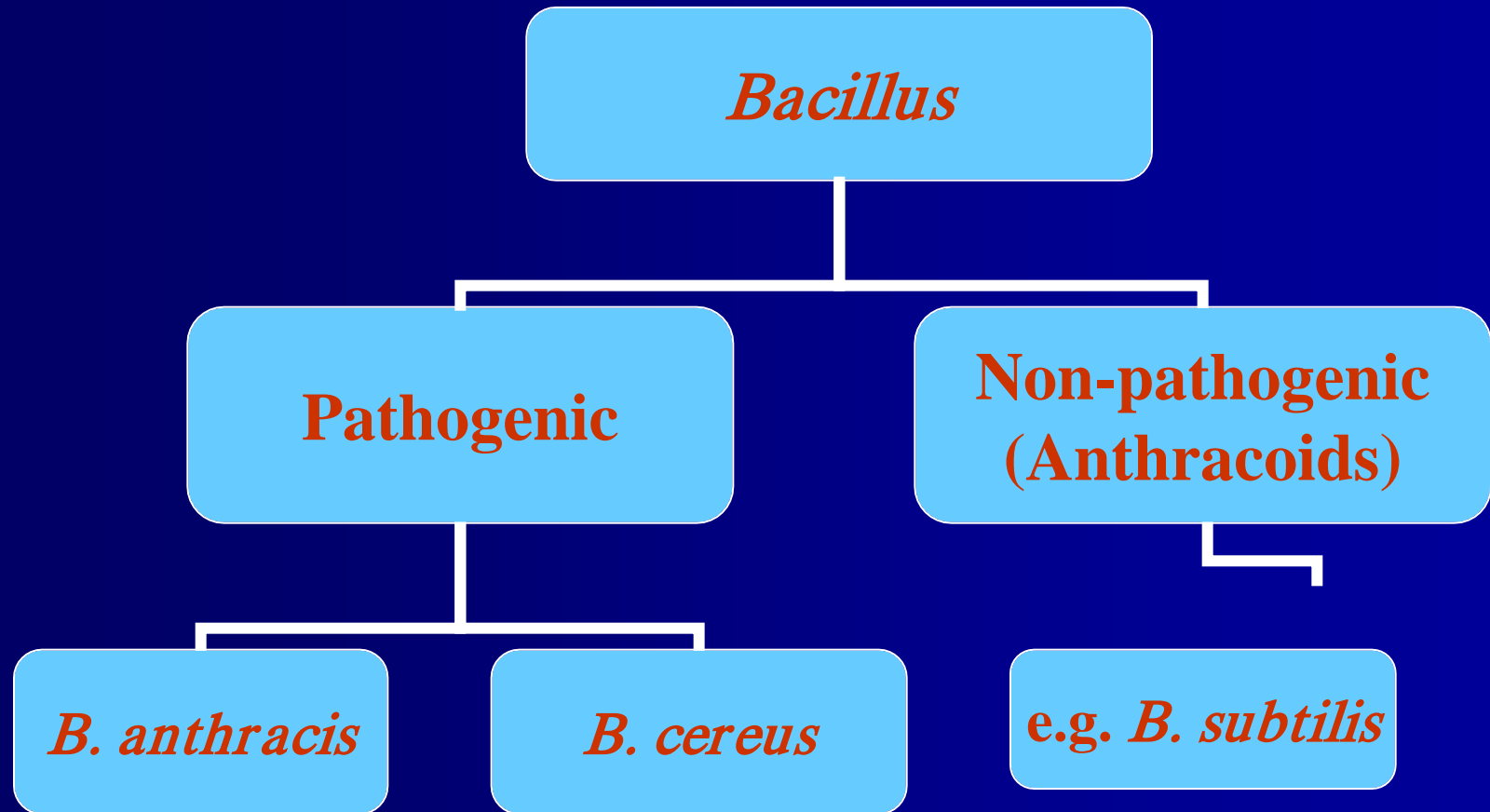
Bacteria colonize burn infection within 24 hrs if appropriate prophylaxis is not given ,with eventual cellulitis of adjacent tissues and septicemia.

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Classification of Gram-Positive



Aerobic Spore Forming *Bacillus spp*



Bacillus Species: General Characteristics

Gram-positive spore-formers ■
vs. non-spore-formers



Bacillus sp.



Corynebacterium sp.

Bacillus species: General Characteristics

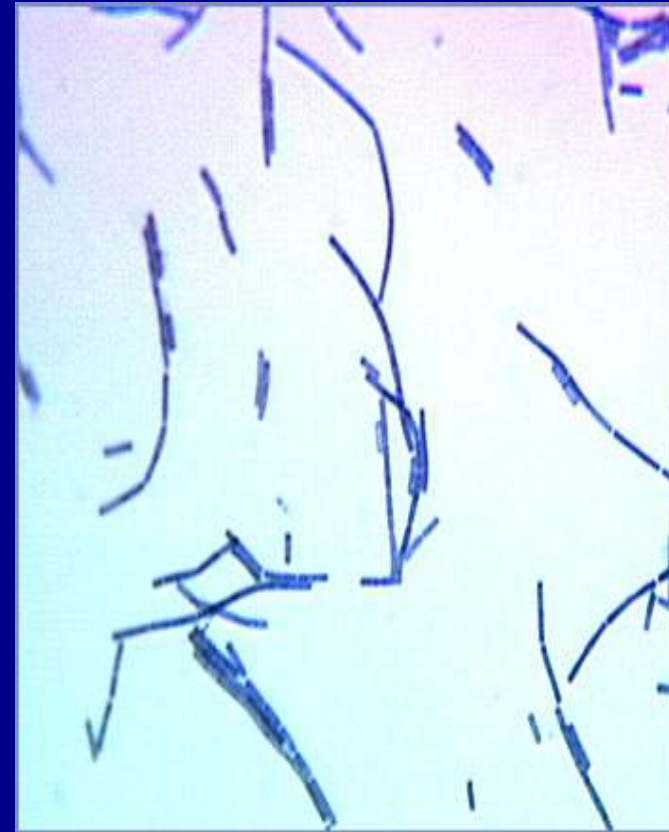
- Found in nature
- Most are saprophytic and are isolated as contaminants
- *Bacillus anthracis* as a major pathogen
- Others are opportunists



Bacillus sp. stained with spore stain

General Characters of *Bacillus spp*

- Very large Gram positive bacilli
- 1-1.2 μm in width x 3-5 μm in length
- Arranged in long chains
- Motile except *B. anthracis*
- Spore forming (outside the host)
- Capsulated (inside the host)
- Non Fastidious
- Facultative anaerobic
- Breakdown glucose by oxidative and fermentative i.e. O+/F+
- Catalase positive
- It is found in soil habitats



Bacillus anthracis: General Characteristics

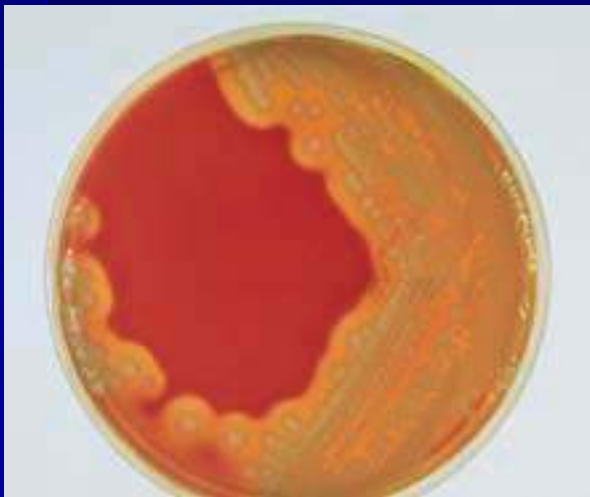
Morphology ■

- Large, sporeforming gram-positive bacilli –
- Spores viable for up to 50 years –
- Nonhemolytic on sheep blood agar –

Other *Bacillus* species

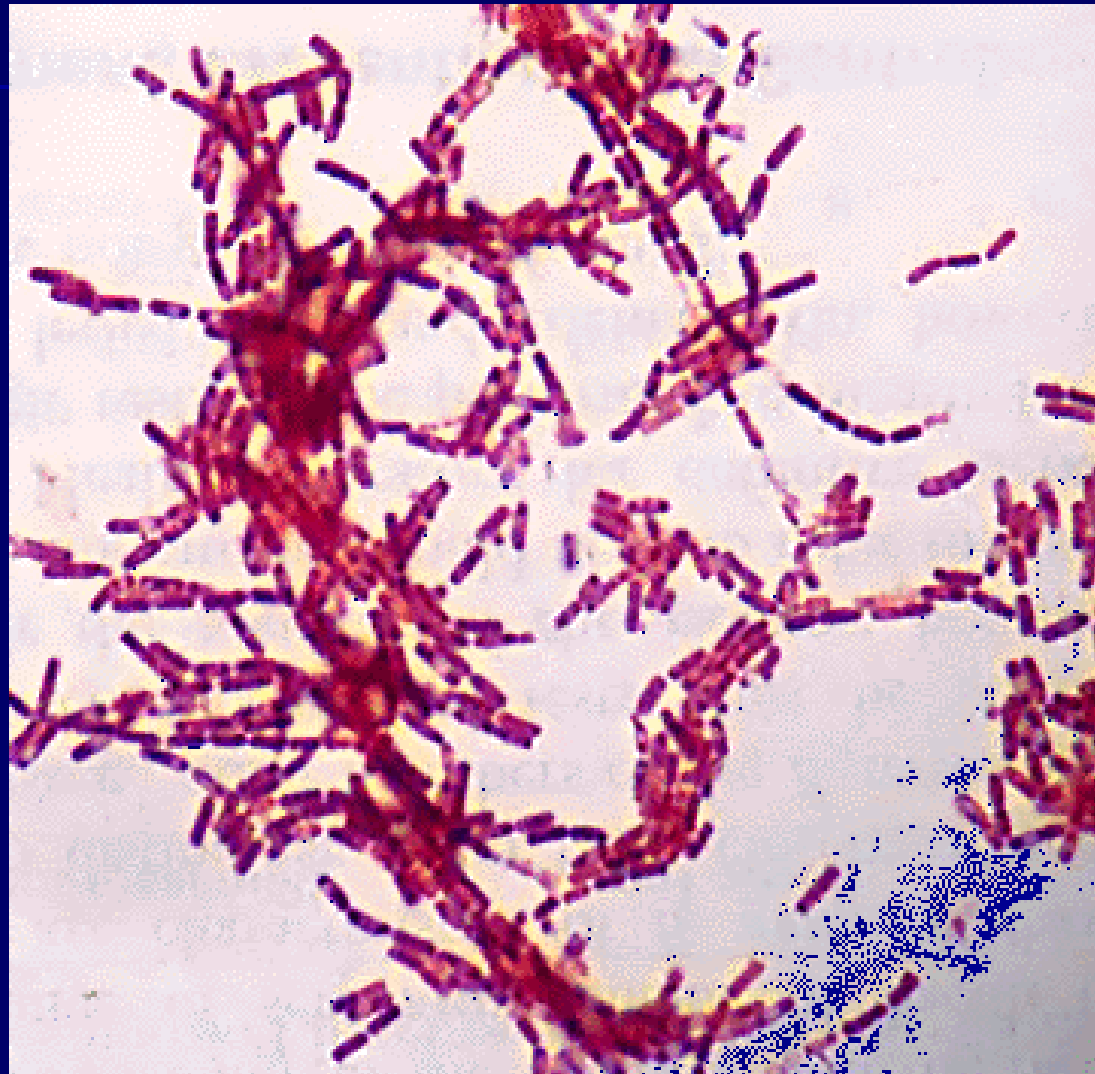
Bacillus subtilis ■

- **Common laboratory contaminant**
- *B. cereus* is a normal inhabitant of soil
- Also isolated from food such as grains and spices
- *B. cereus* causes Two Types of food poisoning



B. cereus colony on blood agar

Gram-Variable Stain of B. cereus with Endospores



Laboratory Diagnosis:

Bacillus anthracis

Microscopic morphology ■

Gram stain: large, square-ended gram-positive rods; may appear end-to-end giving a "bamboo appearance"

Colonial morphology ■

Nonhemolytic on 5% blood agar; raised, large, grayish-white, irregular, fingerlike edges described as "Medusa head" or "beaten egg whites"

Laboratory Identification: *Bacillus anthracis*

Characteristics	<i>B. anthracis</i>	<i>B.cereus</i>
<i>Hemolysis on BAP</i>	=	+
<i>Motility</i>	=	+
<i>String of pearls</i>	+	=
<i>Growth on PEA</i>	=	+
<i>Gelatin hydrolysis</i>	=	+
<i>Susceptibility to Penicillin (10U/ml)</i>	Susceptible	Resistant

Differential characteristics of *B. anthracis* & *B. cereus*

	<i>B. anthracis</i>	<i>B. cereus</i>
Hemolysis	No hemolysis	β -hemolysis
Motility	Non-Motile	Motile

Identification of *Bacillus Spp.*

■ Specimen

- Pastular exudates in malignant pustule
- Sputum in pneumonic anthrax
- Stool in intestinal anthrax (also in food poisoning by *B. cereus*)
 - Stool specimen is emulsified and heated to 80 C to kill non spore forming microorganism

■ Morphology

- Macroscopical (Cultural characteristics)
- Microscopical (Gram Stain, Spore Stain)

Laboratory Characteristics of *Bacillus*

On blood agar ➤

Large, spreading, gray-white colonies, with irregular margins •

Many are beta-hemolytic (helpful in differentiating various *Bacillus* species from *B. anthracis*) •

Spores seen after several days of incubation, but not typically in fresh clinical specimens ➤

Identification of *Bacillus Spp.*

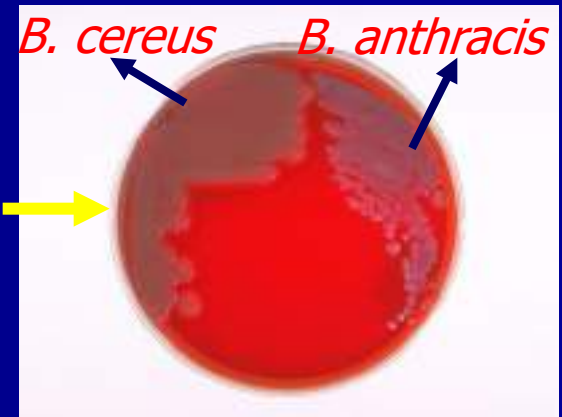
- **Cultural Characteristics**
 - **Grow on nutrient Agar**
 - On ordinary medium
 - Grow aerobically at 37C with characteristic mucoid or smooth colonies, which indicates the pathogenicity of organism (presence of capsule)
 - Rough colonies are relatively avirulent
 - Stab culture on gelatin medium results in inverted fire tree appearance.
 - **Growth on Blood Agar**
 - *Bacillus* species grow well on blood agar showing a double zone of hemolysis
 - *B. anthracis*, which grows well on blood agar without any hemolytic effect.

Cultural Characteristics

Nutrient Agar



Blood Agar



Identification of *Bacillus Spp.*

- **Morphology**

- **Microscopical**

- **Stain**

- **Gram Stain**

- Gram positive bacilli
 - Found in chains
 - Non motile
 - Capsulated inside the host
 - Sporulated outside the host
 - Spore is central, oval and non-bulging



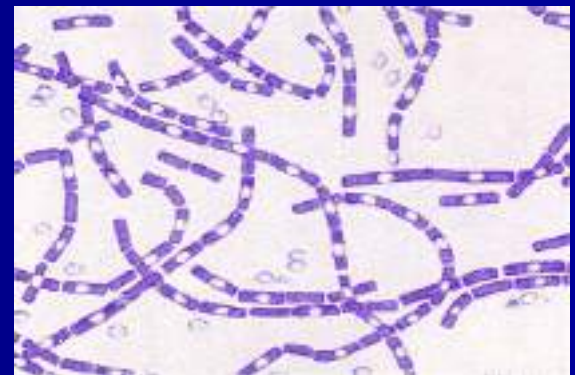
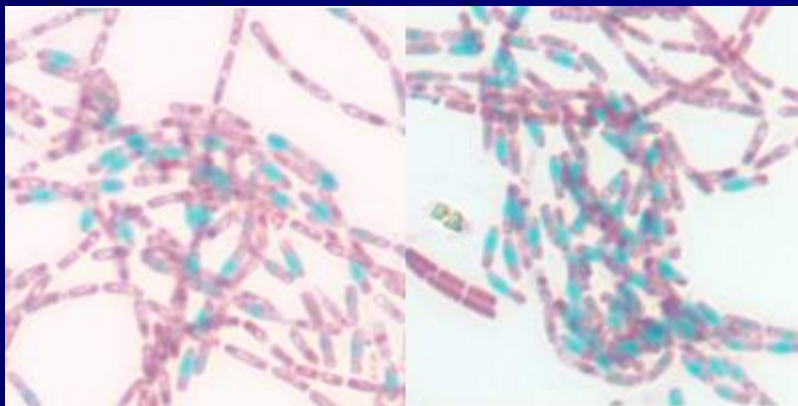
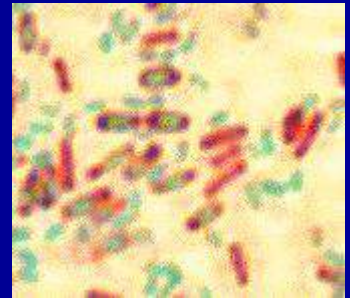
Spore Stain Procedure

1. Make a heat fixed smear of *Bacillus*
2. Place the slide on the slide rack
3. Cover the smear with malachite green stain
4. Apply heat for 3-5 min without boiling and drying of the slide
5. Wash the slide gently in running water about 20 S
6. Counterstain with safranin for one minute
7. Gently rinse with water
8. Gently blot the slide dry, no rubbing, and let it air dry and examine with oil immersion optics.
9. Observe red vegetative cells and sporangia, and green endospores and free spores

Identification of *Bacillus Spp.*

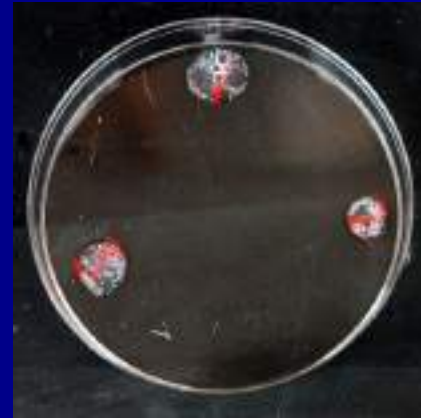
■ Spore Stain

- Bacillus spores are oval & central
- By spore staining technique (Malachite green & safranin) , the **spore** appears **green** while the **vegetative cells** appear **red**.



Biochemical Tests:

1- Catalase Test



- All *Bacillus* species are catalase positive
(Remember staphylococci are catalase positive)

Starch Hydrolysis (Amylase Activity)



■ Principle

- Starch + Iodine \longrightarrow blue color
- Glucose + Iodine \longrightarrow No reaction

- Nutrient Agar containing 1% Starch + M.O $\xrightarrow{\text{Amylase}}$ Glucose
- Appearance of colorless zone around the growth

Iodine

■ Procedure

- Inoculate nutrient agar plate containing 1% Starch with the M.O.
- Incubate the plate at 37 for overnight
- After incubation, flood the plate with Iodine solution

■ Result

- **Activity of amylase is indicated by a clear zone around the growth while the rest of the plate gives blue color after addition of iodine solution**

Clostridia of medical importance

Clostridium
Causing

Tetanus
e.g. *Cl. tetani*

Gas gangrene

Botulism
e.g. *Cl. botulinum*

○ **Antibiotic associated diarrhea**
e.g. *Cl. difficile*

Saccharolytic
e.g. *Cl. perfringens* & *Cl. septicum*

Proteolytic
e.g. *Cl. sporogenes*

Mixed: *Cl. histolyticum*

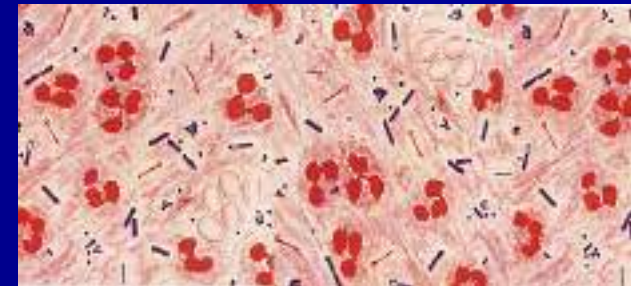
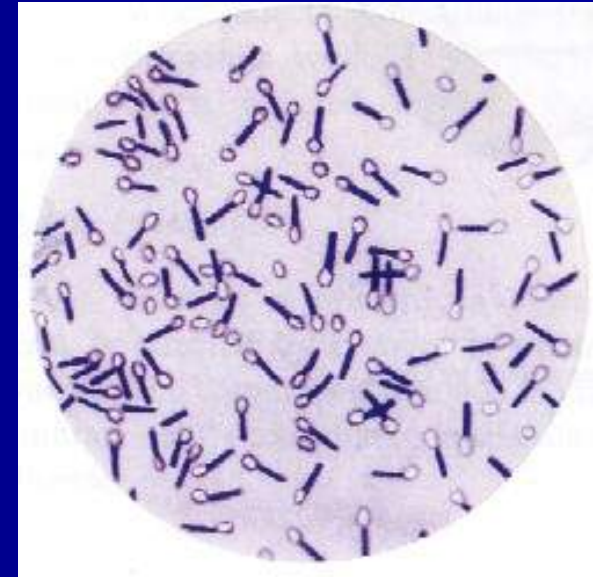
Clostridia

- Large Gram positive
- Straight or slightly curved rods with slightly rounded ends
- Anaerobic bacilli
- Spore bearing
- Spore do not germinate and growth does not normally proceed unless a suitably low redox potential Eh exists
- Saprophytes
- Some are commensals of the animal & human gut which invade the blood and tissue when host die and initiate the decomposition of the corpse (dead body)
- Causes diseases such as gas gangrene, tetanus, botulism & pseudo-membranous colitis by producing toxins which attack the neurons pathways

Clostridium Causing Tetanus

Cl. tetani

- Gram positive, straight, slender rod with rounded ends
- All species form endospore (drumstick with a large round end)
- Fermentative
- Obligate anaerobe
- Motile by peritrichous flagella
- Grows well in cooked meat broth and produces a thin spreading film when grown on enriched blood agar
- Spores are highly resistant to adverse conditions
- Iodine (1%) in water is able to kill the spores within a few hours



Laboratory Diagnosis of Tetanus

- The diagnosis of tetanus depends primarily upon the clinical manifestation of tetanus including muscle spasm and rigidity.
- **Specimen:** Wound exudates using capillary tube
- **Culture:**
 - On blood agar and incubated anaerobically
 - Growth appears as a fine spreading film.
- **Gram stain** is a good method for identifying *Clostridium*
 - *Cl. tetani* is Gram positive rod motile with a round terminal spore giving a drumstick appearance

Clostridium Causing Gas Gangrene

Clostridia causing gas gangrene

Saccharolytic organisms
Cl. perfringens, Cl. septicum
Ferment carbohydrates
Acid and gas are produced

Proteolytic organisms
Cl. sporogenes
Digest proteins with blackening
bad smell production

Mixed saccharolytic & proteolytic
Cl. histolyticum

Saccharolytic Microorganisms

Cl. perfringens
Causing

Gas gangrene

Food poisoning
(Enterotoxin)

Clostridium perfringens

- Large Gram-positive bacilli with stubby ends
- Capsulated
- Non motile (*Cl. tetani* is motile)
- Anaerobic
- Grown quickly on selective media
- **Can be identified by Nagler reaction**

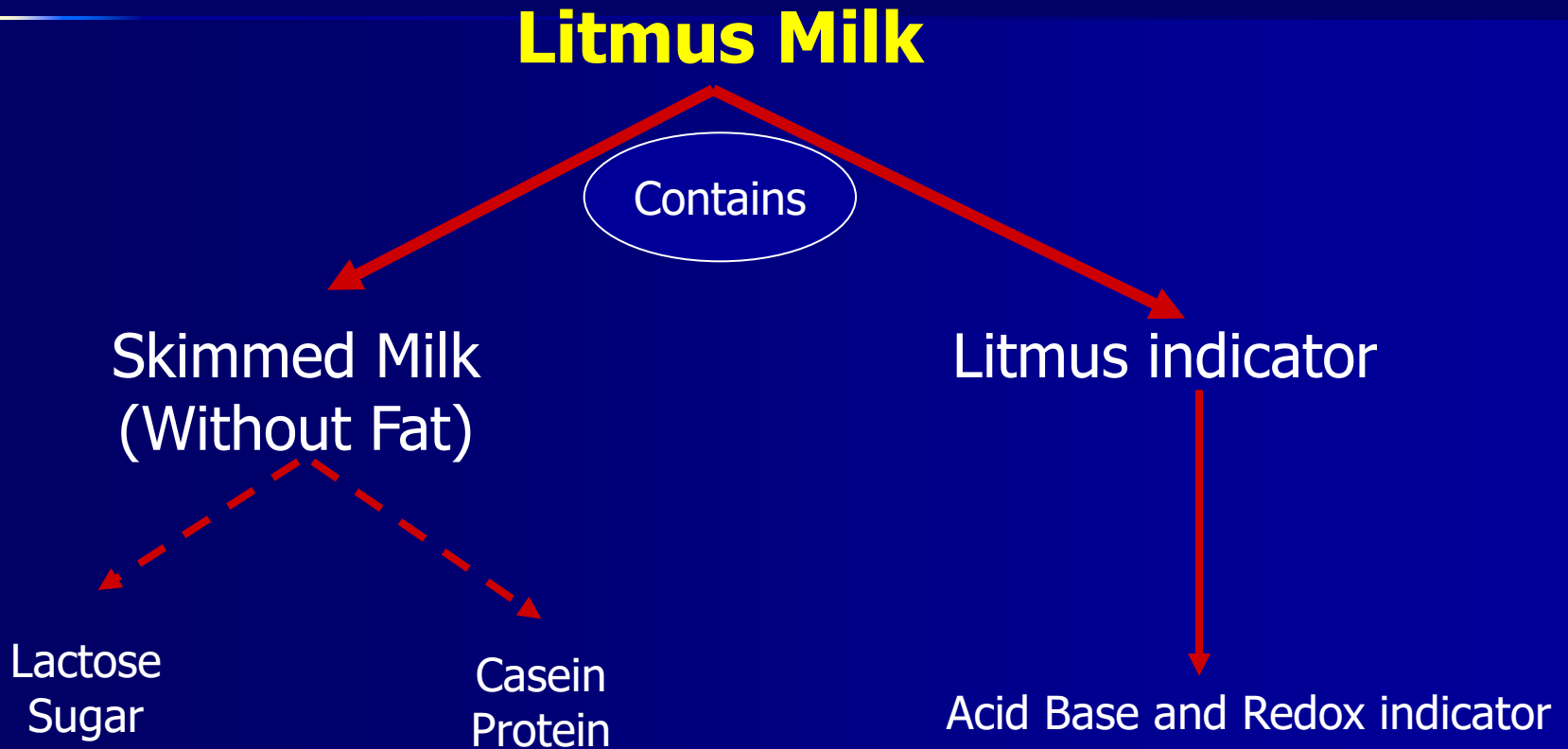
Laboratory Diagnosis

- **Specimen:** Histological specimen or wound exudates
 - Histological specimen transferred aseptically into a sterile screw-capped bottle & used immediately for microscopical examination & culture
 - Specimens of exudates should be taken from the deeper areas of the wound where the infection seems to be most pronounced
- **Microscopical examination (Gram, Spore stain etc)**
 - Gram-positive bacilli, non motile, capsulated & sporulated
 - The spore is oval, sub-terminal & non bulging
 - Spores are rarely observed
- **Culture:** Anaerobically at 37C
 - **On Robertson's cooked meat medium** → blackening of meat will be observed with the production of H₂S and NH₃
 - **On blood agar** → β-hemolytic colonies

Biochemical Tests

- *Cl. perfringnes* characterized by:
 - It ferments many carbohydrates with acid & gas
 - It acidified litmus milk with stormy clot production
 - Nagler reaction is positive

Reaction on Litmus Milk



Reaction on Litmus Milk

1- Acidic Reaction

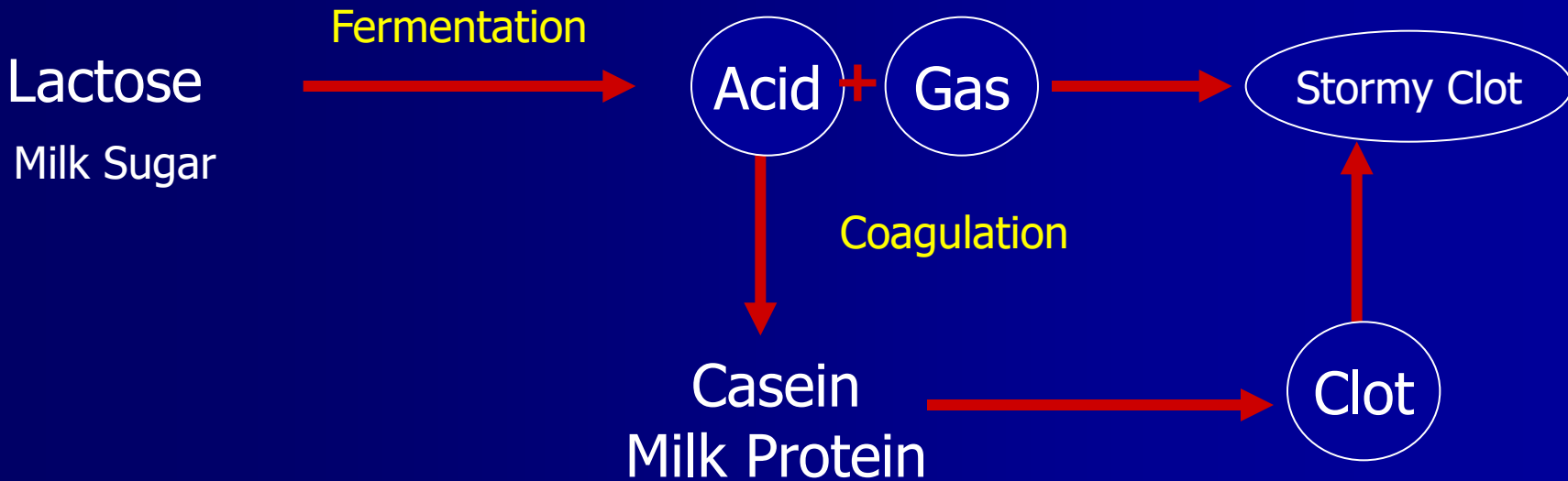


2- Basic Reaction

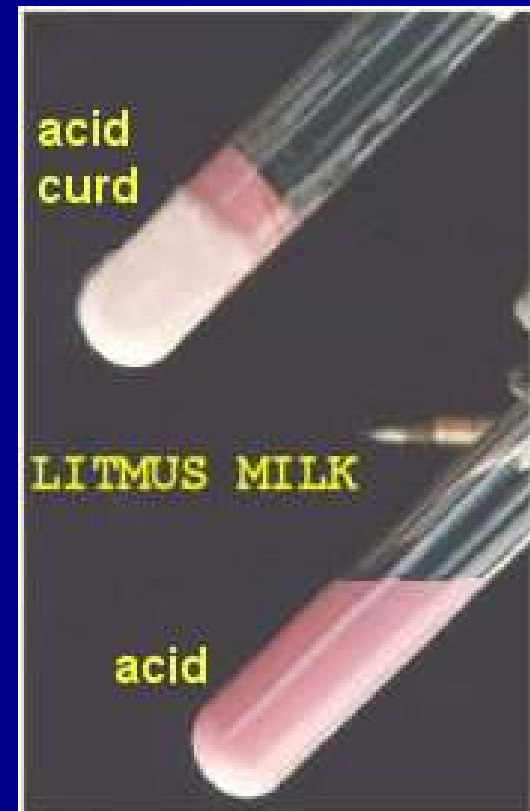


Reaction on Litmus Milk

Stormy Clot Formation



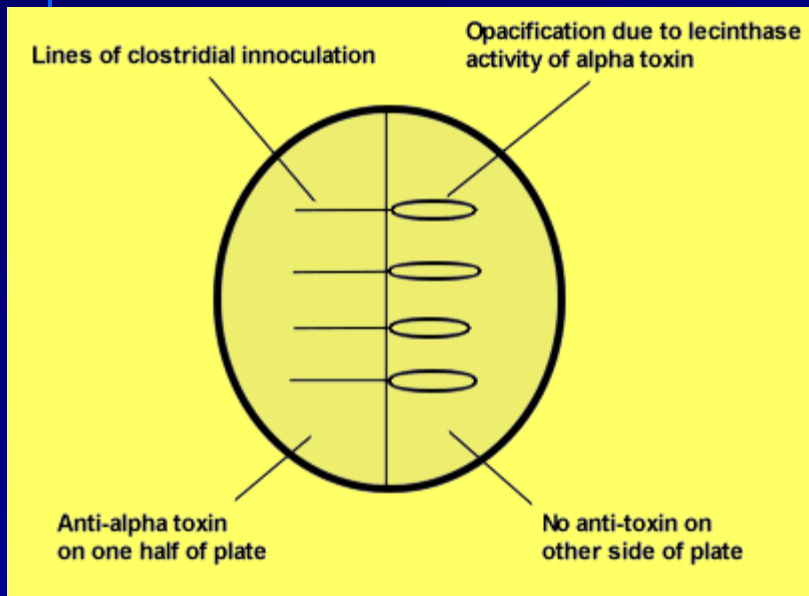
Reaction on Litmus Milk



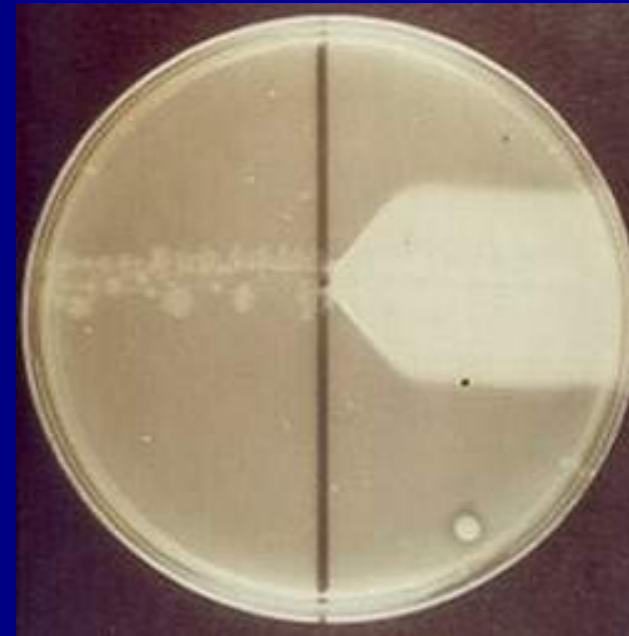
Nagler's Reaction

- This test is done to detect the lecithinase activity
 - The M.O is inoculated on the medium containing human serum or egg yolk (contains lecithin)
 - The plate is incubated anaerobically at 37 C for 24 h
 - Colonies of *Cl. perfringens* are surrounded by zones of turbidity due to lecithinase activity and the effect is specifically inhibited if *Cl. perfringens* antiserum containing α antitoxin is present on the medium

Nagler Reaction



Procedure of Nagler Reaction



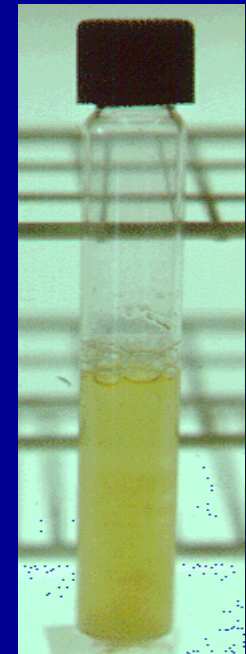
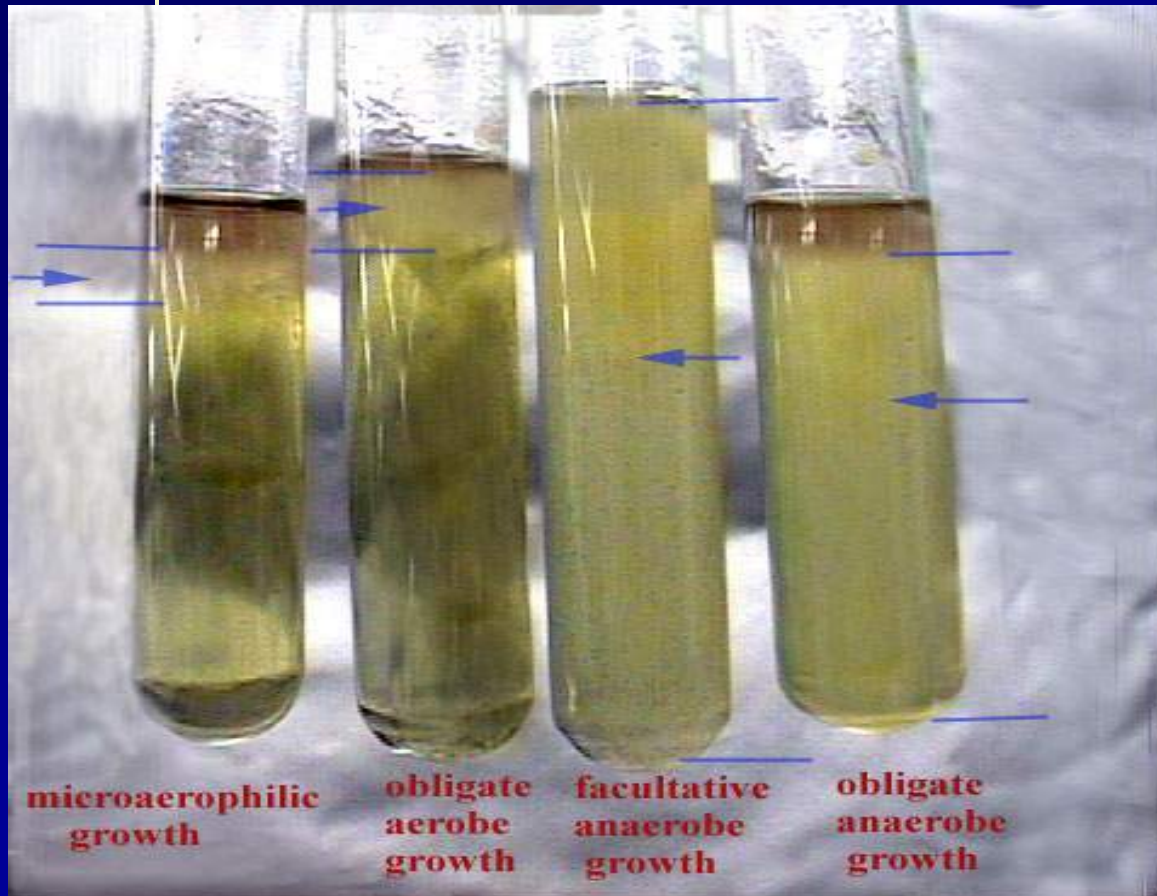
Positive Nagler Reaction

Anaerobic Cultivation

- Culture Media containing reducing agent
 - **Thioglycollate broth**
 - It contains
 - Sodium thioglycollate (Reducing agent)
 - Rezazurin (redox indicator)
 - Low percentage of Agar-Agar to increase viscosity of medium
 - **Cooked Meat Medium**
 - It contains
 - Meat particles (prepared from heart muscles) which contain hematin & glutathione that act as reducing agent

Growth on Fluid Thioglycolate

Clostridium sporogenes
Growing in Thioglycolate
Medium



Reducing agents in the medium absorb oxygen and allow obligate anaerobes to grow

Anaerobic Jar



Candle Jar



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Mycobacterium

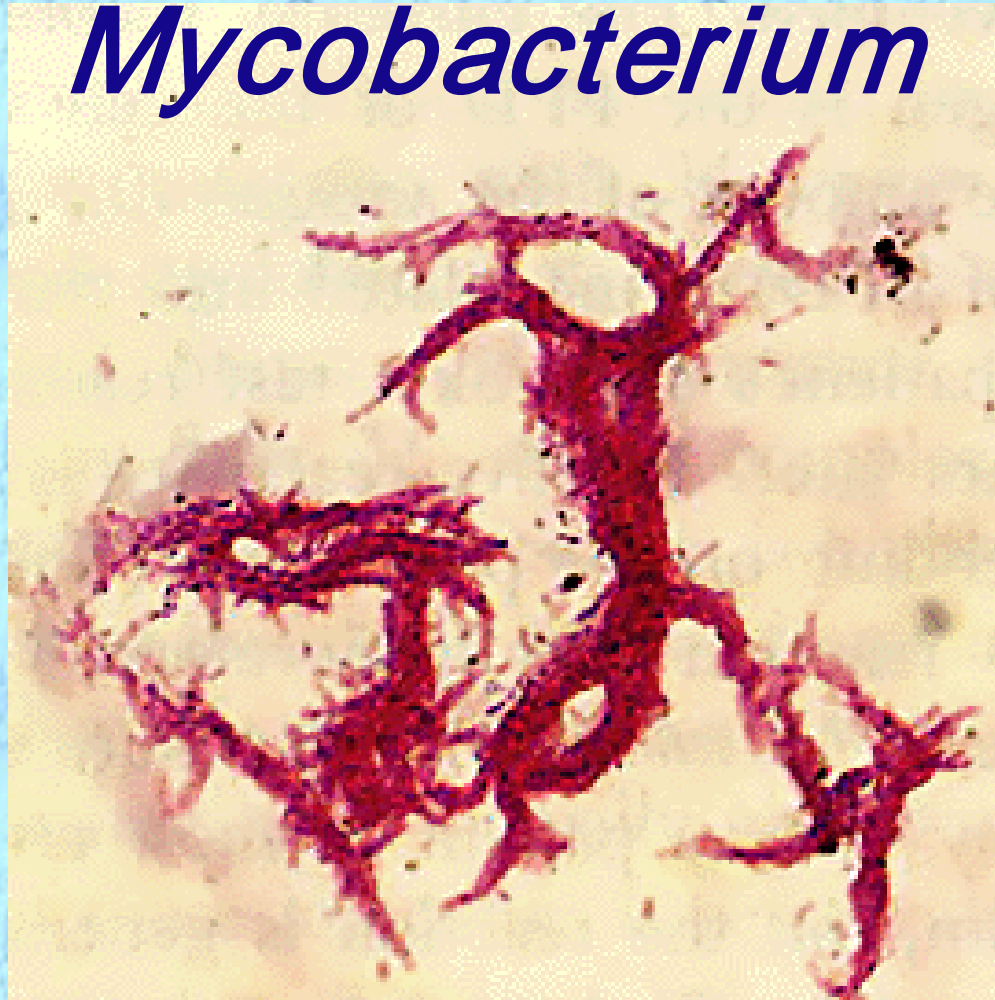
Important Human Pathogens

Mycobacterium tuberculosis

Mycobacterium leprae (uncommon)

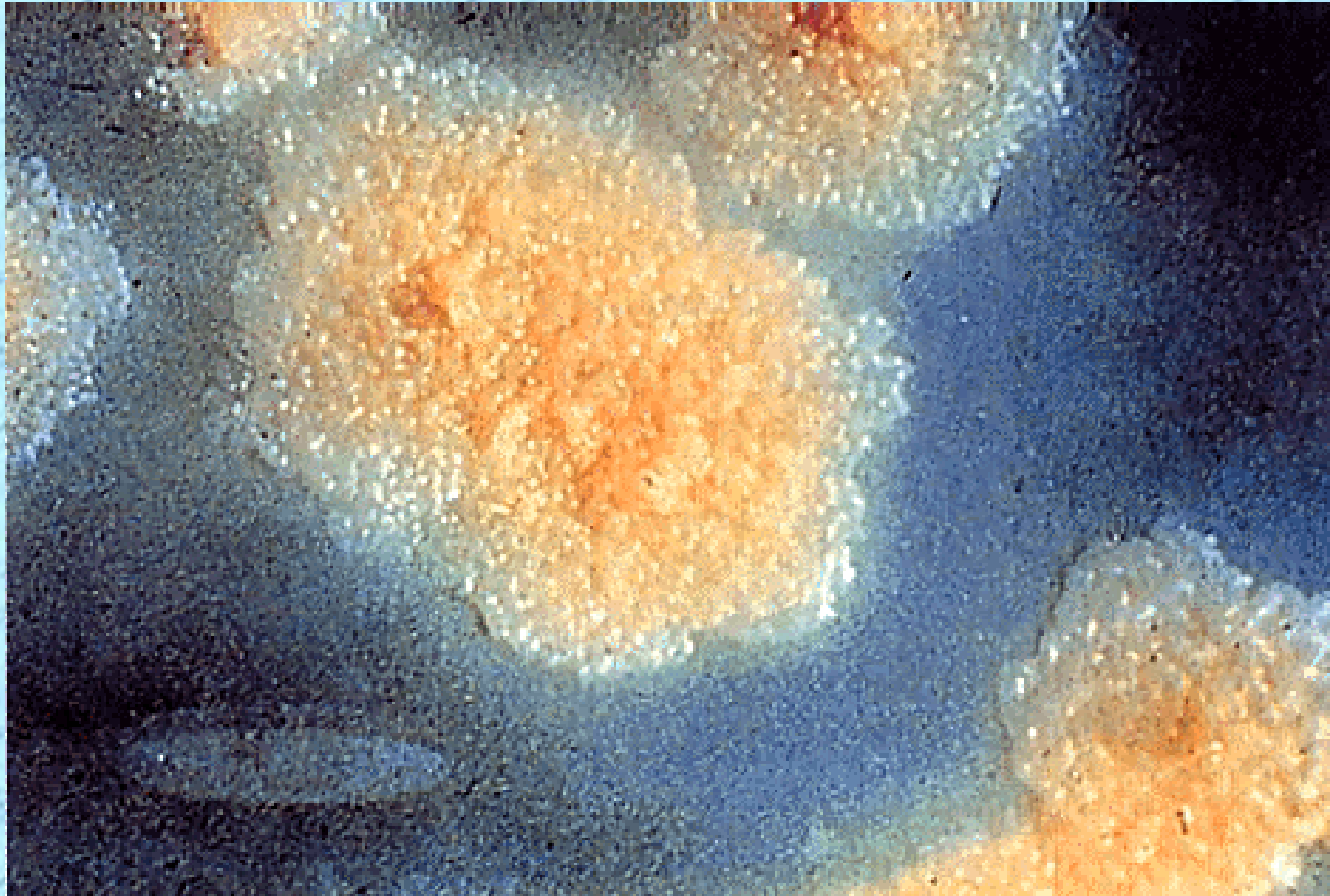
Mycobacterium avium-intracellulare Complex
(MAC) or (M. avium)

Acid-Fast (Kinyoun) Stain of Mycobacterium



NOTE: cord growth (serpentine arrangement) of virulent strains

***Eight Week Growth of
Mycobacterium tuberculosis on
Lowenstein-Jensen Agar***



Mycobacterium tuberculosis

Infections (cont.)

→ Positive PPD + Chest X-Ray +

MDR-TB a serious global health threat →

BCG (bacille Calmette-Guerin) = attenuated *M. bovis* →

REVIEW

Diseases

Primary infection is pulmonary.

Dissemination to any body site occurs most commonly in immunocompromised patients and untreated patients.

Diagnosis

Microscopy and culture are sensitive and specific. Direct detection by molecular probes is relatively insensitive.

Treatment, Prevention, and Control

Multiple-drug regimens and prolonged treatment are required to prevent development of drug-resistant strains.

Regimens recommended for treatment include isoniazid and rifampin for 9 months, with pyrazinamide and ethambutol or streptomycin added for drug-resistant strains.

Prophylaxis for exposure to tuberculosis can include isoniazid for 9 months, rifampin for 4 months, or rifampin and pyrazinamide for 2 months. Pyrazinamide and ethambutol or levofloxacin are used for 6 to 12 months following exposure to drug-resistant *M. tuberculosis*.

Immunoprophylaxis with BCG in endemic countries.

Control of disease through active surveillance, prophylactic and therapeutic intervention, and careful case monitoring.

Laboratory Diagnosis of Mycobacterial Disease

Detection

Skin test

Microscopy

Carbolfuchsin acid-fast stain

Fluorochrome acid-fast stain

Direct nucleic acid probes

Culture

Solid agar-based or egg-based media

Broth-based media

Identification

Morphologic properties

Biochemical reactions

Analysis of cell wall lipids

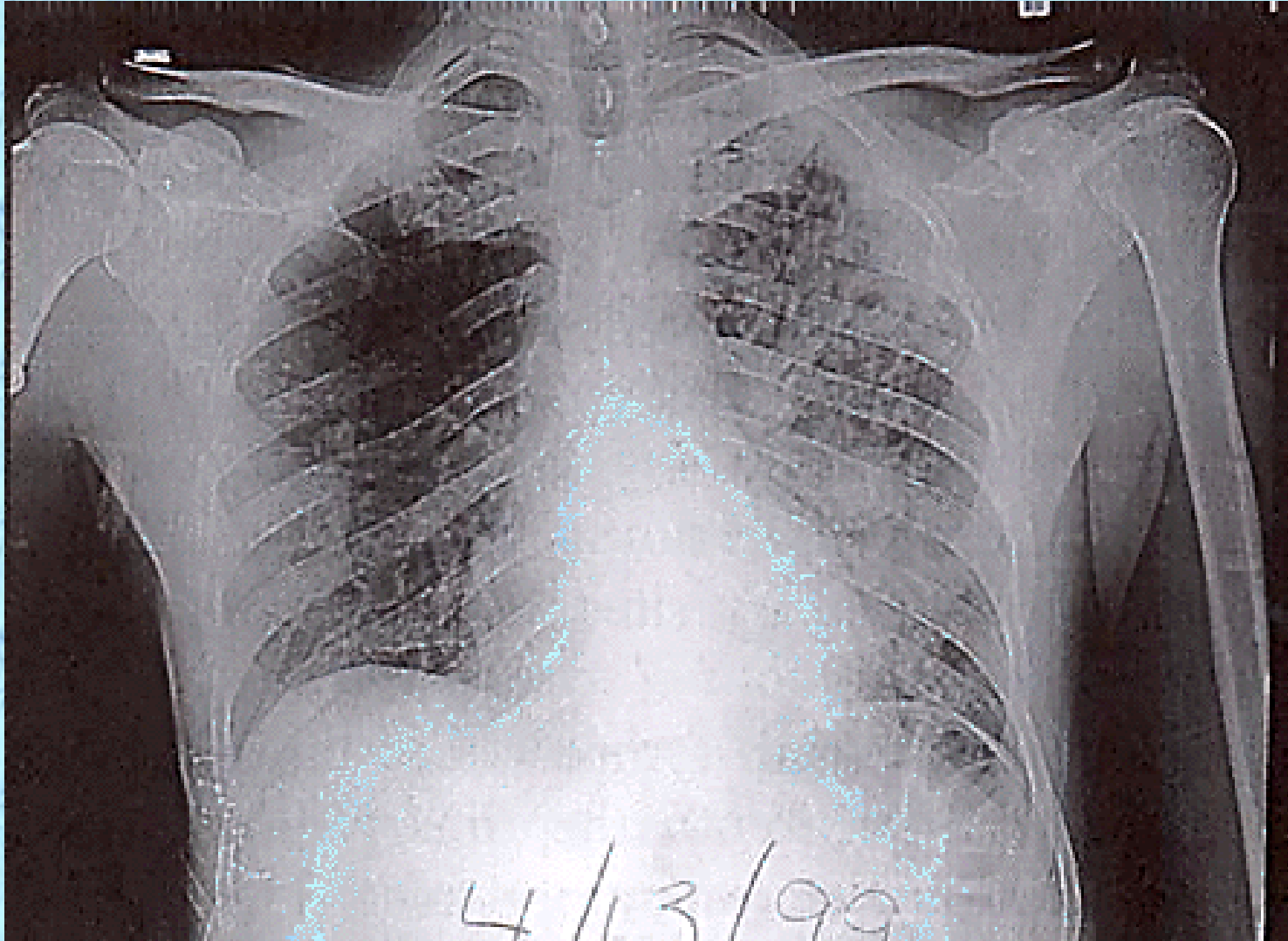
Nucleic acid probes

Nucleic acid sequencing

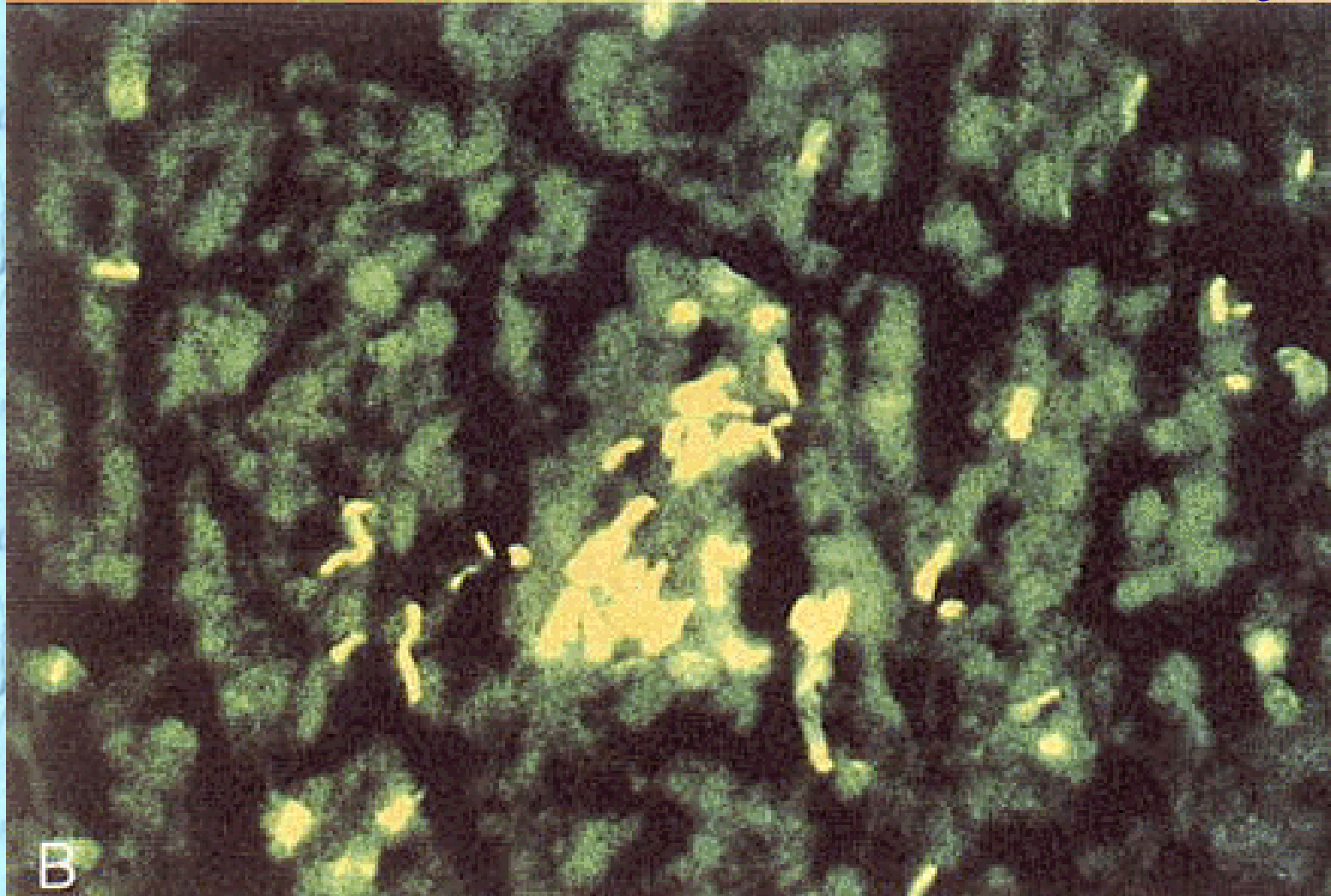
Differential Characteristics of Commonly Isolated Mycobacterium spp.

Organism	Niacin	Nitrate Reductase	Heat-Stable Catalase	Tween-80 Hydrolysis	Iron Uptake	Arylsulfatase	Urease
<i>M. tuberculosis</i>	+	+	-	-		-	+
<i>M. kansasii</i>	-	+	+	+		-	+
<i>M. avium</i> complex	-	-	+/-	-		-	-
<i>M. fortuitum</i>	-	+	+	V	+	+	+
<i>M. chelonae</i>	V	-	V	V	-	+	+

Chest X-Ray of Patient with Active Pulmonary Tuberculosis



Mycobacterium Tuberculosis *Stained with Fluorescent Dye*



Mycobacterium leprae

Mycobacterium leprae Infections (cont.)

Diseases

Tuberculoid form of leprosy.

Lepromatous form of leprosy.

Intermediate forms of leprosy.

Diagnosis

Microscopy is sensitive for the lepromatous form but not the tuberculoid form.

Skin testing required to confirm tuberculoid leprosy.

Culture cannot be used.

Treatment, Prevention, and Control

Dapsone with or without rifampin is used to treat the tuberculoid form of disease; clofazimine is added for the treatment of the lepromatous form. Therapy is prolonged.

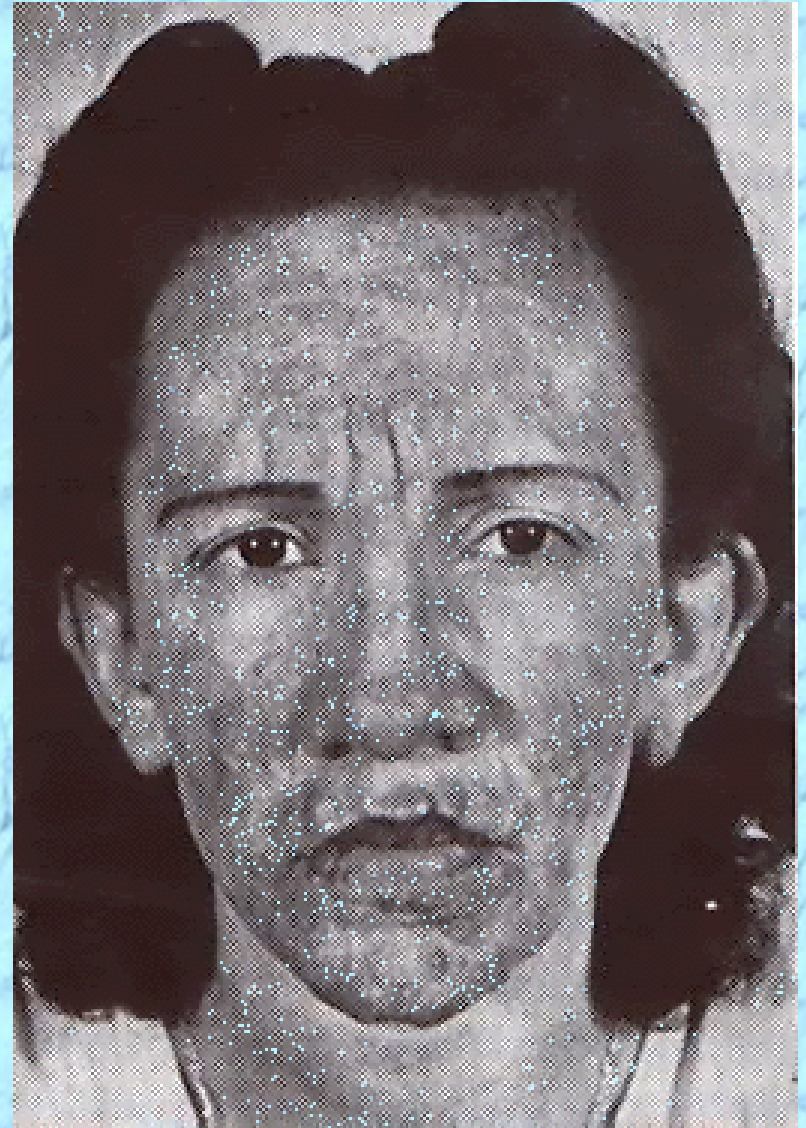
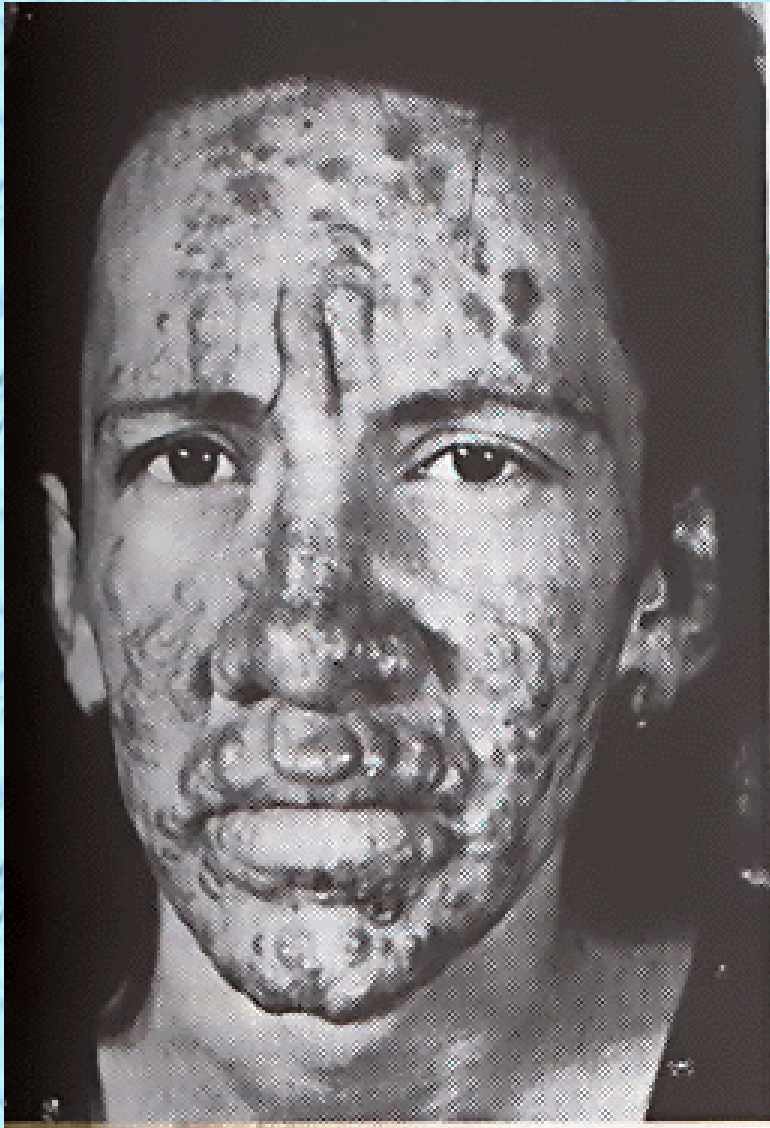
Dapsone is recommended for long-term prophylaxis in treated patients.

Disease is controlled through the prompt recognition and treatment of infected people.

Lepromatous Leprosy (Early/Late Stages)

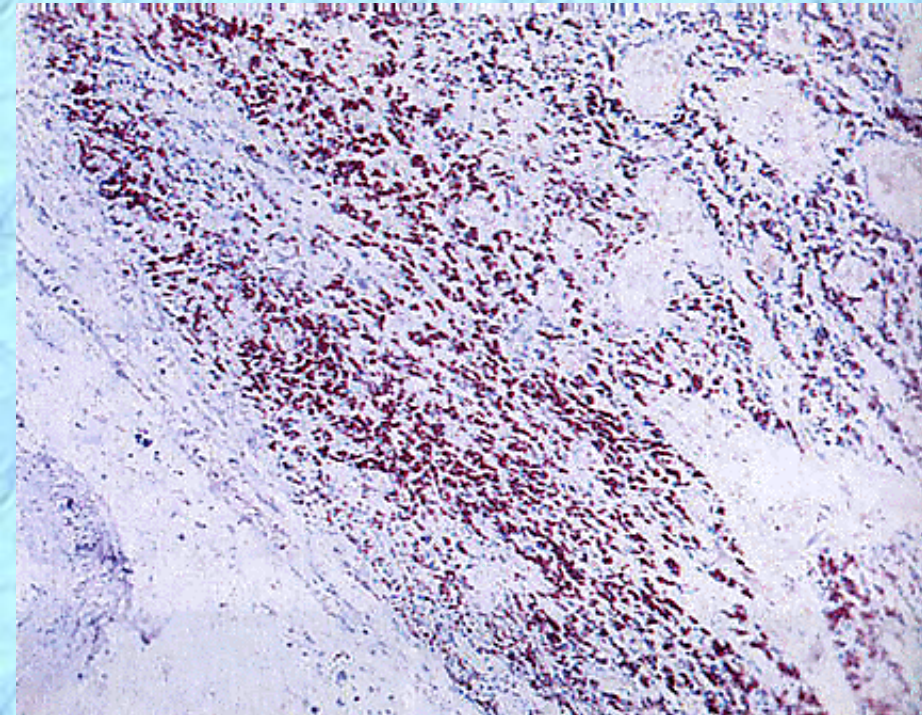


Lepromatous Leprosy Pre- and Post-Treatment

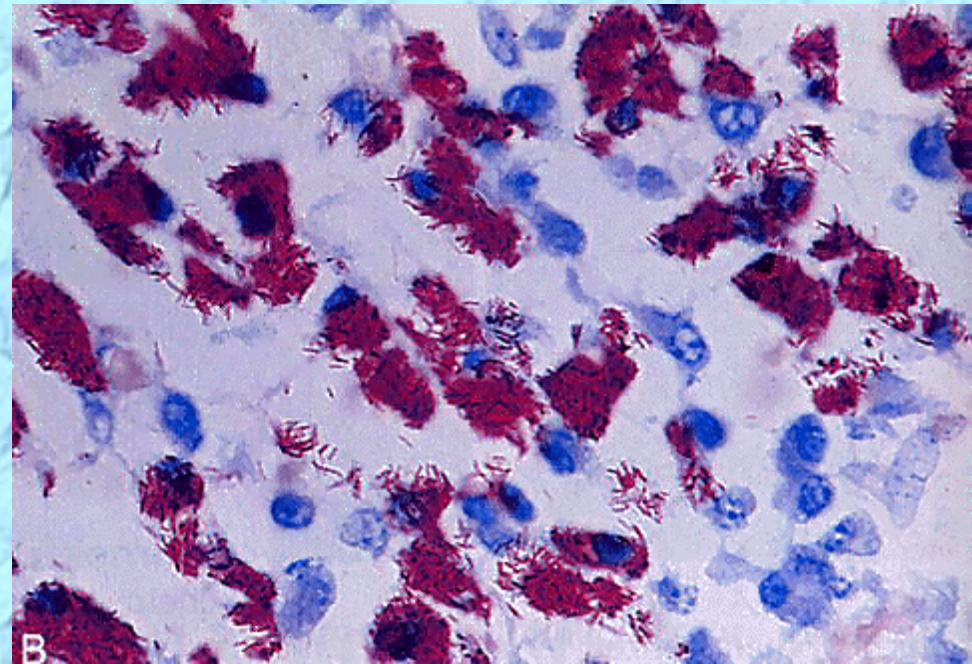


***Mycobacterium avium-
intracellulaire Complex
(MAC)***

Mycobacterium avium-intracellulaire in Tissue Specimens



Low Magnification



High Magnification

Mycobacterium avium-intracellulaire Infections

Diseases

Asymptomatic colonization.

Chronic localized pulmonary disease.

Disseminated disease, particularly in patients with AIDS.

Diagnosis

Microscopy and culture are sensitive and specific.

Treatment, Prevention, and Control

Infections treated for prolonged period with clarithromycin or azithromycin combined with ethambutol and rifabutin.

Prophylaxis in patients with AIDS who have low CD4+ cell count consist of clarithromycin or azithromycin or rifabutin.

Prophylaxis has dramatically reduced the incidence of disease in patients with AIDS.

Aerobic Actinomycetes

Aerobic Actinomycetes: Nocardia species

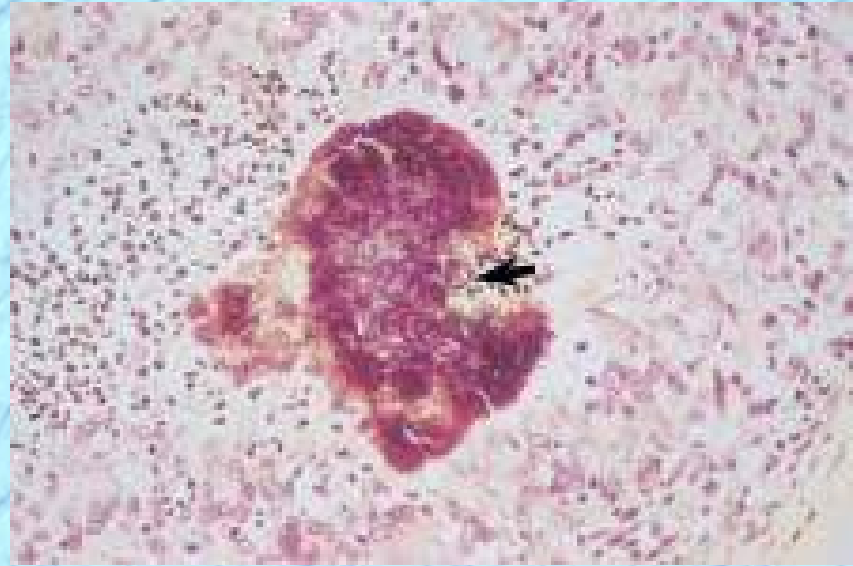
- **General Characteristics**
 - ✓ **Aerobic, gram-positive, filamentous rods, sometimes resembling branched hyphae**
 - ✓ **Weakly acid-fast and may stain gram-variable**
 - ✓ **Morphologically resemble fungi, both in culture and in types of infections produced**
 - ✓ **Generally found in the environment and mostly affect immunocompromised individuals**

Aerobic Actinomycetes: Nocardia, Actinomadura, and Streptomyces species

- **Significant *Nocardia* species**
 - ✓ *N. asteroides*
 - ✓ *N. braziliensis*
 - ✓ *N. caviae*
- ***Actinomadura* species**
 - ✓ *A. madurae*
 - ✓ *A. pelletieri*
- ***Streptomyces* species**

Aerobic Actinomycetes: Nocardia, Actinomadura, and Streptomyces species

- **Clinical infections**
 - ✓ Pulmonary form
 - ✓ Mycetomas



**Sulfur granules
collected from
draining sinus**

typical in

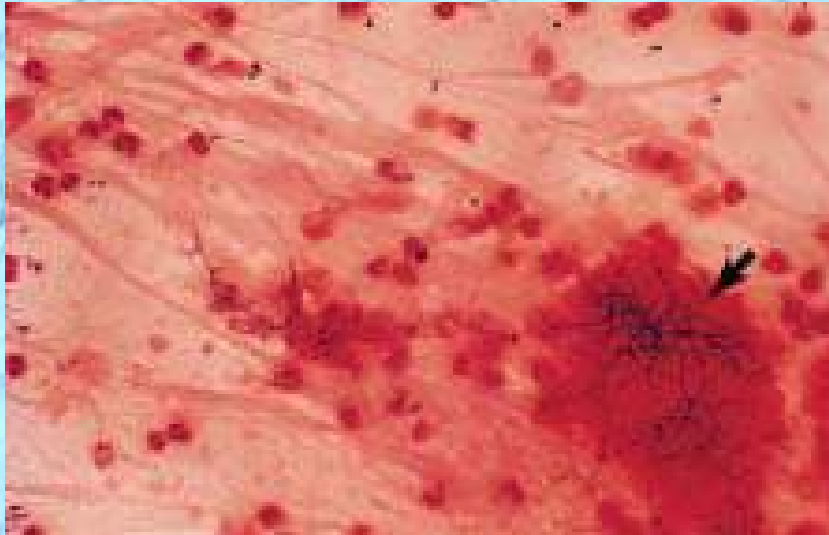
Laboratory Diagnosis: Nocardia, Actinomadura, and Streptomyces species

- **Microscopy**
 - ✓ **Gram-positive branching filaments are seen in direct smears from sputum or aspirated material**
 - ✓ **May show beading appearance**



Gram-stained smear of sputum showing Gram-positive branched

Laboratory Diagnosis: ***Nocardia, Actinomadura, and Streptomyces*** ***species***



- Expectorated sputum with purulence
- Gram-positive filamentous bacilli
- Suspicious for actinomycetes

Laboratory Diagnosis:

Nocardia, Actinomadura, and Streptomyces Species

- **Cultural characteristics**
 - ✓ Chalky, matte, dry, crumbly appearance
 - ✓ May be pigmented
- **Identification**
 - ✓ Utilization of carbohydrates
 - ✓ Hydrolysis of casein, tyrosine, and xanthine



Chalky, white colonies on blood agar plate isolated from

Laboratory Diagnosis:

Nocardia, Actinomadura, and Streptomyces Species

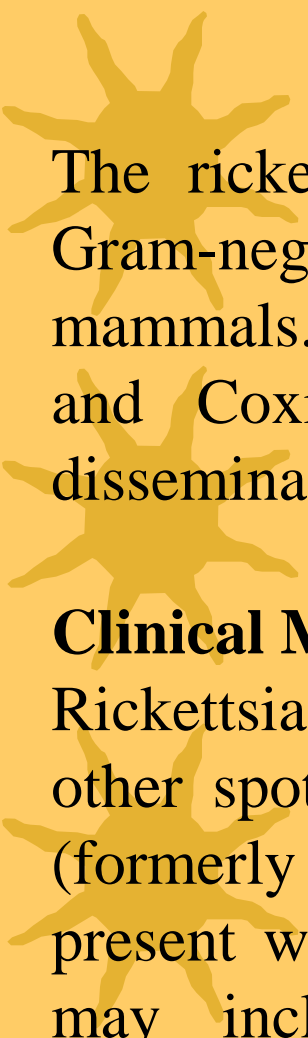


- **Sputum smear, partially acid-fast bacilli, consistent with *Nocardia* sp.**
- ***Actinomadura* and *Streptomyces* sp. are not acid-fast**

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Rickettsiae



The rickettsiae are a diverse collection of obligately intracellular Gram-negative bacteria found in ticks, lice, fleas, mites, chiggers, and mammals. They include the genera Rickettsiae, Ehrlichia, Orientia, and Coxiella. These zoonotic pathogens cause infections that disseminate in the blood to many organs.

Clinical Manifestations

Rickettsia species cause Rocky Mountain spotted fever, rickettsialpox, other spotted fevers, epidemic typhus, and murine typhus. Orientia (formerly Rickettsia) tsutsugamushi causes scrub typhus. Patients present with febrile exanthems and visceral involvement; symptoms may include nausea, vomiting, abdominal pain, encephalitis, hypotension, acute renal failure, and respiratory distress.

Structure, Classification, and Antigenic Types

Rickettsia species are small, Gram-negative bacilli that are obligate intracellular parasites of eukaryotic cells. This genus consists of two antigenically defined groups: spotted fever group and typhus group, which are related; scrub typhus rickettsiae differ in lacking lipopolysaccharide, peptidoglycan, and a slime layer, and belong in the separate, although related, genus *Orientia*.

Pathogenesis

Rickettsia and *Orientia* species are transmitted by the bite of infected ticks or mites or by the feces of infected lice or fleas. From the portal of entry in the skin, rickettsiae spread via the bloodstream to infect the endothelium and sometimes the vascular smooth muscle cells. *Rickettsia* species enter their target cells, multiply by binary fission in the cytosol, and damage heavily parasitized cells directly.

Host Defenses

T-lymphocyte-mediated immune mechanisms and cytokines, including gamma interferon and tumor necrosis factor alpha, play a more important role than antibodies.

Diagnosis

Rickettsioses are difficult to diagnose both clinically and in the laboratory. Cultivation requires viable eukaryotic host cells, such as antibiotic-free cell cultures, embryonated eggs, and susceptible animals. Confirmation of the diagnosis requires comparison of acute- and convalescent-phase serum antibody titers.





MYCOPLASMA

- ★ **Smallest free-living** micro organisms, **lack cell wall.**
- ★ **Size varies from spherical shape(125-250nm to longer branching filaments 500-1000 nm in size. Many can pass through a bacterial filter.**
- ★ **1st member of this group – isolated by Nocard & Roux (1898) – caused bovine pleuropneumonia.**
- ★ **Later, many similar isolates were obtained from animals, human beings, plants & environmental sources – called as “pleuropneumonia like organisms”(PPLO).**





MYCOPLASMA

- ★ **Eaton (1944) first isolated the causative agent of the disease in hamsters and cotton rates.**
- ★ **Also known as Eaton agent.**
- ★ **1956- PPLO replaced by Mycoplasma.**
 - **Myco : fungus like branching filaments**
 - **Plasma : plasticity**
- ★ **highly pleomorphic – no fixed shape or size - Lack cell wall.**





Morphology and Physiology



- ★ **Small genome size (*M. pneumoniae* is ~800 Kbp)**
 - **Require complex media for growth**
- ★ **Facultative anaerobes**
 - **Except *M. pneumoniae* - strict aerobe**
- **No cell wall means these are resistant to penicillins, cephalosporins and vancomycin, etc.**
- **Grow slowly by binary fission**
- ★ **Doubling time can be as long as 16 hours, extended incubation needed**



Morphology and Physiology cont'



- ***M. pneumoniae* - glucose**
- ***M. hominis* - arginine**
- ***U. urealyticum* - urea (buffered media due to growth inhibition by alkaline media)**
- ***M. genitalium* - difficult to culture**



Mycoplasmas of Humans

★ **Parasitic**

1. **Established pathogens: *M. pneumoniae***

2. **Presumed pathogens: *M. hominis*,
*U. urealyticum***

3. **Non pathogenic: *M. orale*, *M. buccale*,
M. genitalium, *M. fermentans***

★ **Saprophytic** – present mainly on skin & in mouth.





Mycoplasma pneumoniae



★ Also called **Primary Atypical Pneumonia/ Walking pneumonia.**



★ **Seen in all ages**



★ **Incubation period: 1-3 wks**

★ **Transmission: airborne droplets of nasopharyngeal secretions, close contacts (families, military recruits).**



Laboratory Diagnosis - *M. pneumoniae*

★ Microscopy

- Difficult to stain
- This process can help eliminate other organisms

★ Culture (definitive diagnosis)

- Sputum (usually scant) or throat washings
- Special transport medium needed
 - Must suspect *M. pneumoniae*
- May take 2-3 weeks or longer, 6 hour doubling time with glucose and pH indicator included
- Incubation with antisera to look for inhibition.





Laboratory Diagnosis

★ **Specimens** – throat swabs, respiratory secretions.

★ **Microscopy** –

1. **Highly pleomorphic**, varying from small spherical shapes to longer branching filaments.

2. **Gram negative**, but better stained with **Giemsa, Dienes' stain, crystal-fast violet, orcein** or **fluorochroming with nucleic acid stain as acridine orange**





Laboratory Diagnosis

★ Isolation of Mycoplasma (Culture) –

1. **Semi solid enriched medium** containing **20%** horse or human serum, yeast extract & **DNA**. **Penicillium & Thallium acetate** are selective agents.

(serum – source of cholesterol & other lipids)

2. Incubate **aerobically** for **7 -12 days** with **5–10% CO₂** at **35-37°C**. (temp range **22- 41°C**, parasites **35- 37°C**, saprophytes – lower temp)



Laboratory Diagnosis



3. Typical **“fried egg”** appearance of **colonies** - Central opaque granular area of growth extending into the depth of the medium, surrounded by a flat, translucent peripheral zone.
4. Colonies best seen with a hand lens after staining with **Diene’s method**.
5. Produce **beta hemolytic** colonies, can agglutinate guinea pig erythrocytes.



Mycoplasma colonies with Diene's stain

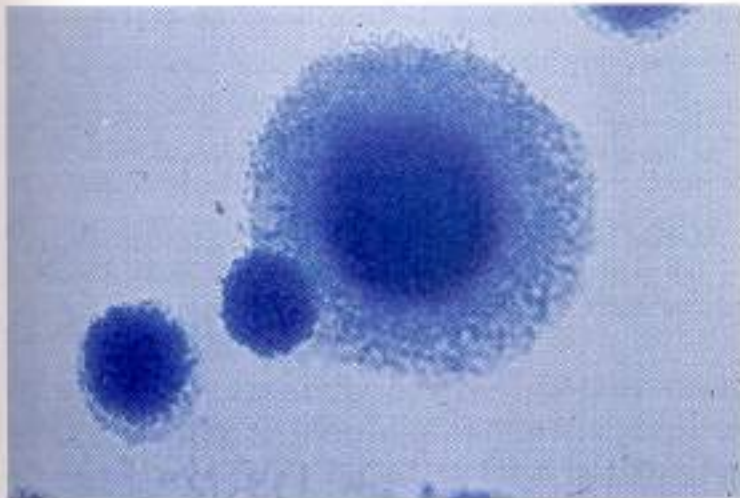


Figure 21-16 _____
Diene's stain of *Mycoplasma* spp. colonies demonstrating typical "fried egg" appearance.

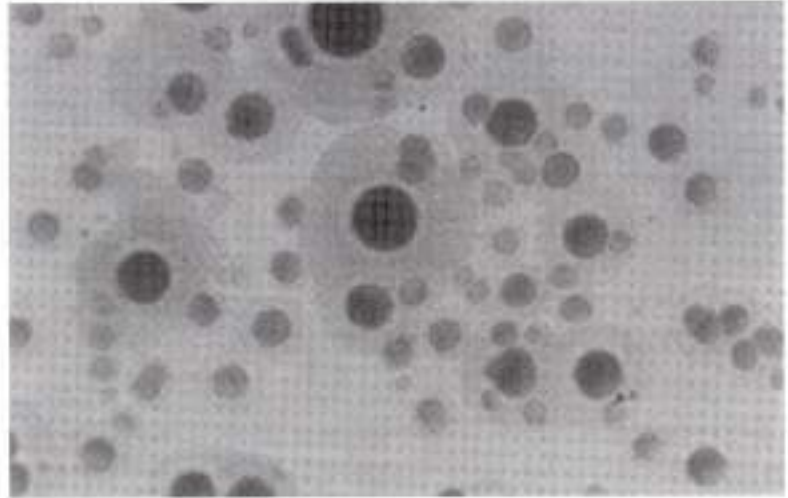
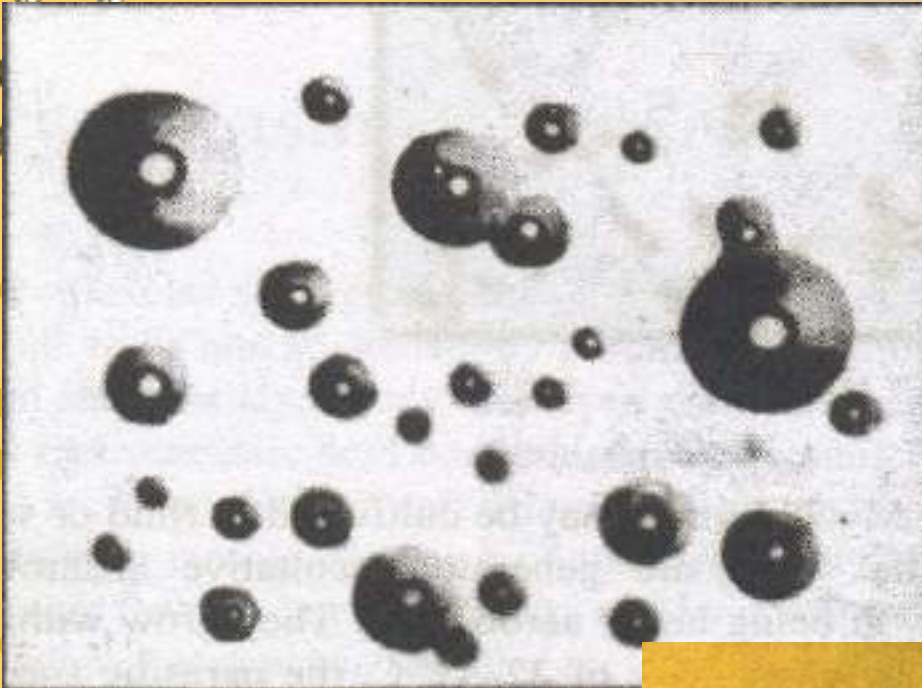
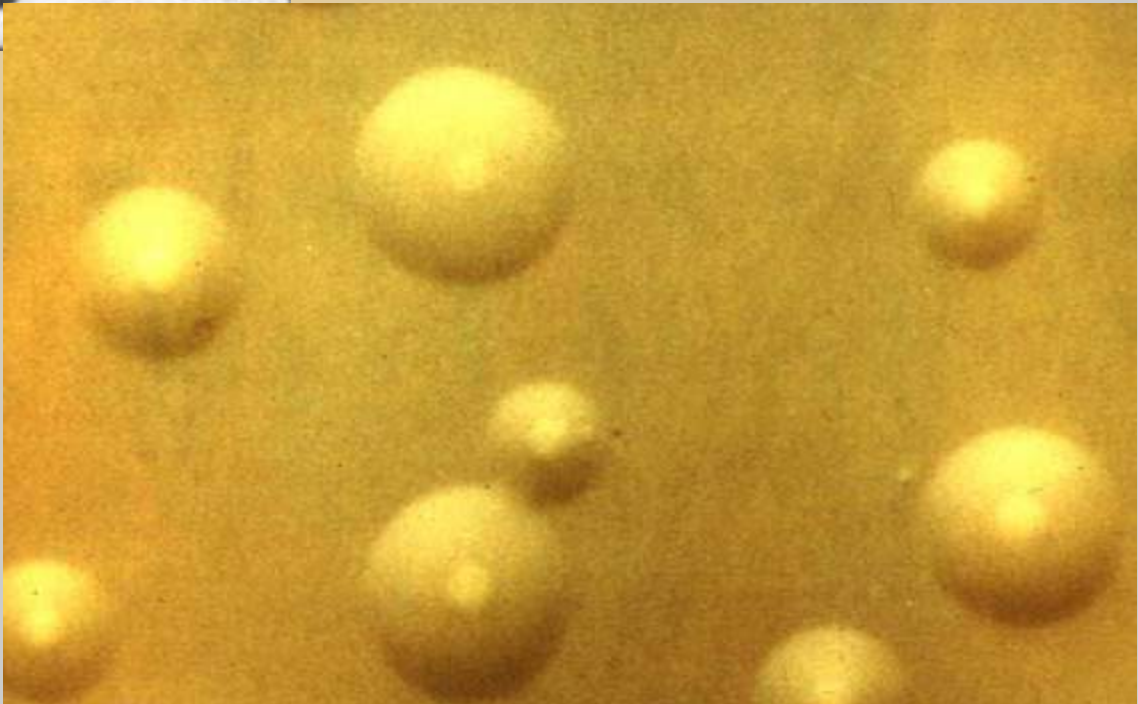


Figure 21-17 _____
Typical mixed sizes of *Mycoplasma* organisms on primary isolation media: *Mycoplasma salivarium*. (Courtesy Bionique Testing Laboratories, Saranac Lake, N.Y.)



Fried egg colonies



Except for *M. pneumoniae* colonies which have a granular appearance, described as being mulberry shaped





Identification of Isolates



★ **Growth Inhibition Test** – inhibition of growth around discs impregnated with specific antisera.



★ **Immunofluorescence** on colonies transferred to glass slides.



★ **Molecular diagnosis**

- PCR-based tests are being developed and these are expected to be the diagnostic test of choice in the future.
- These should have good sensitivity and be specific



Identification of Isolates

★ **Serological diagnosis**

1. Specific tests – IF, HAI

2. Non specific serological tests – cold agglutination tests (Abs agglutinate human group O red cells at low temperature, 4°C).

1:32 titer or above is significant.





Genital Infections



★ **Caused by *M. hominis* & *U. urealyticum***

★ **Transmitted by sexual contact**



★ **Men** - Nonspecific urethritis, proctitis, balanoposthitis & Reiter's syndrome

★ **Women** – acute salpingitis, PID, cervicitis, vaginitis



★ **Also associated with infertility, abortion, postpartum fever, chorioamnionitis & low birth weight infants**



Mycoplasma & HIV infection

- ★ **Severe & prolonged infections in HIV infected & other immunodeficient individuals**





Mycoplasma as cell culture contaminants



- ★ **Contaminates continuous cell cultures maintained in laboratories**
- ★ **Interferes with the growth of viruses in these cultures.**
- ★ **Mistaken for viruses.**
- ★ **Eradication from infected cells is difficult.**





Treatment and Prevention

M. pneumoniae

★ Treatment

- **Tetracycline in adults (doxycycline) or erythromycin (children)**
 - **Newer fluoroquinolones (in adults)**
- **Resistant to cell wall synthesis inhibitors.**

★ Prevention

- **Avoid close contact**
- **Isolation is not practical due to length of illness**
- **No vaccine, although attempted**



Chlamydia

- Classification – order Chlamydiales – contains one medically important genus – *Chlamydia*
 - Are obligate intracellular parasites
 - Cell walls are similar to the cell walls of G-B, but lack muramic acid
 - Have a complex developmental cycle
 - The infectious form is called an elementary body (EB) which is circular in form and is taken into the cell by induced phagocytosis.
 - Inside the phagocytic vesicle replication takes place

Chlamydia

- Over the next 6-8 hours, the EB reorganizes into the noninfectious, but metabolically active reticulate body (RB) which is larger and less dense than the EB.
- For 18-24 hours the RB synthesized new materials and divides by binary division to form inclusion bodies that reorganize and condense into EBs.
- Between 48-72 hours, the cell lyses and releases the EB which begin the cycle again.

Chlamydia life cycle

***Chlamydia* developmental cycle**

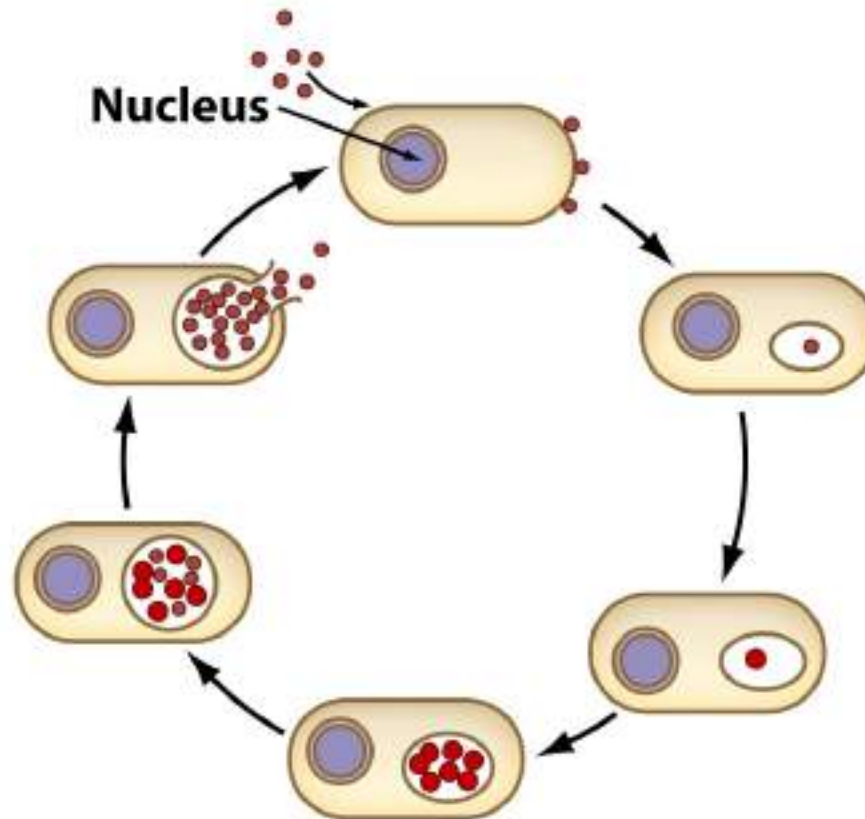


Figure 18.43b Microbiology: An Evolving Science
© 2009 W. W. Norton & Company, Inc.

Chlamydia

- Are energy parasites that use ATP produced by the host cell
- A Giemsa stain can be used to visualize chlamydial inclusions in tissues.
- Identification
 - Direct methods – stain tissues with Giemsa or use a direct fluorescent antibody technique.
 - The most sensitive method is to culture the organisms in tissue cultures and then stain the infected tissue culture cells

Chlamydia in tissues

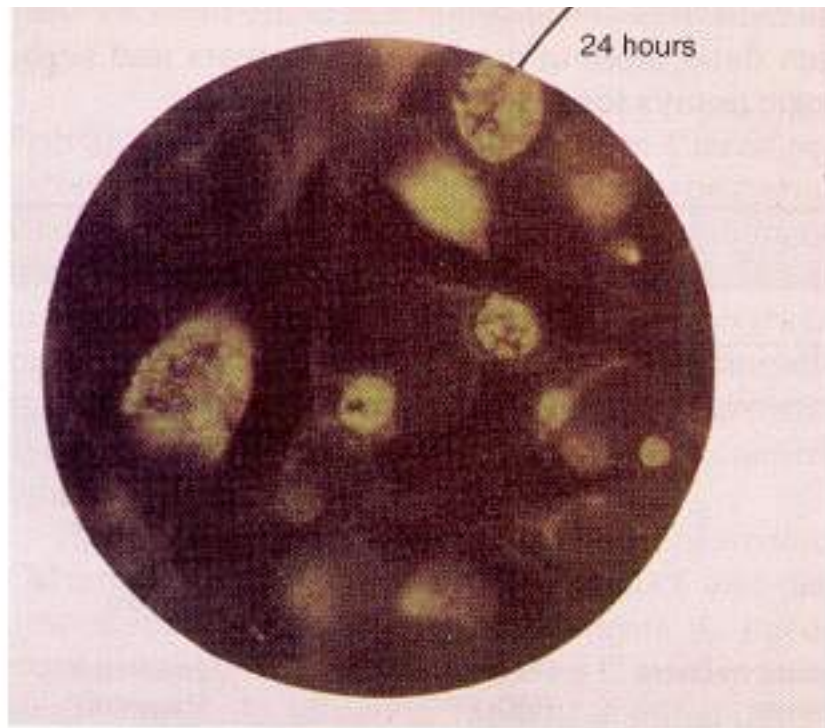


Figure 21-2

Chlamydia spp. growth cycle highlighting reticulate bodies (RBs), sometimes referred to as *initial bodies*. (Courtesy Syva-Microtrak, Palo Alto, Calif.)

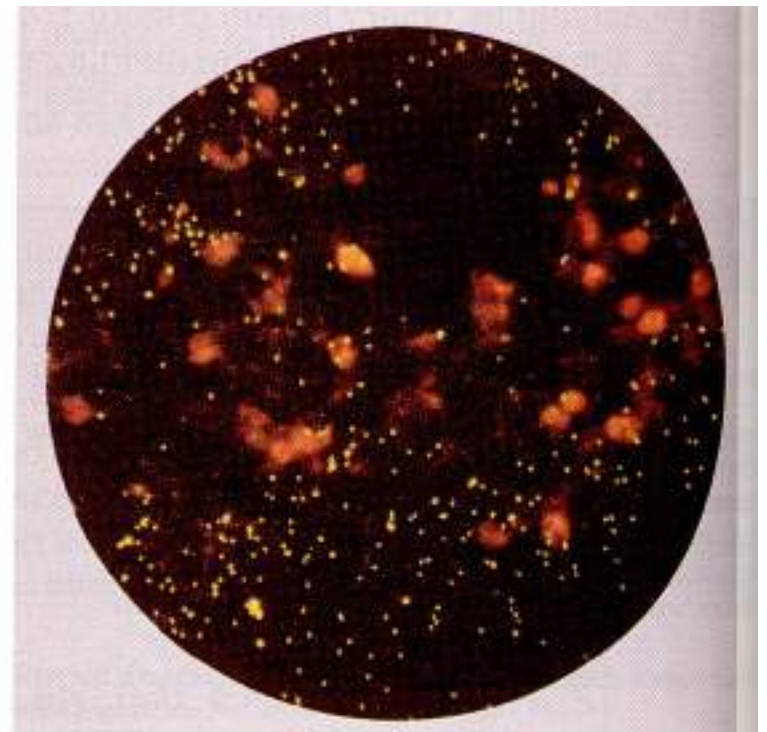
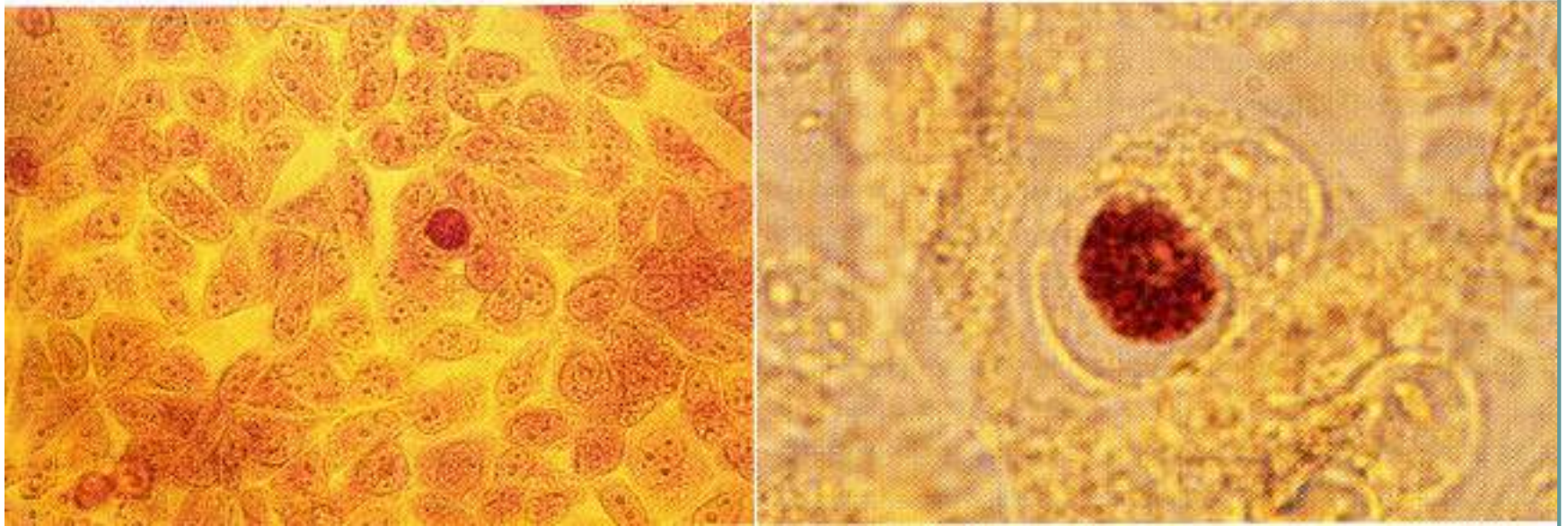


Figure 21-3

Elementary bodies (EBs) and cells in *Chlamydia trachomatis*-positive direct specimen. (Courtesy Syva Microtrak, Palo Alto, Calif.)

Chlamydia inclusion bodies



Chlamydia

- A complement fixation serological test is available as are DNA based tests.
- Virulence factors
 - Toxicity from attachment and penetration
- Clinical significance
 - Chlamydia trachomatis – serotypes A-K and L_{1,2,3}; the serotype determines the clinical manifestation.
 - Genital tract infection (serotypes D-K) – is the major cause of nongonococcal urethritis; is sexually transmitted and frequently found concomitantly with *N. gonorrhoeae*
 - In males symptoms include urethritis, dysuria and it sometimes progresses to epididymitis

Chlamydia

- In females symptoms include mucopurulent cervical inflammation which can progress to salpingitis and PID.
- Inclusion conjunctivitis – this occurs in both newborns and adults and a genital tract infection is the source of the infection (serotypes D-K); is a benign, self-limited conjunctivitis which heals with no scarring
 - Newborns – are infected during the birth process and the infection manifests 1-2 weeks after birth as a mucopurulent discharge that lasts 2 weeks and then subsides.
 - Some may develop an afebrile, chronic pneumonia

Chlamydia

- In adults – causes an acute follicular conjunctivitis with little discharge.
- Trachoma (serotypes A-C) – is the single, greatest cause of blindness in underdeveloped countries.
 - Transmission is by direct contact and in poor, less developed countries children may be infected in the first three months of life.
 - Chronic infection and reinfection are common and result in conjunctival scarring and corneal vascularization.
 - The scars contract causing the upper lid to turn in so that the eyelashes cause corneal abrasions.
 - This leads to secondary bacterial infections and results in blindness.

Trachoma



Chlamydia

- Lymphogranuloma venereum (serotypes $L_{1, 2, 3}$) is a venereal disease that occurs in poor, tropical areas.
 - Upon infection, widespread dissemination takes place and a primary, painless lesion (either a vesicle or an ulcer) occurs at the site of entry within a few days.
 - This heals with no scarring.
 - A secondary stage occurs 2-6 weeks later with symptoms of regional suppurative lymphadenopathy (buboes) that may drain for a long time and be accompanied by fever and chills.
 - Arthritis, conjunctival, and CNS symptoms may also occur.
 - A tertiary stage may occur and is called the urethrogenital perineal syndrome.
 - This is characterized by structural changes such as non-destructive elephantiasis of the genitals and rectal stenosis.

Chlamydia

- *Chlamydia psittaci* – naturally infects avian species and non-primate animals causing mild to severe illness.
 - In man causes psittacosis (ornithosis) and is acquired by contact with an infected animal.
 - Infection can range from subclinical to fatal pneumonia.
 - Most commonly causes an atypical pneumonia with fever, chills, dry cough, headache, sore throat, nausea, and vomiting.

Chlamydia

- Treatment/antimicrobial susceptibility
 - *C. trachomatis* –
 - *Trachoma* – systemic tetracycline, erythromycin; long term therapy is necessary
 - Genital tract infections and conjunctivitis – tetracyclines and erythromycin
 - *C. psittaci* – same as above

Treponema

is a genus of spiral-shaped bacteria. The major treponeme species of human pathogens is *Treponema pallidum*, whose subspecies are responsible for diseases such as syphilis, bejel, and yaws. *Treponema carateum* is the cause of pinta. *Treponema paraluis-cuniculi* is associated with syphilis in rabbits. *Treponema succinifaciens* has been found in the gut microbiome of traditional rural human populations.

Treponema pallidum is a helically shaped bacterium with high mobility consisting of an outer membrane, peptidoglycan layer, inner membrane, protoplasmic cylinder, and periplasmic space. It is often described as Gram negative, but its outer membrane lacks lipopolysaccharide, which is found in the outer membrane of other Gram-negative bacteria. It has an endoflagellum (periplasmic flagellum) consisting of four main polypeptides, a core structure, and a sheath.

The flagellum is located within the periplasmic space and wraps around the protoplasmic cylinder. *T. pallidum*'s outer membrane has the most contact with host cells and contains few transmembrane proteins, limiting antigenicity while its cytoplasmic membrane is covered in lipoproteins. The outer membrane's treponemal ligands main function is attachment to host cells, with functional and antigenic relatedness between ligands. The genus *Treponema* has ribbons of cytoskeletal cytoplasmic filaments that run the length of the cell just underneath the cytoplasmic membrane.

Clinical significance

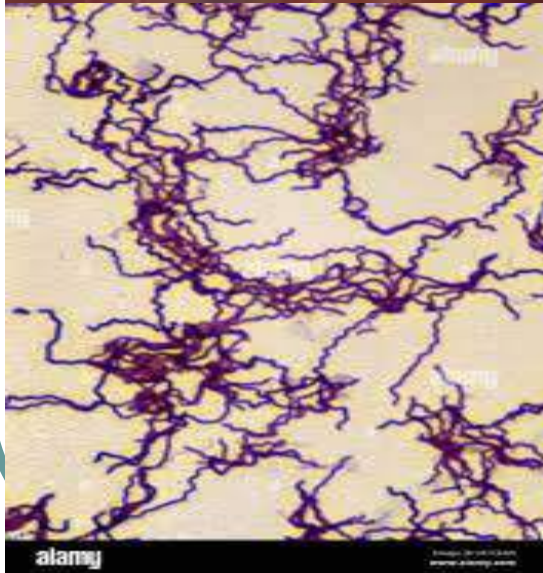
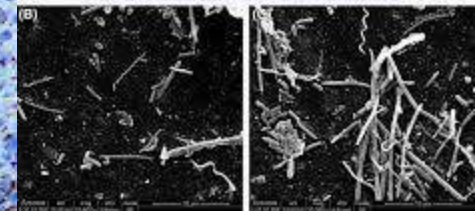
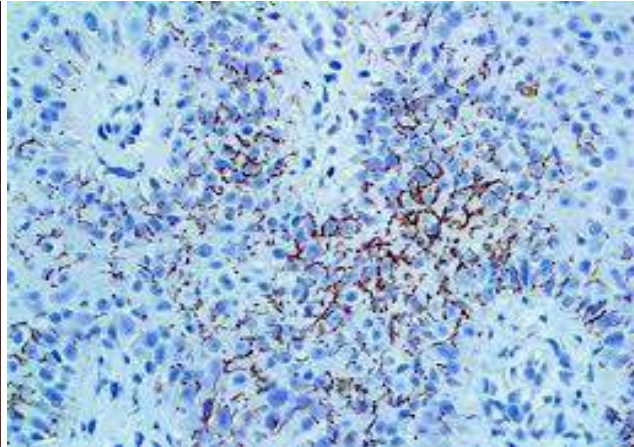
The clinical features of syphilis, yaws, and bejel occur in multiple stages that affect the skin. The skin lesions observed in the early stage last for weeks or months. The skin lesions are highly infectious, and the spirochetes in the lesions are transmitted by direct contact. The lesions regress as the immune response develops against *T. pallidum*. The latent stage that results lasts a lifetime in many cases. In a minority of cases, the disease exits latency and enters a tertiary phase, in which destructive lesions of skin, bone, and cartilage ensue. Unlike yaws and bejels, syphilis in its tertiary stage often affects the heart, eyes, and nervous system as well.

Laboratory identification

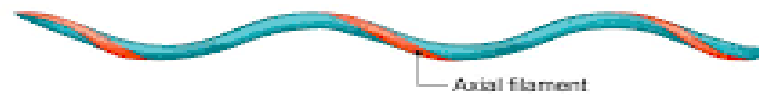
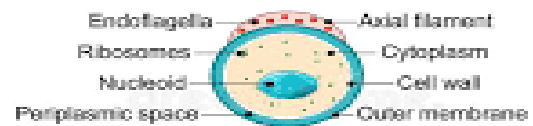
Micrograph showing *T. pallidum* (black and thin) – Dieterle stain
Treponema pallidum was first microscopically identified in syphilitic chancres by Fritz Schaudinn and Erich Hoffmann at the Charité in Berlin in 1905. This bacterium can be detected with special stains, such as the Dieterle stain. *T. pallidum* is also detected by serology, including nontreponemal VDRL, rapid plasma reagin, treponemal antibody tests (FTA-ABS), *T. pallidum* immobilization reaction, and syphilis TPHA test

Successful long-term cultivation of *T. pallidum* subspecies *pallidum* in a tissue culture system has been reported in 2018.

However, because *T. pallidum* cannot be grown in a pure culture



Treponema



Treponema denticola (oral trepanoma)

is a Gram-negative, obligate anaerobic, motile and highly proteolytic spirochete bacterium. *T. denticola* is associated with the incidence and severity of human periodontal disease. *Treponema denticola* is one of three bacteria that form the Red Complex, the other two being *Porphyromonas gingivalis* and *Tannerella forsythia*. Together they form the major virulent pathogens that cause chronic periodontitis. Having elevated *T. denticola* levels in the mouth is considered one of the main etiological agents of periodontitis. *T. denticola* is related to the syphilis-causing obligate human pathogen,

Adherence and cytotoxicity

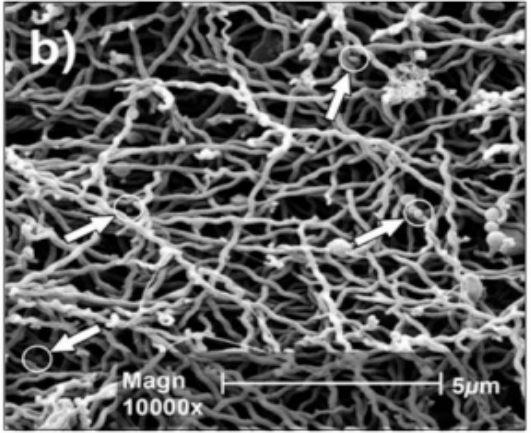
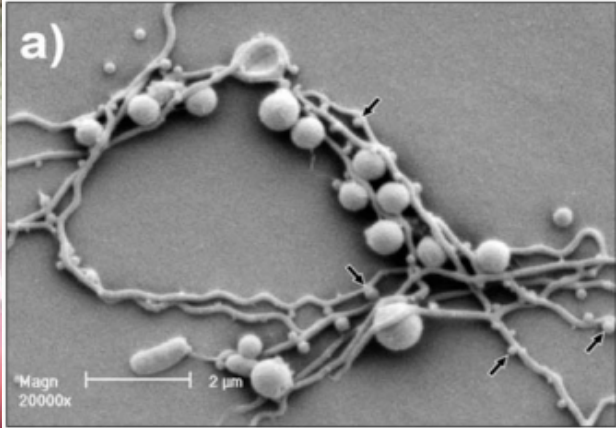
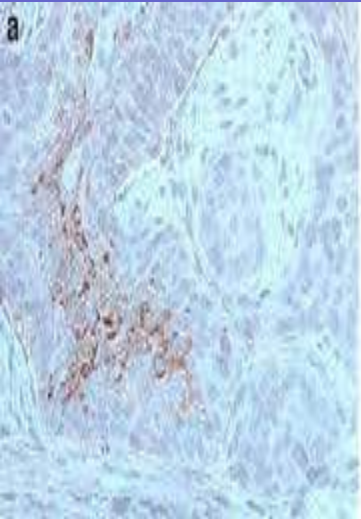
The main site for *T. denticola* habitation in the oral cavity is the gingival crevice. These spirochetes attach to proteins (including fibronectin and collagen) of local gingival fibroblasts, binding to their plasma membrane. A 53-kDa surface protein on *T. denticola* is responsible for transporting its components into the host cell, exhibiting a cytotoxic effect. Accumulation of *T. denticola* in this manner facilitates the disease-causing process, including membrane blebbing and red blood cell lysis

role in Periodontal disease

Treponema denticola, a subgingival oral spirochete has been associated with many periodontal disease conditions such as: the early stage of periodontitis, acute pericoronitis (infection under the gum tissue covering a partially erupted tooth), as well as necrotising ulcerative gingivitis (severe inflammation of the gum more common in immunocompromised patients). It relates to lesions limited to gingival tissue. Clinical evidence includes periodontal pockets contain large numbers of treponema denticola together with other proteolytic gram negative bacteria, playing an important role in the development of periodontal disease. The toxic products of these bacteria, especially treponema denticola may damage the surface lining periodontal cells making them more prone to damage as well as lysis. Treponema denticola attaches to fibroblasts and epithelial cells as well as to extracellular matrix components which are found in periodontal tissues and release its own bacterial contents.

Oral cancer

Treponema denticola is a potential etiological bacterial agent for oral cancer. It encourages oncogenesis (process in which healthy cells become cancer cells) and therefore the progression of oral cancer through chronic inflammation advancing invasiveness of the cancer cells. This results in the ceasing of cell apoptosis (inhibition of controlled cell death – a safety mechanism within cells to stop more damage from occurring), resulting in rapid growth and multiplication of cancer cells. This suppresses the immune system stopping the body from recognising the cancerous cells and as a result more cancer-promoting substances are produced. The presence of *T. denticola* along with other periodontal pathogens and bacterial diversity within the oral cavity are important factors contributing to cancerous cells (including precancerous gastric lesions).



Dental Caries, dental plaque

The most frequent and economically-important condition in humans resulting from interactions with our normal flora is probably dental caries. Dental plaque, dental caries result from actions initiated and carried out by the normal bacterial flora. **Dental plaque**, which is material adhering to the teeth, consists of bacterial cells (60-70% the volume of the plaque), salivary polymers, and bacterial extracellular products. Plaque is a naturally-constructed biofilm, in which the consortia of bacteria may reach a thickness of 300-500 cells on the surfaces of the teeth. These accumulations subject the teeth and gingival tissues to high concentrations of bacterial metabolites, which result in dental disease.

Caries is defined as localized destruction of the tissues of the tooth by bacterial fermentation of dietary carbohydrate.

Etiology:1- host factors (tooth,saliva)

2-diet (mainly intake of fermentable carbohydrate)

3-plaque microorganisms (supragingival plaque)

Plaque metabolism:

The main source of nutrition for oral bacteria is saliva. Although the carbohydrate content of saliva is generally low ,increased level are seen after meal .To make use of these transients increase in food levels , oral bacteria have developed a number of regulatory mechanisms , which act at three levels :

1- transport of sugar into organisms

2-the glycolytic pathway

3- conversion of pyruvate into metabolic end products

- The dominant bacterial species in dental plaque are *Streptococcus sanguis* and *Streptococcus mutans*, both of which are considered responsible for plaque

- Plaque formation is initiated by a weak attachment of the streptococcal cells to salivary glycoproteins forming a pellicle on the surface of the teeth. This is followed by a stronger attachment by means of extracellular sticky polymers of glucose (glucans) which are synthesized by the bacteria from dietary sugars (principally sucrose). An enzyme on the cell surface of *Streptococcus mutans*, glycosyl transferase, is involved in initial attachment of the bacterial cells to the tooth surface and in the conversion of sucrose to dextran polymers (glucans and fructans) which form plaque. Glucan used a major bacterial food source ,in soluble fructan contribute to plaque matrix while facilitating the adhesion and aggregation of plaque bacteria and serve as ready ,extracellular food source.

-some sucrose is transported into bacteria as disaccharide or disaccharide phosphate which is metabolized intracellularly by sucrose phosphate hydrolase into glucose and fructose .During glucolysis glucose degraded by bacteria via Embden-Myeyerhof pathway with production two molecule of pyruvate from each molecule of glucose .

-different species produce acid at different rates . *Streptococcus mutans* is most acidogenic and aciduric (acid tolerant) ,reduce plaque pH to low levels below 5.5 initiate the process of enamel demineralization.

PLAQUE HOMEOSTASIS:

- The bacterial composition of plaque remains relatively stable despite regular exposure to minor environmental conditions . This stability (microbial homeostasis) is due in part to a dynamic balance of both synergistic and antagonistic microbial interactions

-Homeostasis can break down, leading to shifts in the balance of the microflora, thereby predisposing sites to disease. For example, the frequent exposure of plaque to low pH leads to inhibition of acid-sensitive species and the selection of organisms with an aciduric physiology, such as mutans streptococci and lactobacilli. Similarly, plaque accumulation around the gingival margin leads to an inflammatory host response and an increased flow of gingival crevicular fluid. The subgingival microflora shifts from being mainly Gram-positive to being comprised of increased levels of obligate anaerobic, a saccharolytic Gram-negative organisms. It is proposed that disease can be prevented or treated not only by targeting the putative pathogens but also by interfering with the processes that drive the breakdown in homeostasis.

- Thus, the rate of acid production following sugar intake could be reduced by fluoride, alternative sweeteners, and low concentrations of antimicrobial agents, while oxygenating or redox agents could raise the pH of periodontal pockets and prevent the growth and metabolism of obligately anaerobic species.

The role of *Streptococcus mutans*

The evidence for the etiological role of *Streptococcus mutans* in dental caries include the following :

1-correlation of bacteria counts in saliva and plaque with prevalence and incidence of caries .

2-this bacteria can often isolated from the tooth surface immediately after development of caries

3-positive correlation between the progression of carious lesion and bacteria count

4-production of extracellular poly saccharide from sucrose (which help cement the plaque organisms together and to the tooth surface)

5-ability to initiate and maintain growth to continue acid production at low pH value

6-ability to attain critical pH for enamel demineralization more rapidly than other common plaque bacteria

7-ability to produce intracellular polysaccharide (IPSs) as glycogen ,which may act as food source for use when dietary carbohydrate are low.

-**Actinomyces spp** are associated with the development of root surface caries (root lesion differ from enamel caries in that calcified tissue are softened without obvious cavitation) .

The flow rate and composition of Saliva:

-The mechanical washing action of saliva is a very effective mechanism in the removal of food debris and unattached oral microorganisms.

-It has a high buffering capacity ,which tend to neutralize acids produce by plaque bacteria on tooth surfaces.

-It has supersaturated with calcium and phosphorus ions, which are important in the re-mineralization of white spot lesions .

-Saliva also act as a delivery vehicles for fluoride.

Prevention of dental caries :

The major approaches to prevention dental caries are:

1-Sugar substitutes: stopping or reducing between meal consumption of carbohydrates ,or substituting non-cariogenic artificial sweeteners ,e.g. sorbitol, xylitol or lycasin.

2-Fluoride: making the tooth structure less soluble to acid attack by using fluoride. This can delivered to the tooth tissue in ways. When administered systematically during childhood ,it is incorporated during melogenesis.The best vehicle is domestic water supply (concentration 1ppm) failing this tablets ,topical application of fluoridated gel or fluoridated toothpaste may be used.

3-Sealants: to protect susceptible areas of tooth (e.g. pit and fissures) that cannot easily be kept plaque –free by routine oral hygiene measure.

4-Reducing cariogenic flora: So that even in the presence of sucrose,acid production will be minimal (e.g. oral hygiene aids ,antimicrobial agents ,and possibly immunization)

5-Probiotics: replacement therapy of cariogenic bacteria by organisms with low or no cariogenic potential.

Control of cariogenic plaque flora:

-Control may be achieved by :

1-mechanical cleansing (flossing ,interdental brushes,wood sticks)

2-antimicrobial therapy(chlorhexidine 0.2% mouth wash disrupts cell wall and cell membrane permeability of bacteria)

3-immunization and replacement therapy

a-Active immunization against dental caries :

using either cell wall –associated antigens (I/II) or glucosyl transferase (extracellular enzyme)from mutans streptococci is effective in reducing experimental dental caries in rats and monkeys.The vaccine may be produce its protective by :

-inhibition of the microbial colonization of enamel by secretory immunoglobulin A(IgA)

-Interference with bacterial metabolism

-enhancement of phagocytic activity in the gingival crevice area due to the opsonization of mutans streptococci with IgA ,IgG antibodies.

- the vaccination trials on humans have been unsuccessful because of fears of possible side effects ,which would be unacceptable as caries is not a life threatening disease (The antibodies that develop after immunization with most antigens of mutans streptococci tend to cross react with heart tissue ,and the possibility that heart damage could result has made human vaccine trials very difficult).

-A caries vaccine could,however,be useful for developing countries with limited dental services and increasing prevalence of caries, and for prevention of disease in high risk groups,for instance ,children with mental or physical disabilities.

b-Passive immunization:

-Experimental studies indicate that when the natural levels of oral mutans streptococci are suppressed by chlorhexidine, topical application of monoclonal antibodies against antigen I\II of mutans streptococci prevent recolonization by organisms.

-Transgenic plants could be used to produce dimeric antibodies with specificity to antigen I\II of streptococci that are stable in the mouth and persist for longer periods than monomeric antibody.

Gram-Negative Anaerobes

Gram-Negative Bacilli

1. *Bacteroides*—The *Bacteroides* species

are very important anaerobes that cause human infection. They are a large group of bile-resistant, non-spore-forming, slender gram negative rods that may appear as coccobacilli.

Many species previously included in the genus *Bacteroides* have been reclassified into the genus *Prevotella* or the genus *Porphyromonas*.

Those species retained in the *Bacteroides* genus are members of the *B fragilis* group (~20 species). *Bacteroides* species are normal inhabitants of the bowel and other sites.

Normal stools contain 10¹¹ *B fragilis* organisms per gram (compared with 10⁸/g for facultative anaerobes). Other commonly isolated members of the *B fragilis* group

include *Bacteroides ovatus*, *Bacteroides distasonis*, *Bacteroides vulgatus*, and *Bacteroides thetaiotaomicron*.

Bacteroides species are most often implicated in intra-abdominal infections, usually under circumstances of disruption of the intestinal wall as occurs in perforations related to surgery or trauma, acute appendicitis, and diverticulitis. These infections are often polymicrobial; anaerobic cocci, *Clostridium* species, and *Eubacterium* may also be found. Both *B. fragilis* and *B. thetaiotaomicron* are implicated in serious intrapelvic infections such as pelvic inflammatory disease and ovarian abscesses.

B. fragilis group species are the most common species recovered in some series of anaerobic bacteremia, and these organisms are associated

with a very high mortality rate. *B fragilis* is capable of elaborating numerous virulence factors, which contribute to its pathogenicity and mortality in the host.

Bacteroides fragilis

Habitat and transmission

Bacteroides species are the most predominant flora in the intestine (10¹¹ cells per gram of faeces), far outnumbering *Escherichia coli*. They cause serious anaerobic infections such as intra-abdominal sepsis, peritonitis, liver and brain abscesses, and wound infection.

Characteristics

Strictly anaerobic, Gram-negative, non-motile, non-spore-forming bacilli, but may appear pleomorphic. The polysaccharide capsule is an important virulence factor.

Culture and identification

These organisms have stringent growth requirements; they demonstrate slow growth on blood agar and appear as grey to opaque, translucent colonies. They grow well in

Robertson's cooked meat medium supplemented with yeast extract. Identified by biochemical tests, growth inhibition by bile salts, antibiotic resistance tests and gas-liquid chromatographic analysis of fatty acid end products of glucose metabolism.

Pathogenicity

strains. Consequently, many *Bacteroides* infections are **polymicrobial** in nature.

Treatment

Sensitive to metronidazole and clindamycin. Resistant to penicillins, first-generation cephalosporins and aminoglycosides. Penicillin resistance is due to β -lactamase production.



2. *Prevotella*—The *Prevotella* species are gram-negative bacilli and may appear as slender rods or coccobacilli. Most commonly isolated are *Prevotella melaninogenica*, *Prevotella bivia*, and *Prevotella disiens*. *P. melaninogenica* and similar species are found in infections associated with the upper respiratory tract. *P. bivia* and *P. disiens* occur in the female genital tract. *Prevotella* species are found in brain and lung abscesses, in empyema, and in pelvic inflammatory disease and tubo-ovarian abscesses. In these infections, the prevotellae are often associated with other anaerobic organisms that are part of the normal microbiota—particularly peptostreptococci, anaerobic gram positive rods, and *Fusobacterium* species—as well as gram positive and gram-negative facultative anaerobes that are part of the normal microbiota

3. *Porphyromonas gingivalis*

belongs to the phylum Bacteroidota and is a nonmotile, Gram-negative, rod-shaped, anaerobic, pathogenic

bacterium. It forms black colonies on blood agar. It is found in the oral cavity, where it is implicated in periodontal disease as well as in the upper gastrointestinal tract, the respiratory tract and the colon. It has been isolated from women with bacterial vaginosis.

The role of *P. gingivalis* in the development of periodontal disease can be attributed to the multiple virulence factors such as production of capsule that contribute to its defense and destruction against epithelial cells . An important form of evasion for *P. gingivalis* is its capsule, which prevents phagocytosis. The presence of fimbriae surrounding the bacteria allows for adhesion to the epithelial cells promoting colonization. Important virulence factors for *P. gingivalis* are the proteases, which have the ability to disrupt complement activity, degrade immunoglobulins, cleave matrix proteins, and inhibit iron transport. Other virulence factors include endotoxin, collagenase, phospholipase A, hemolysin, and fibrolysin.

Lipopolysaccharide found in Gram-negative bacteria, provides *P. gingivalis* with its endotoxin properties. Hemagglutinins are also produced by *P. gingivalis* resulting in agglutination of host red blood cells

4 Tannerella forsythia

Tannerella forsythia is an anaerobic gram-negative member of the Cytophaga-Bacteriodes family, that aids in the development of periodontal diseases and belongs to the red complex bacteria. *Tannerella forsythia* is pleomorphic, non-motile, spindle-shaped, and gram-negative rods *Tannerella forsythia* is commonly located in the supragingival and subgingival sites They have an inner and outer membrane below a distinctive outer layer (S layer). The production of endotoxin, fatty acid and methylglyoxal are considered virulence factors of *Tannerella forsythia*⁽⁹⁴⁾ . Additional virulence factors have been shown in research studies such as, a trypsin-like protease, sialidase, BspA(Bacteroid surface protein), alpha-D-glucosidase, hemagglutinin and an apoptosis-inducing activity

4-Fusobacterium:

Fusobacterium species are member of the family Bacteroidaceae. gram negative saccharolytic obligate anaerobes that are catalase negative ,non spore forming and non motile. *Fusobacterium* inhabits the mucous membranes of humans and animals, serving as a pathogen to both. *Fusobacterium nucleatum* is an oral **bacterium**, indigenous to the human **oral cavity**, that plays a role in **periodontal disease**. This organism is commonly recovered from different monomicrobial and mixed infections in humans and animals. It is a key component of periodontal **plaque** due to its abundance and its ability to coaggregate with other species in the oral cavity.*Fusobacterium nucleatum* is one of the dominant bacteria found in the mouth although it is confined principally to the gingival sulcus .It is also the most common species of the genus isolated from fusospirochaetal infections at other sites in humans ,although the lack of choline binding protein confines the organism to the mouth and not the nasopharynxFusobacteria isolated from patients with periodontitis include *F.varium* ,*F.nucleatum* ,*F.periodonticum*, *F.alocis* and *F.sulci*.

Fusobacteria are differentiated from the bacteroides by their production of major amounts of n-butyric acid alone; iso-butyric and iso-valeric acids are not produced. The bacteroides vary in the fatty acids produced, but do not produce n-butyric acid alone. *Fusobacterium varium* has phosphatase activity, esculin hydrolysis, indole, production propionate from lactate and ONPG activity negative but bile resistance, production propionate from threonine and production gas from glucose are positive. Many extracellular products that may contribute to pathogenicity have been identified. Fusobacterial lipopolysaccharide endotoxin, like that of facultative gram-negative bacteria, but unlike *Bacteroides* spp. and other gram-negative anaerobic genera, contains readily detectable keto deoxyoctanoate and appears to endow virulence properties, as do neutrophil-cytotoxic substances, verotoxin, and DNAase.

Metronidazole, piperacillin/tazobactam, ticarcillin/clavulanate, amoxicillin/sulbactam, ampicillin/sulbactam, ertapenem, imipenem, meropenem, clindamycin, and ceftiofur are all

used therapeutically to treat infections associated with *Fusobacterium*. *Fusobacterium* may be resistant to penicillin and there is widespread resistance to erythromycin and other macrolides

5- Veillonella

Veillonella is a gram negative, strict anaerobic, non-spore-forming coccus-shaped bacterium. It is found in the gut of humans and dental plaque. It cannot metabolize carbohydrates, but instead uses organic acids like lactate. The lipopolysaccharide has been found as a major virulence factor in some of these diseases. Oral *Veillonella* is one of the predominant hydrogen sulfide (H₂S)-producing bacteria in the tongue coating. *Veillonella* species are generally susceptible to betalactam antibiotics, clindamycin, and metronidazole. However, *Veillonella* species are generally resistant to tetracycline and are only intermediately susceptible to erythromycin

ا.د هديل مزهر يونس

ا.م.د جتین عزالدین علي

Introduction to Virology

- Are smallest form of microorganism and effect most other forms of life :animals ,plants,human ,bacteria.
- small size (10-100nm)
- consist either DNA or RNA but never both single strand or double strand ,linear or circular
- outside the cells of susceptible host ,viruses lack ribosomes-the protein-synthesis apparatuses.
- The are obligate intracellular parasite.

Viral structures

- ◆ Viruses consist of nucleic acid core containing viral genome surrounded by protein shell called a capsid.
- ◆ The entire structure is referred to nucleocapsid this may be naked or enveloped within lipoprotein sheath derived from host cell membrane.
- ◆ The protein shell of capsid consist of repeating unit of one or more protein molecule to form structural units called capsomere.

Nucleocapsid morphology(symmetry)

- ◆ **A. Helical** : the capsomer surround the viral nucleic acid in the form of a helix or spiral to form tubular nucleocapsid(.e.g.mammalian RNA viruses
- ◆ **B. Icosahedral**: the protein molecule are symmetrically arranged of icosahedral (20-side solid ,each face being an equilateral triangle) Herpes viruses are example.
- ◆ **C. Complex**: This is exhibited by few families of viruses ,retroviruses ,poxviruses.

Classification

- ◆ The viruses are classified according to:
- ◆ 1-symmetry
- ◆ 2-presence and absence of an envelope
- ◆ 3-nucleic acid composition(DNA,RNA)
- ◆ 4-number of nucleic acid strands and their polarity

DNA viruses

- papovaviruses (papillomaviruses , polyomaviruses)(non envelope double strand)
- Adenoviruses(non envelope double strand)
- herpsviruses(envelope double strand)
- poxviruses (envelope double strand)
- parvoviruses(envelope single strand)
- Hepadnaviruses (non-envelope double strand)

RNA viruses

- ◆ Picornaviruses
(polioviruses, echoviruses, coxsackiviruses)(non[-envelope single strand)
- ◆ Orthomyxoviruses(envelope single strand)
- ◆ Paramyxoviruses (envelope single strand)
- ◆ Retroviruses(lentiviruse, oneoviruse)(envelope single strand)
- ◆ Coronaviruses(envelope helical strand)
- ◆ Togaviruses, arenaviruses, rhabdoviruses, filoviruses

Viroid's and prion

- ◆ New classes of viruses are viroid's and prions
- ◆ Viroid's cause disease in plants and composed of naked covalently linked closed circle ssRNA less than 300-400 nucleotide length.
- ◆ Viroid's not associated with human disease.
- ◆ Prions not have either DNA or RNA
- ◆ Cause vaculation of cell sponge like appearance called spongiform
- ◆ Prion have ability to replicate itself by long incubation period up to 20 years
- ◆ Prion highly resistance to heat,chemical agents,irradiation

Latent viruses relative to dentistry

- ◆ Herpes simplex viruses (HSV) the site of latency trigeminal ganglion
- ◆ Varicella –zoster viruses (VZV) in sensory ganglia
- ◆ Epstein –Barr viruses (EBV) in epithelial cell and B lymphocyte
- ◆ Cytomegaloviruses (CMV) in salivary gland cells
- ◆ Papillomaviruses in epithelial cell
- ◆ Human immunodeficiency viruses (HIV) in lymphocyte and other CD4 cells.
- ◆ Hepatitis viruses

B. Comparison to bacteria

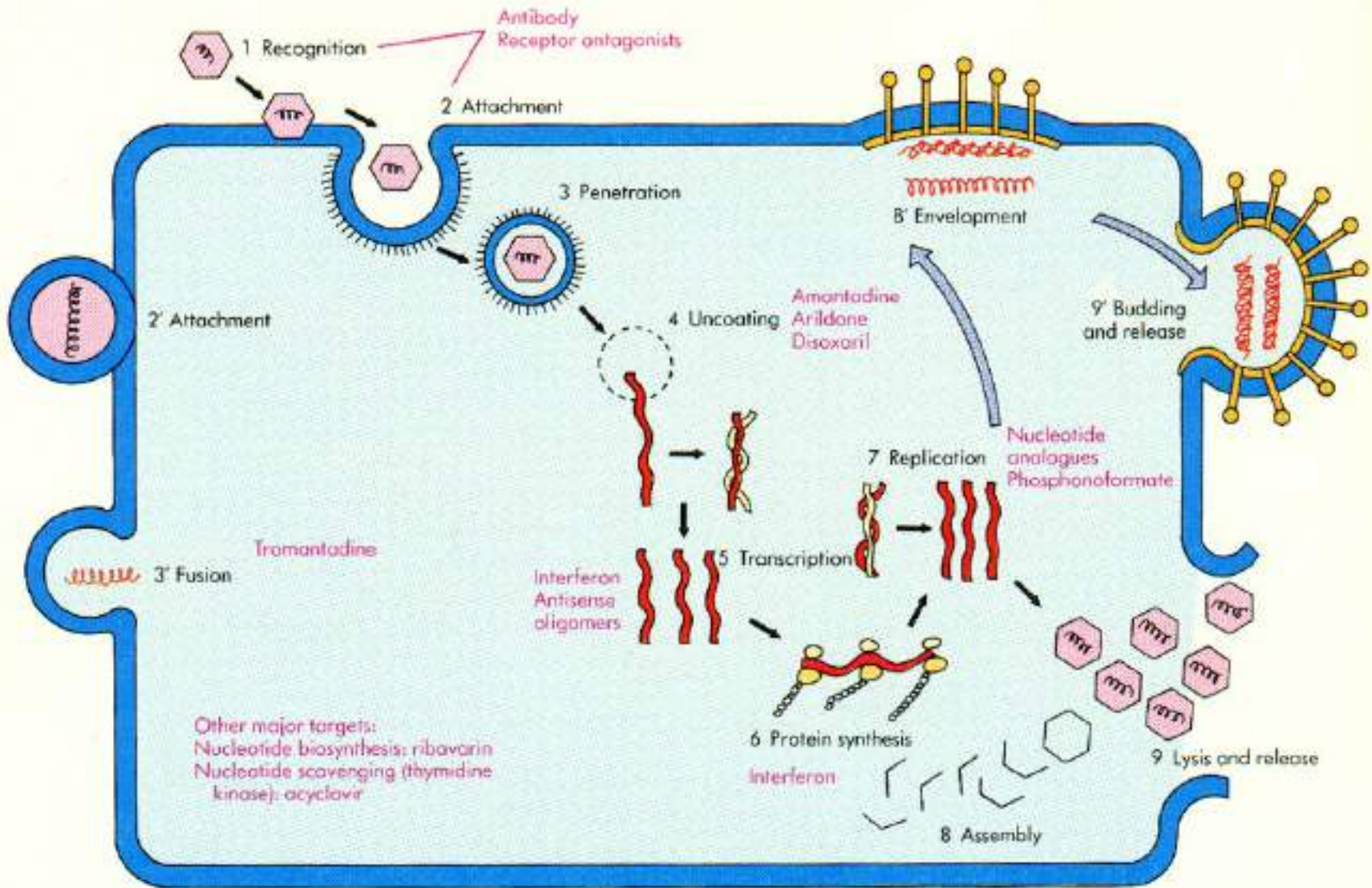
1. overall

	Bacteria	Virus
◆		
◆ Intracellular parasite	(no)	yes
◆ Plasma membrane	yes	no
◆ Binary fission	yes	no
◆ Filterable	no	yes
◆ Possess DNA & RNA	yes	no
◆ ATP production	yes	no
◆ Ribosomes	yes	no
◆ Antibiotic sensitive	yes	no

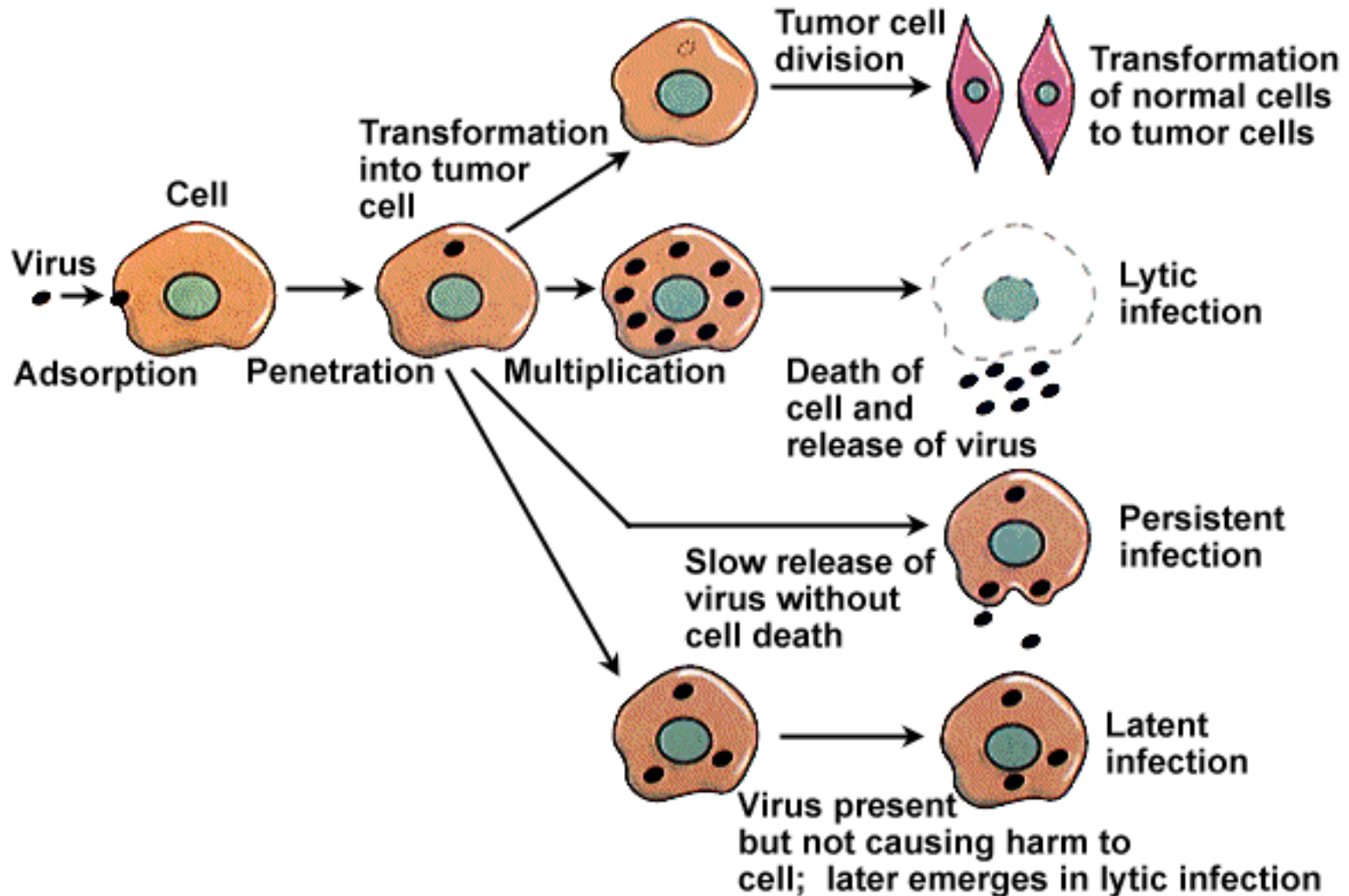
How viruses multiply (replication)

- Adsorption or Attachment(through envelope protein as heamagglutinine,to certain receptors like glycoprotein or glycolipid on host cell)
- Penetration or uptake achieved by :
 - a- Endocytosis ,b- fusion ,or c- translocation
- Uncoating
- Transcription(mRNA for synthesis enzyme ,proteins)
- Synthesis of viral components (structural, proteins and non structural, nucleic acids)
- Assembly by incorporation of nucleic acid into capsomere –procapsid. occur in cell nucleus or cytoplasm ,plasma membrane
- Maturation
- Release through gradual budding , or sudden rupture in envelope viruses

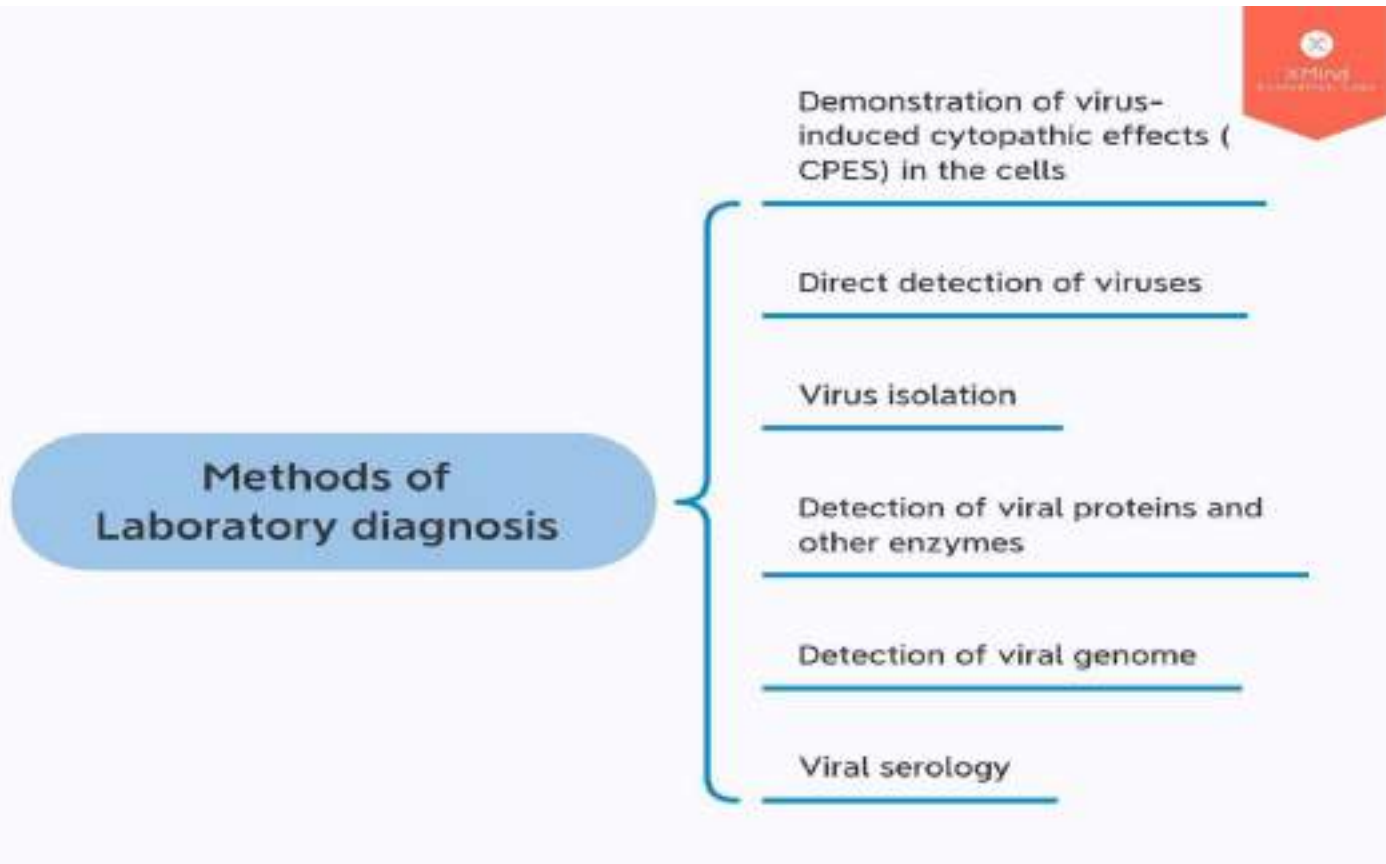
Animal Viruses



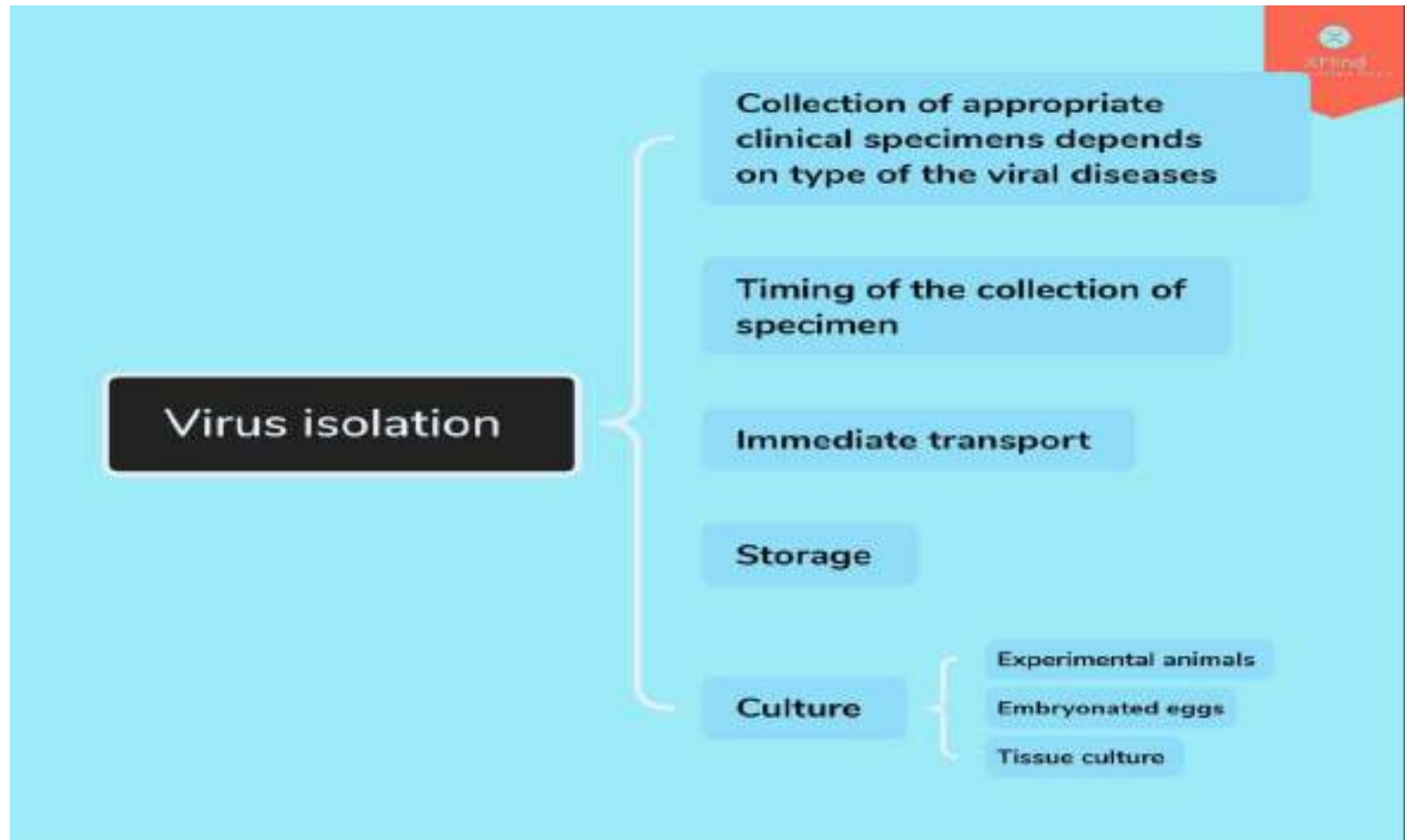
Summary of effects of viral infection on cells



Methods of lab diagnosis



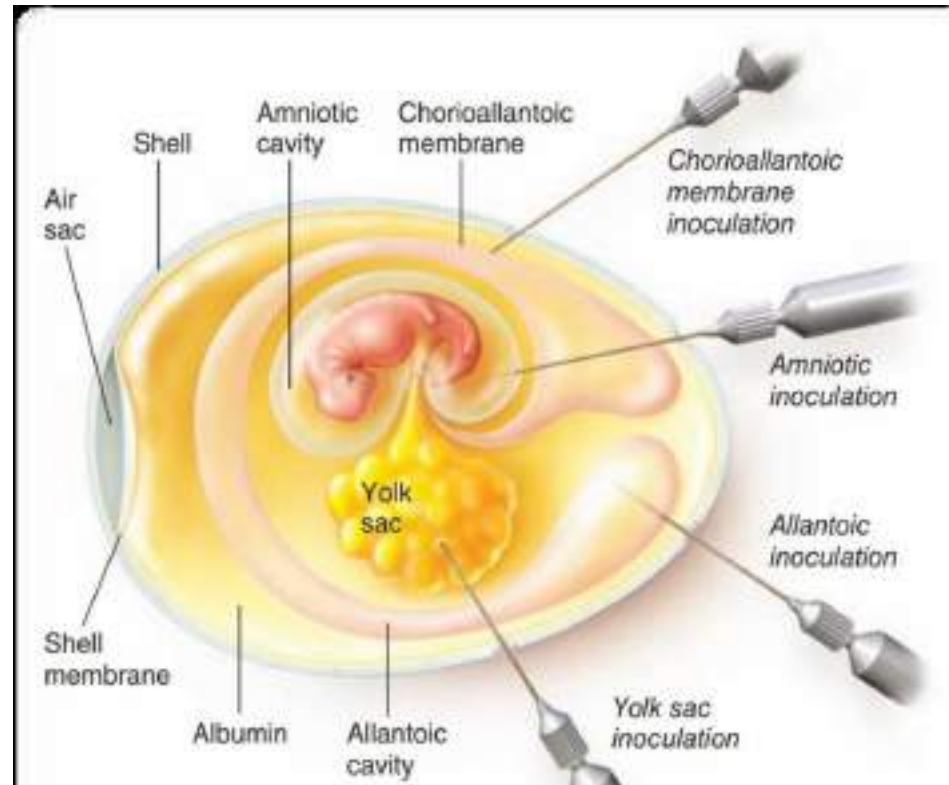
Viruses isolation

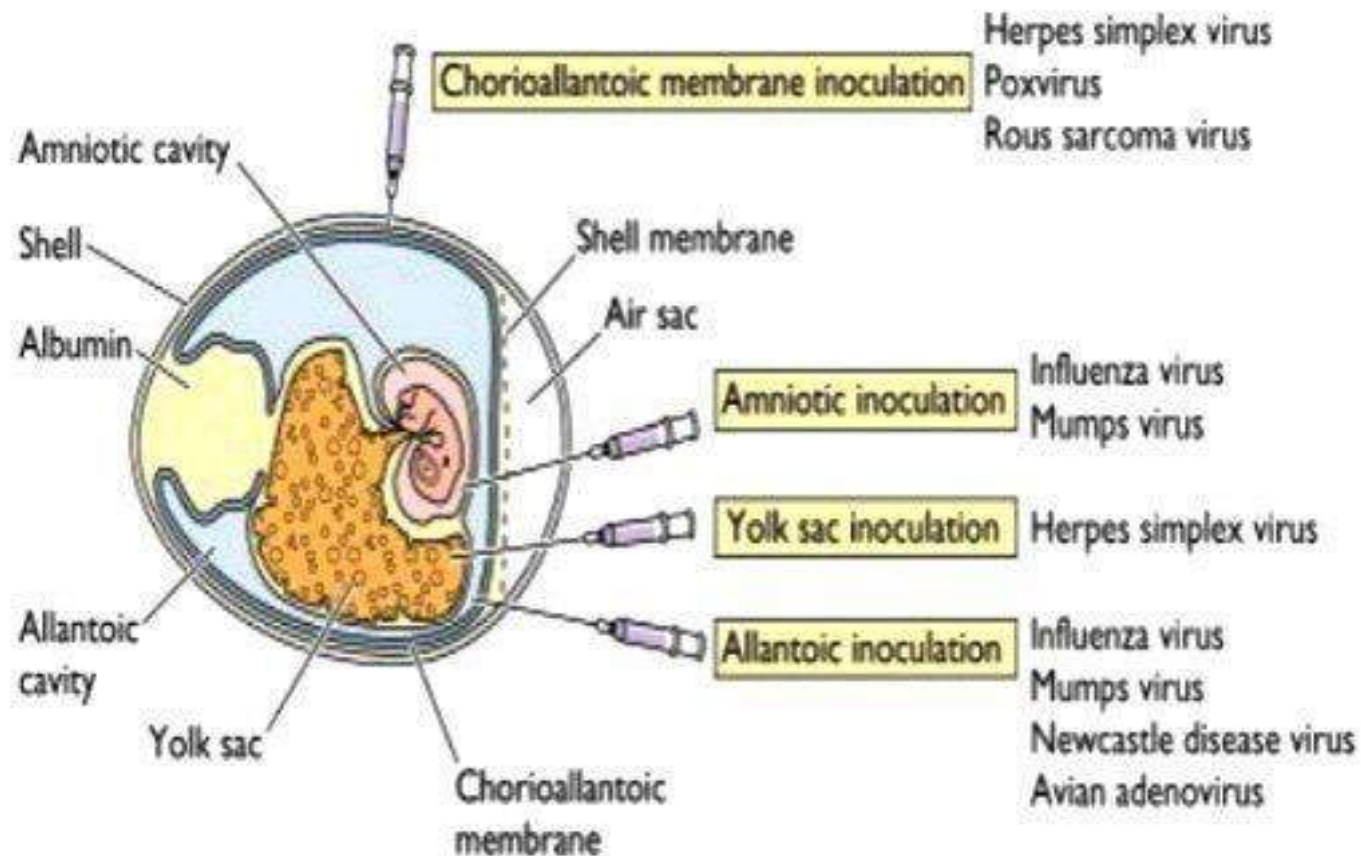


Animal inoculation

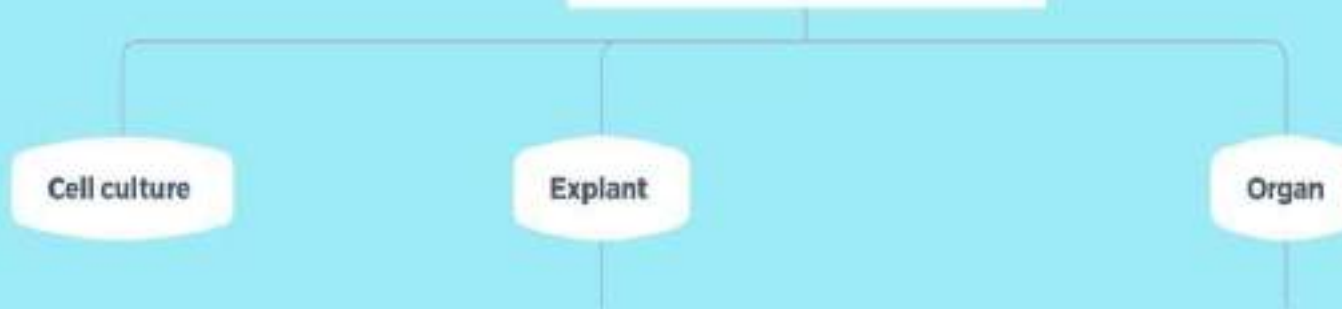


- Embryonated egg





Tissue culture

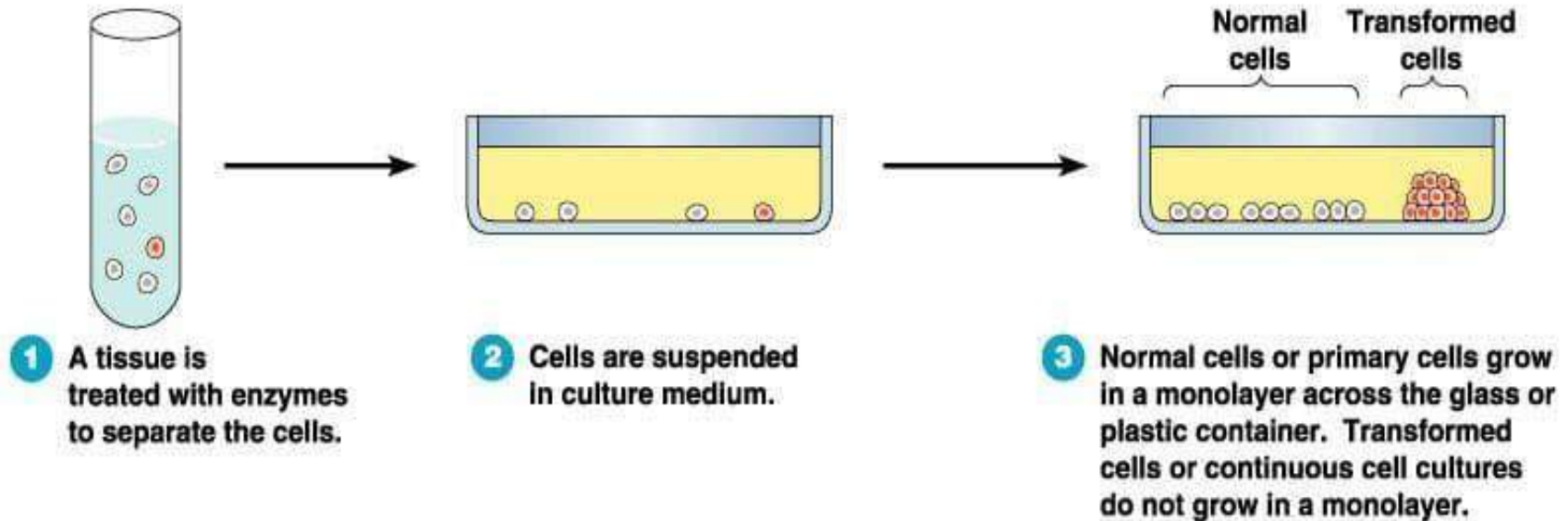


- Fragments of minced tissue can be grown as 'explant' embedded in plasma clots.
- They may be cultivated in suspension.
- Example: Adenoid tissue explants cultures used for isolation of adenovirus.

- Small bits of organs can be maintained in vitro for days and weeks ,preserving their original architecture and function.
- Useful for the viruses which appear to be highly specialized parasites of certain organs.
- For example: tracheal rings for isolation of coronavirus. 1

CELL CULTURE

- It is a process by which cells are grown under controlled conditions, outside of their natural environment.
- For this, cell lines can be obtained from human, animal or mosquito.



TYPES OF CELL CULTURE:

- Based on their origin, chromosomal characters and number of generation 3 types:
 - Primary cell culture
 - Diploid cell strains
 - Continuous cell lines

Because no one cell culture type can support the growth of all medically relevant viruses, virology laboratories must maintain several different cell culture types.

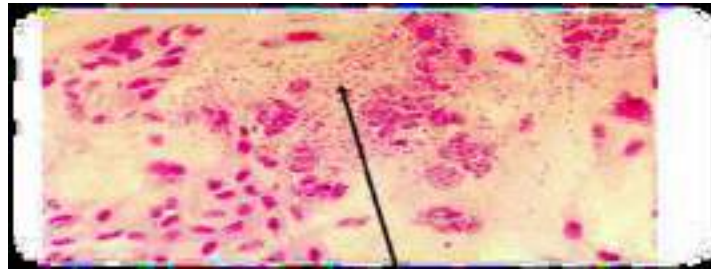
- The **minimum requirements** are:
 - Primary monkey kidney cell line: used for the isolation of respiratory and enteroviruses
 - Human fibroblast line : Used for the isolation of cytomegalovirus (CMV), varicella-zoster virus (VZV), and rhinoviruses.
 - A continuous human epithelial cell line such as HEp-2: required for the isolation of RSV

DETECTION OF VIRUS GROWTH IN CELL CULTURE

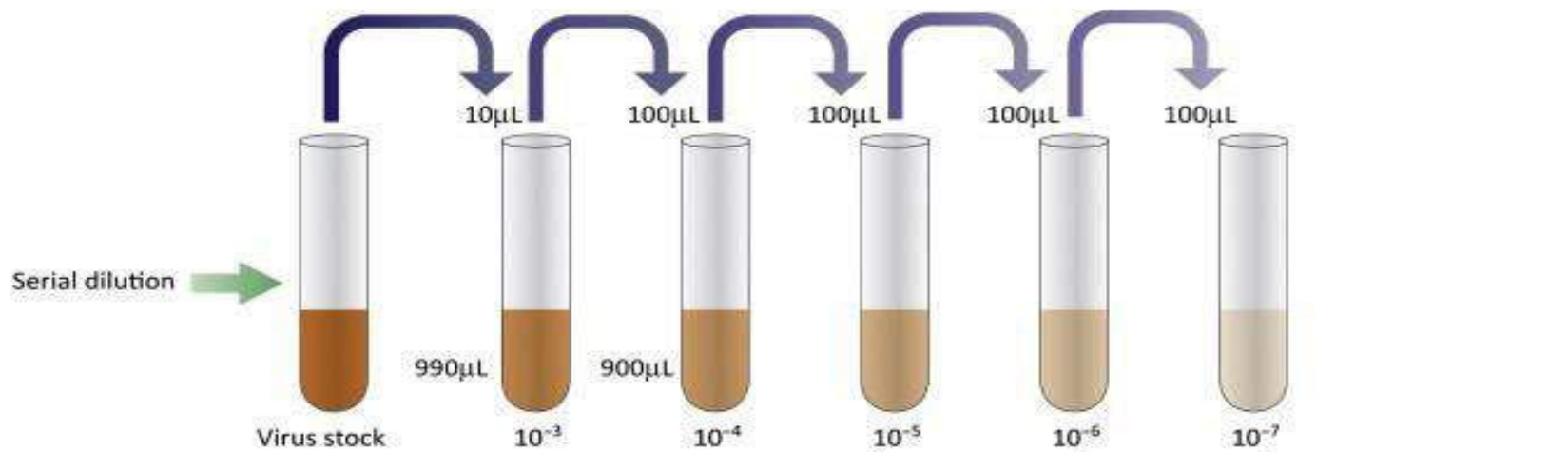
- Cytopathic effect



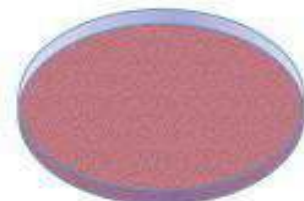
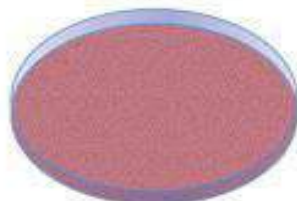
- Hemadsorption



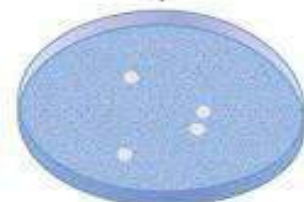
- Interference
- Transformation
- light microscopy
- Immunofluorescence
- Electron microscopy



- 1 Mix virus dilution with cells. Plate. Overlayer cells with agarose.



- 2 Remove agarose layer. Stain cells to visualize plaques in the monolayer.



- 3 Virus titer is determined by counting plaques and multiplying by the dilution factor. Plaque counts from at least 3 replicates at each dilution should be averaged.



Too numerous to count

16 plaques

4 plaques

DETECTION OF VIRAL PROTEINS AND OTHER ENZYMES

- Enzyme-linked immunosorbent assay (ELISA)
- direct immunofluorescence assay
- and radioimmunoassay (RIA)

DETECTION OF VIRAL GENOME

- The methods for detection of viral genome include
 - (a) DNA probes,
 - (b) dot blot or Southern blot analysis,
 - (c) Northern blot or RNA:DNA probe hybridization,
 - (d) polymerase chain reaction (PCR),
 - (e) reverse transcriptase PCR (RT PCR)

VIRAL SEROLOGY

- These include;
 - hemagglutination inhibition (HI) test,
 - neutralization test (NT),
 - indirect fluorescent antibody (IFA) test,
 - ELISA, RIA, latex agglutination test (LAT), Western blot.
- **The viral serology has following uses:**
 - used to identify the virus and its strain or serotype.
 - It is used to determine whether viral infection is an acute or chronic infection, or primary infection or reinfection
 - It is used for diagnosis of viral infections that cause diseases of long duration.
 - It is used for diagnosis of infections caused by viruses that are difficult to culture.

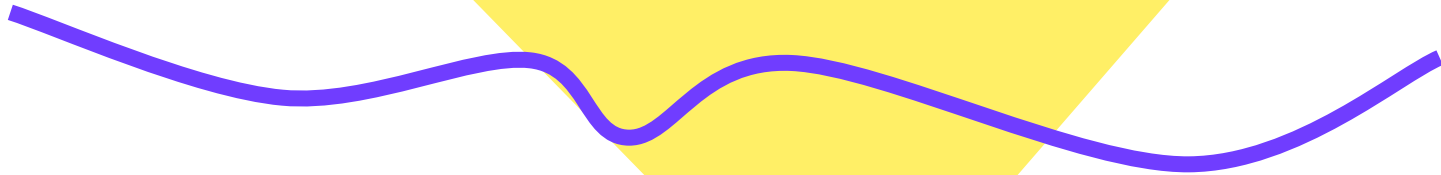
The limitations of serological tests in viral diseases are the following:

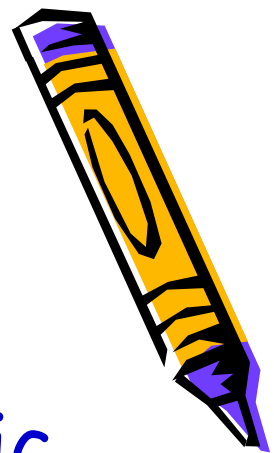
1. The presence of antiviral antibody in serum only indicates infection but cannot determine whether it is recent or old. Demonstration of IgM antibodies or demonstration of a fourfold increase in the antibody titer between acute and convalescent sera indicates only recent infection.
2. The serological tests may be associated with false-positive or false-negative reactions. The serological cross-reaction may occur between different viruses, giving rise to false positive reactions. Formation of immune complexes in serum may give rise to false-negative reaction as observed in viral infection caused by hepatitis B virus.



INTRODUCTION TO MEDICAL MYCOLOGY

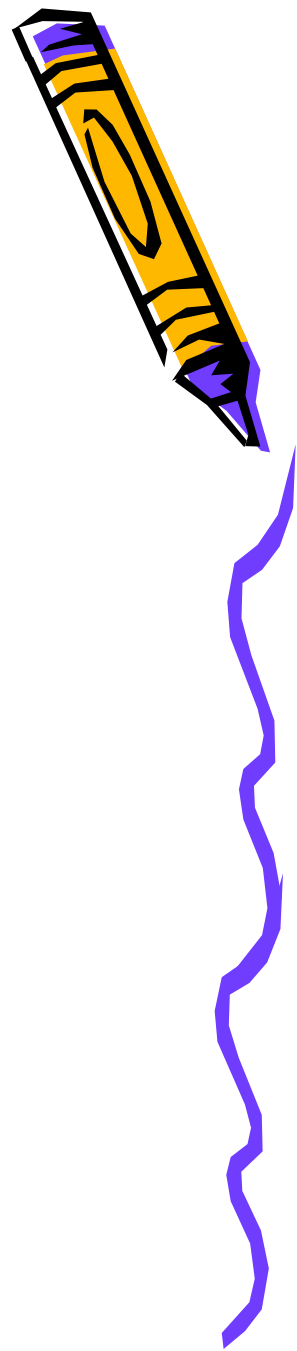
prof dr hadeel m younis
dr chateen I ali





- Fungi (yeast & molds) are **eukaryotic** organisms whereas bacteria are **prokaryotic**, they differ regarding;





- Size-diameter

4 μm -----1 μm

- Nucleus.

- Cytoplasm

- Cell membrane,

Sterol---absent in bacteria

- Cell wall,

Chitin ----peptidoglycane

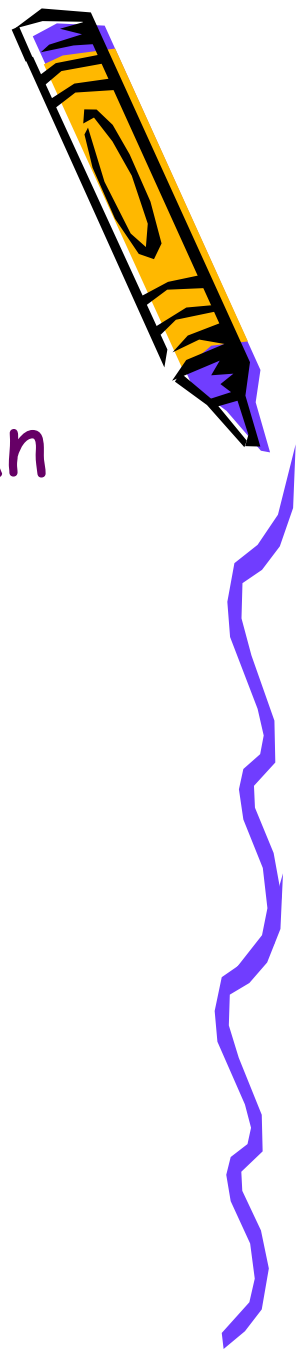
- Thermal dimorphism.

- Metabolism.



Fungal cell wall

- Consists of chitin not peptidoglycan like bacteria.
- Thus fungi are insensitive to antibiotics as penicillins.



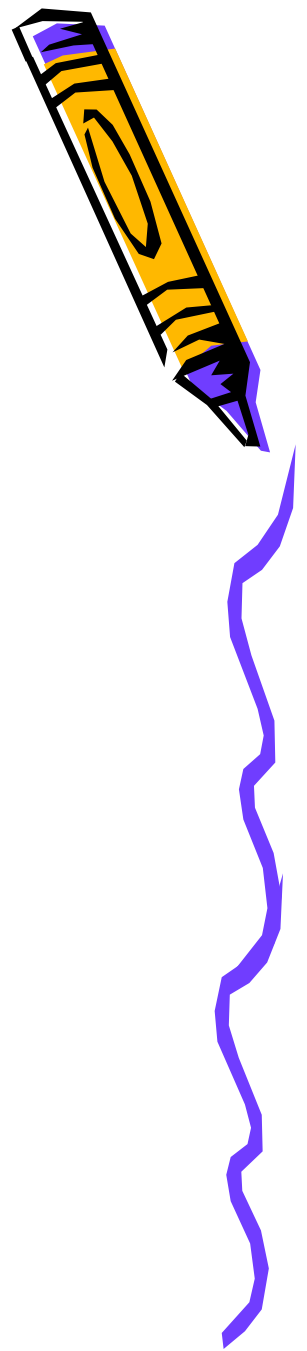


- **Chitin** is a polysaccharide composed of long chain of n-acetyleglucosamine.
- Also the fungal cell wall contain other polysaccharide, **B-glucan**, which is the site of action of some antifungal drugs.



Fungal cell membrane

- Consist of ergosterol rather than cholesterol like bacterial cell membrane.
- Ergosterol is the site of action of antifungal drugs, amphotericin B & azole group



Atmospheric & carbon source requirements

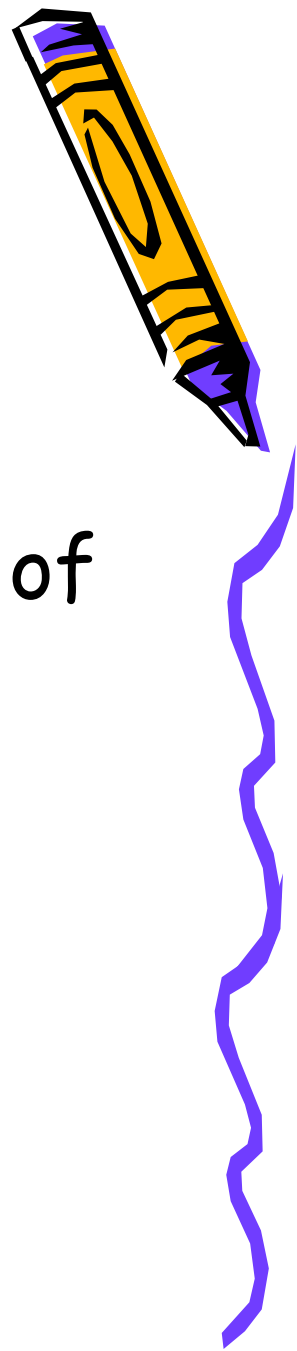


- Most fungi are obligatory aerobes, some are facultative anaerobes, but none are **obligatory anaerobes**.
- All fungi require a performed organic source of carbon -association with decaying matter.

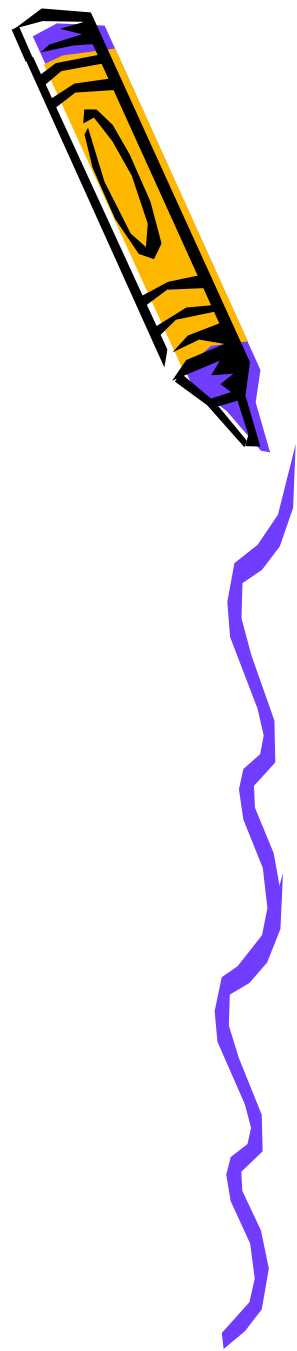


Natural habitat

- The environment.
- Exception *Candida albicans* is part of normal human flora.



Morphology of Fungi

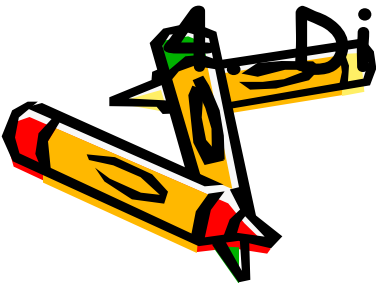


1. Filamentous fungi (molds)

2. Yeasts

3. Yeast-like fungi

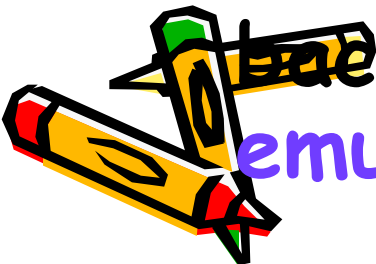
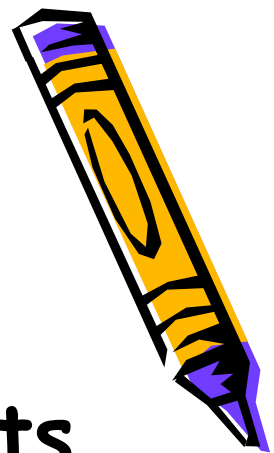
Dimorphic fungi



Filamentous Fungi

1. The basic morphological elements of filamentous fungi are long branching filaments or **hyphae**, which intertwine to produce a mass of filaments or **mycelium**

2. Colonies are strongly **adherent** to the medium and unlike most bacterial colonies **cannot be emulsified in water**.



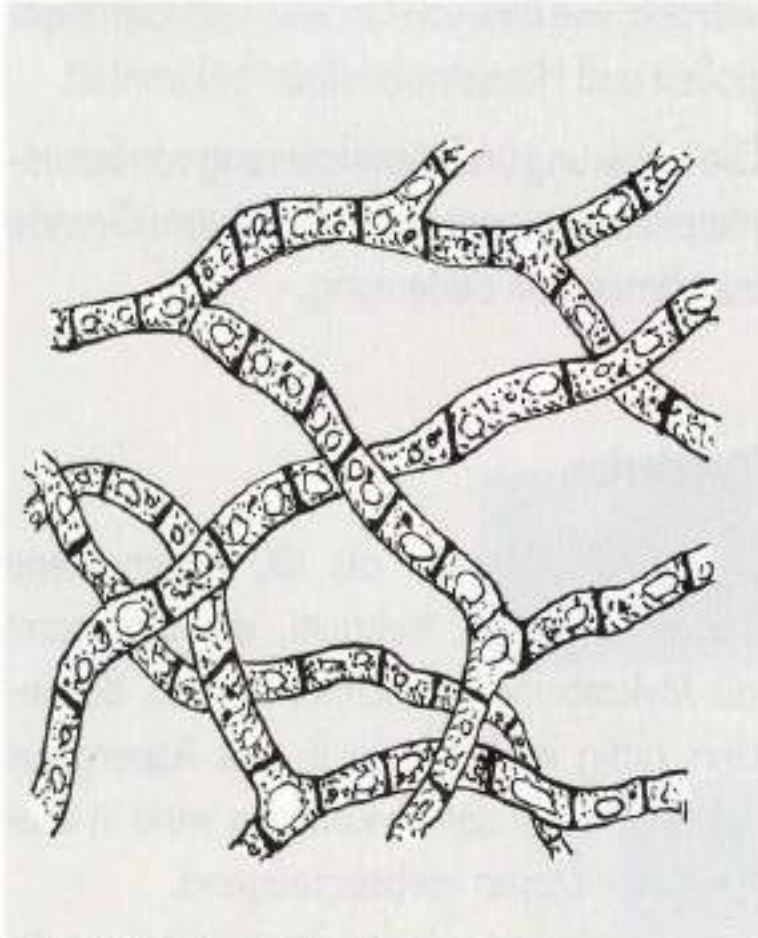
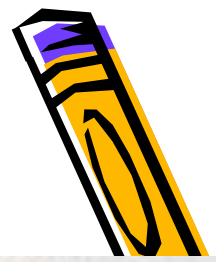


Abb. 47: Septiertes Myzel

mycelium: septate

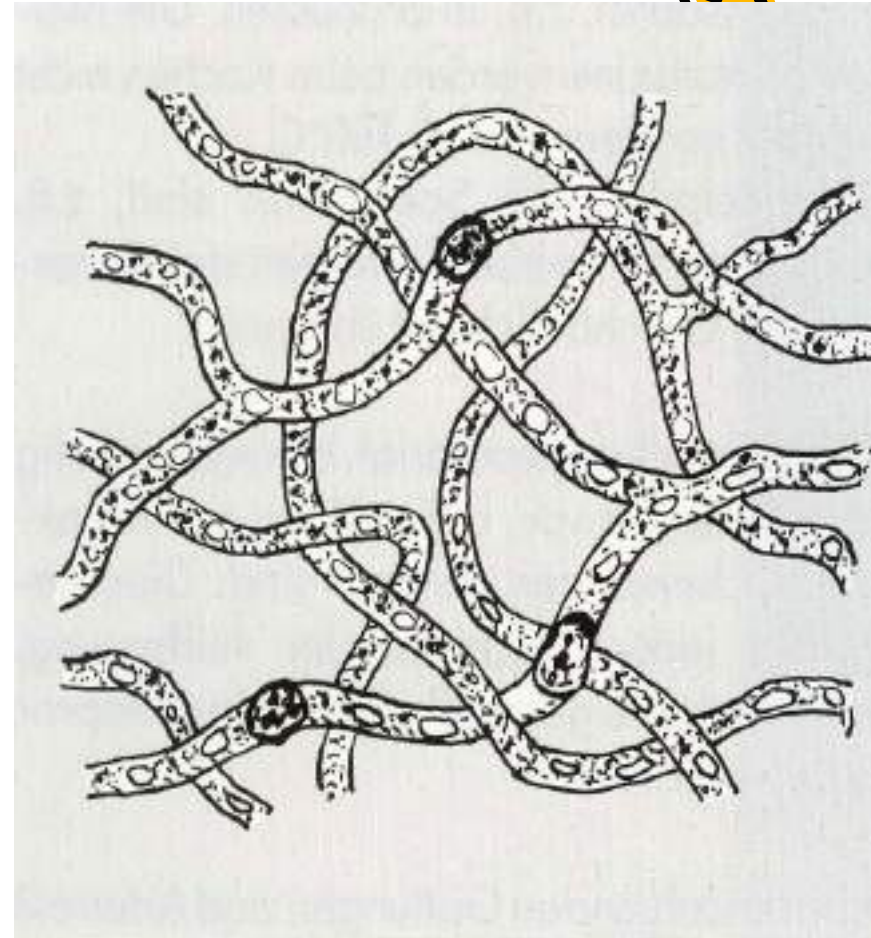
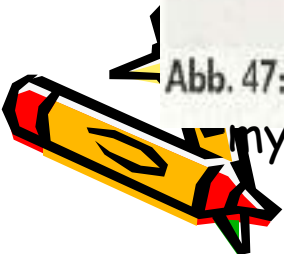


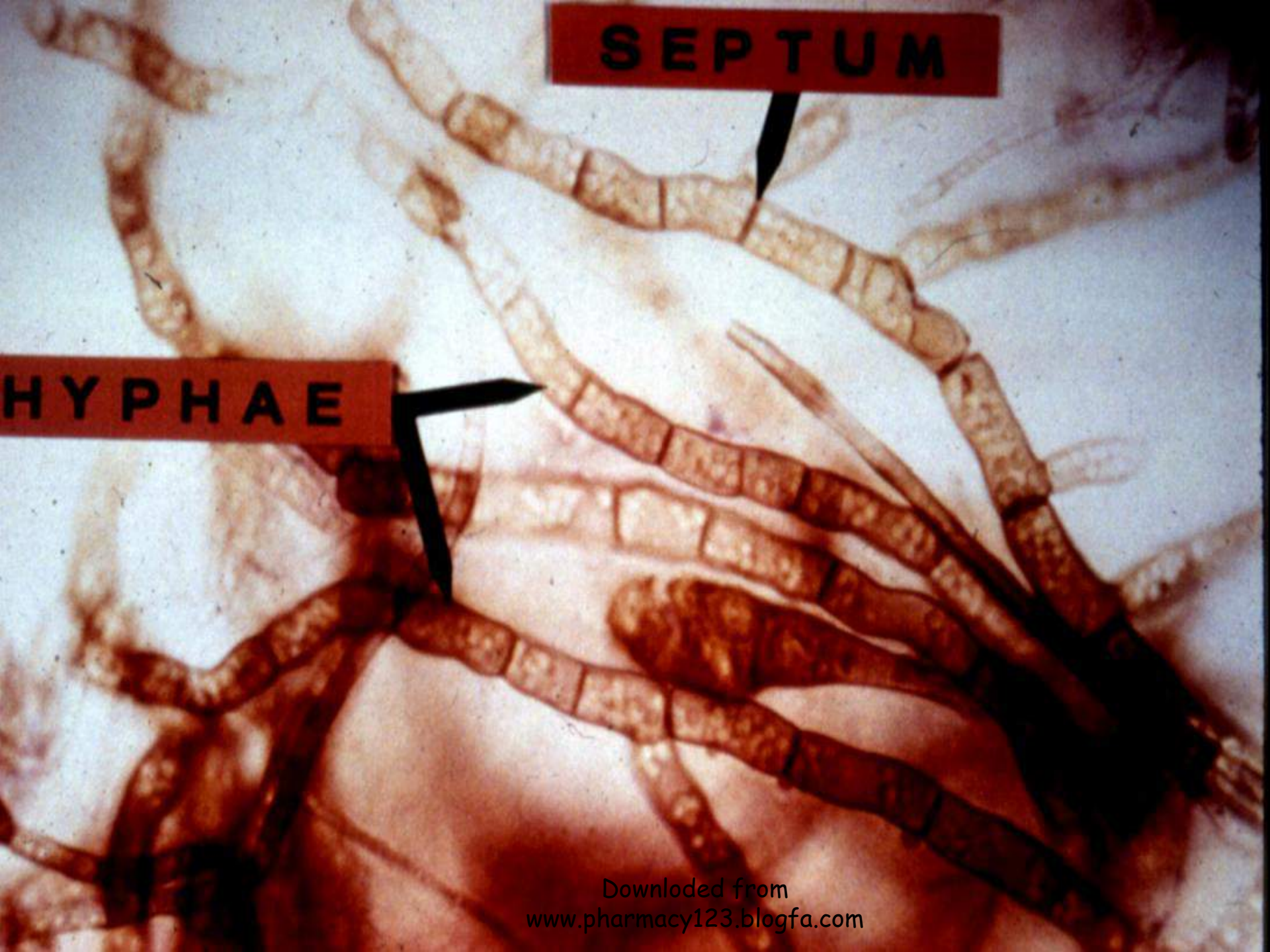
Abb. 48: Unseptiertes Myzel

mycelium: non septate

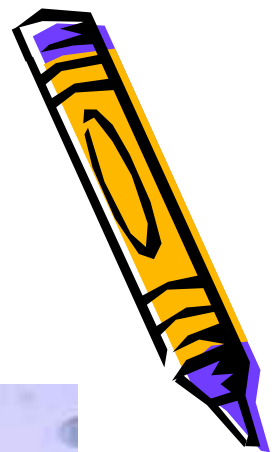
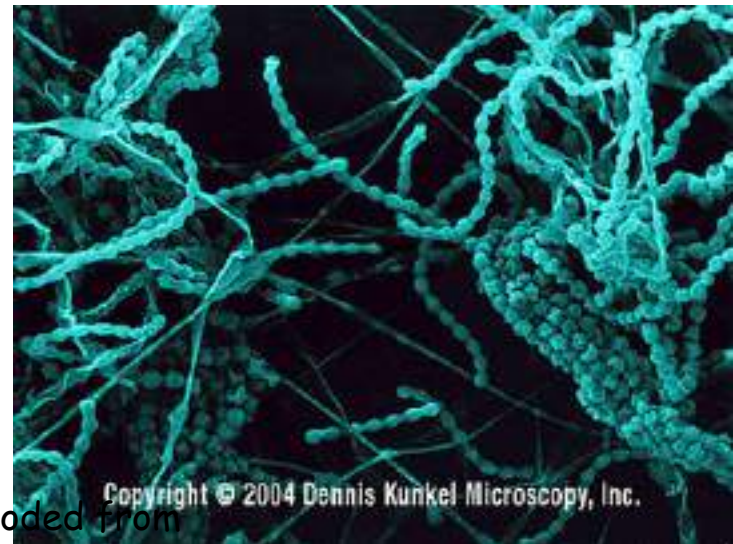
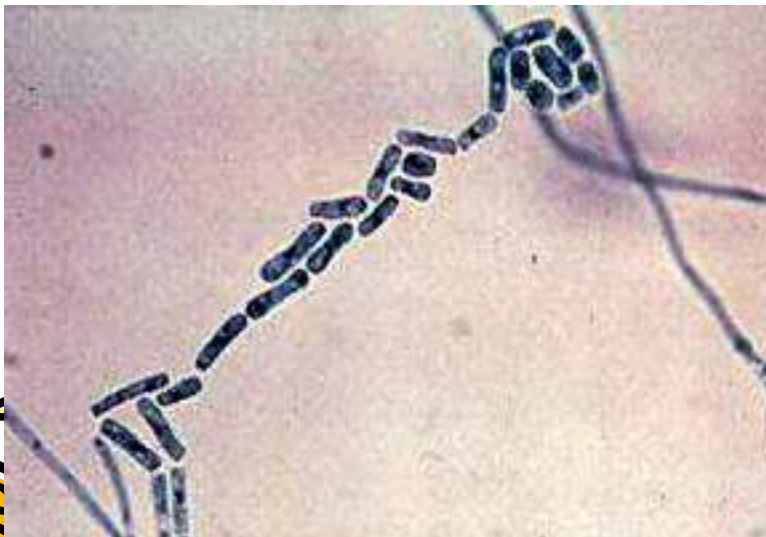
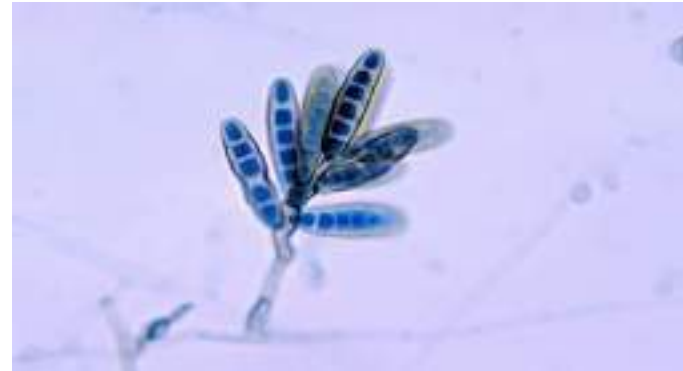


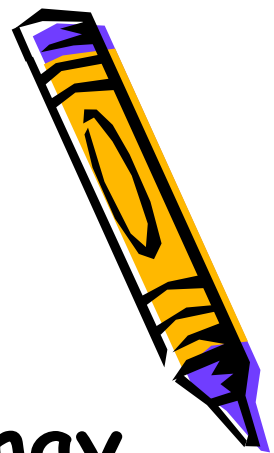
SEPTUM

HYPHAE



Mycelia & Conidia



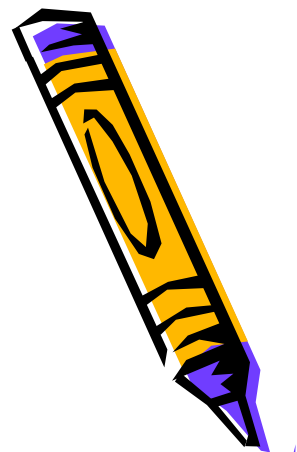
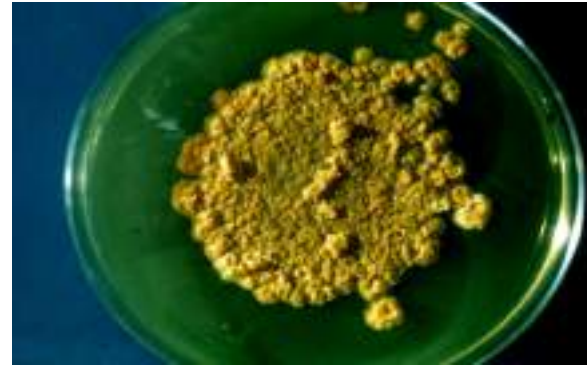


3. The surface of these colonies may be **powdery**, or may show a **cottony** aerial mycelium.

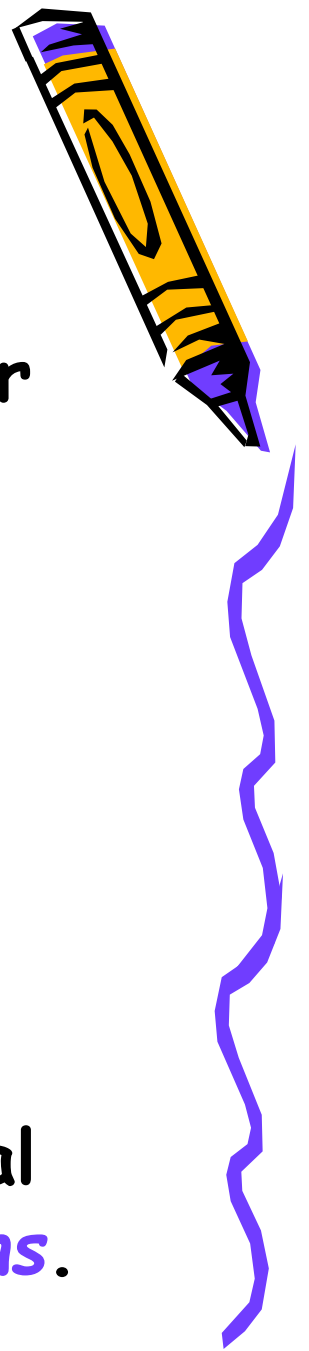
4. **Pigmentation** of the colony itself and of the underlying medium is frequently present.



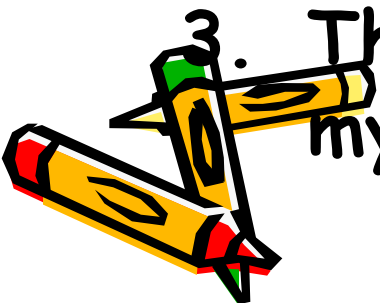
Colony Morphology



Yeasts

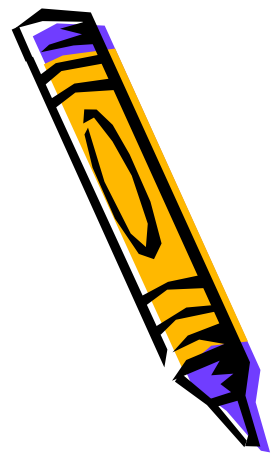
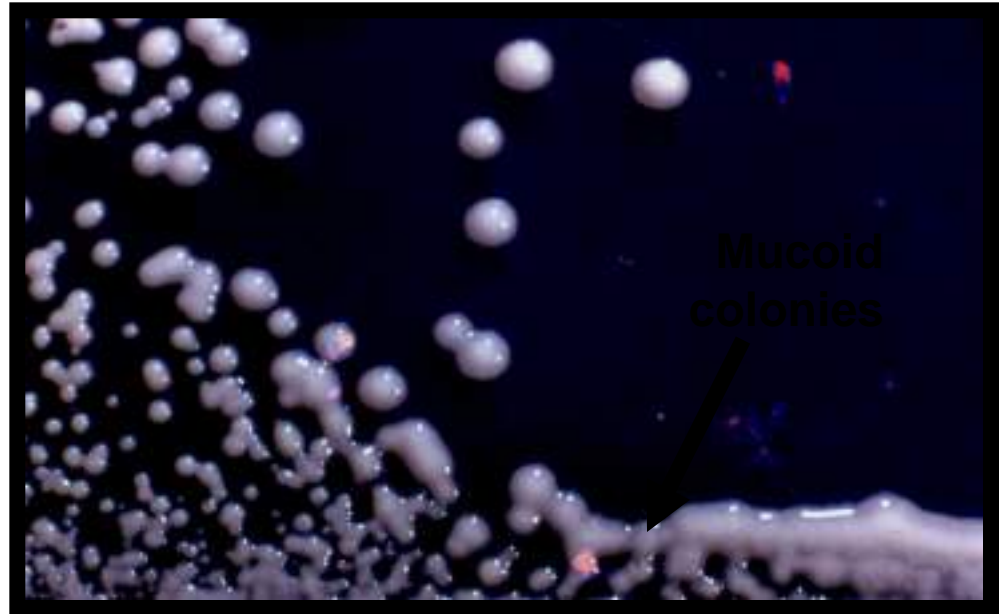


1. These occur in the form of round or oval bodies which reproduce by the formation of buds known as **blastospores**.
2. Yeasts colonies resemble bacterial colonies in appearance and in **consistency**.
3. The only pathogenic yeast in medical mycology is ***Cryptococcus neoformans***.



Yeast colonies

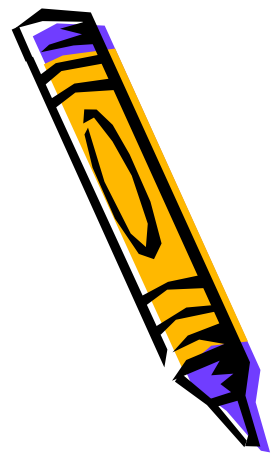
Cryptococcus neoformans



Cryptococcus neoformans



Yeast-Like

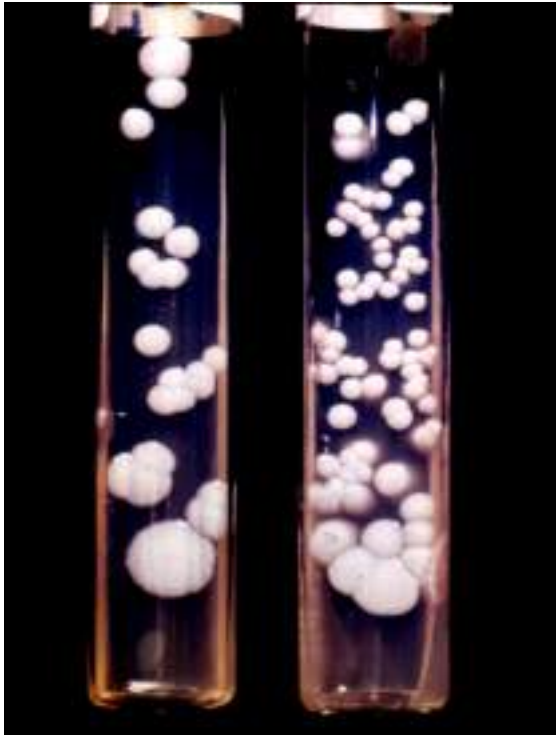


1. These are fungi which occur in the form of budding **yeast-like cells** and as chains of elongated unbranched filamentous cells which present the appearance of broad septate hyphae. these hyphae intertwine to form a **pseudomycelium**.

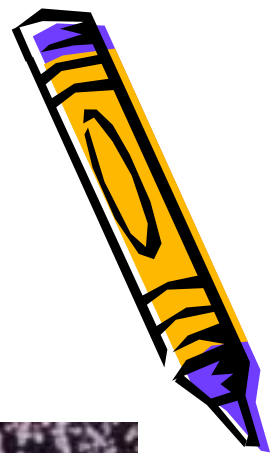


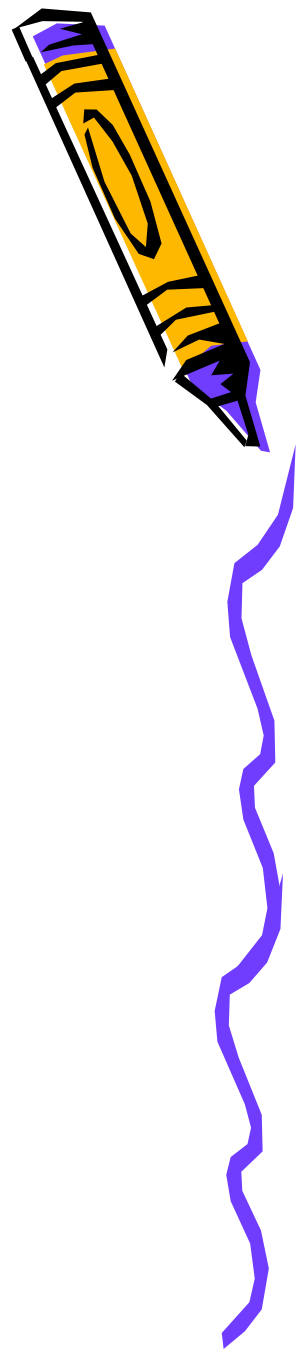
The yeast like fungi are grouped together in the genus **Candida**.

Candida Colonies



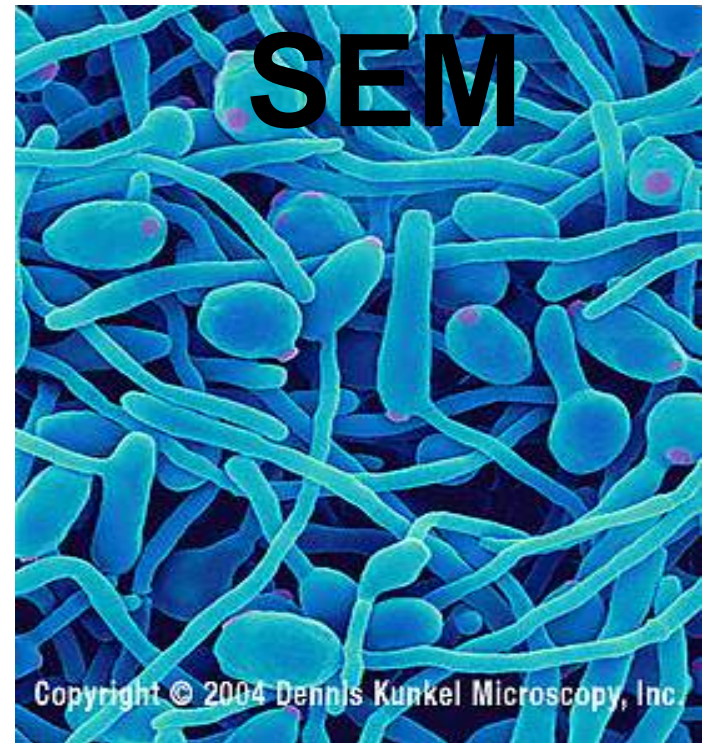
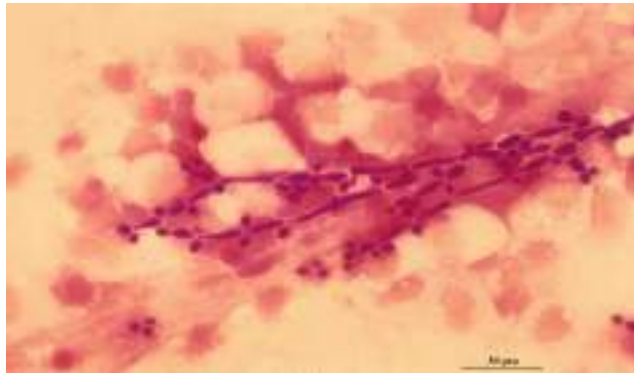
Candida albicans





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Candida albicans



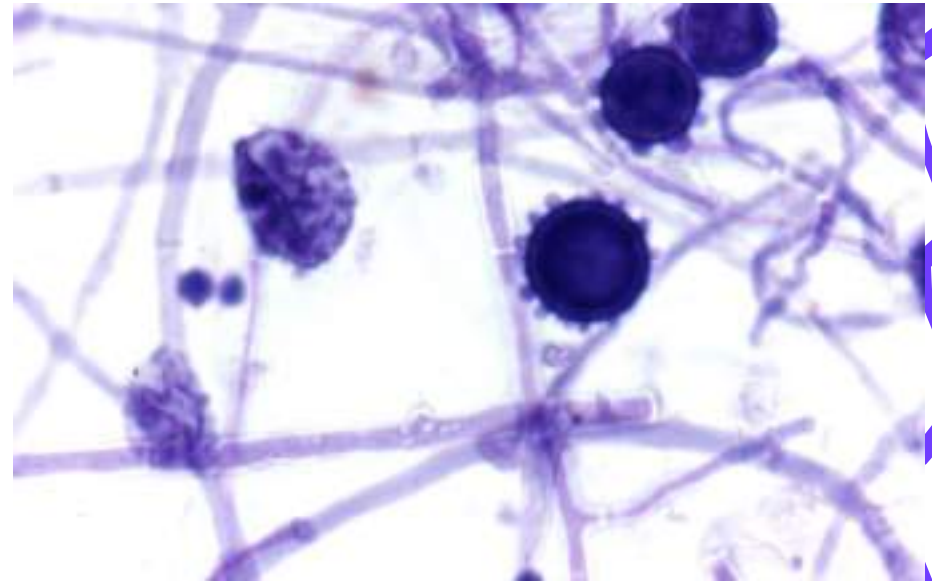
Thermally Dimorphic Fungi

These are fungi which exhibit a filamentous **mycelial** morphology (saprophytic phase) when grown at room temperature **27°C**, but have a typical **yeast** morphology (parasitic phase) inside the body and when grown at **37°C** in the laboratory (e.g. Histoplasmosis).



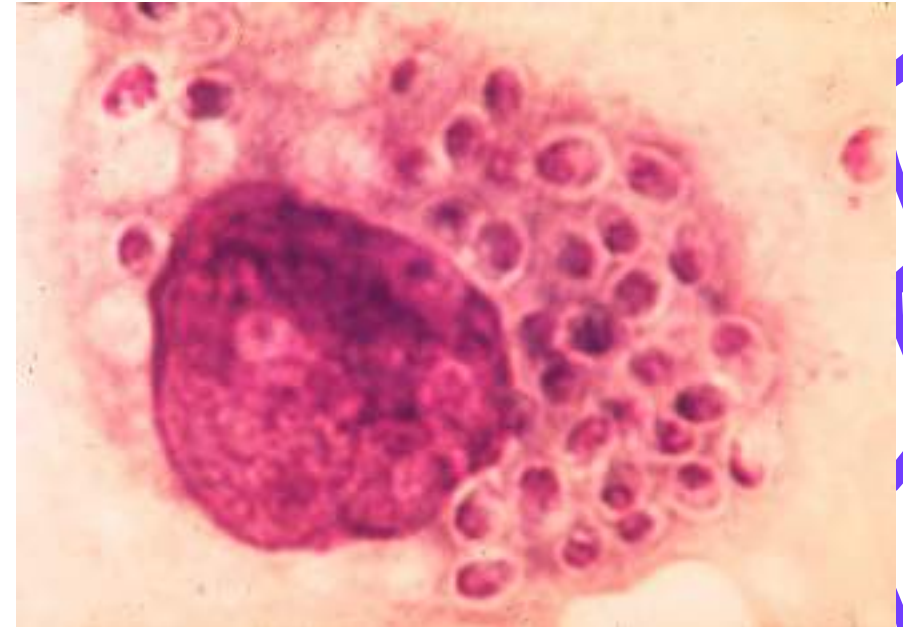
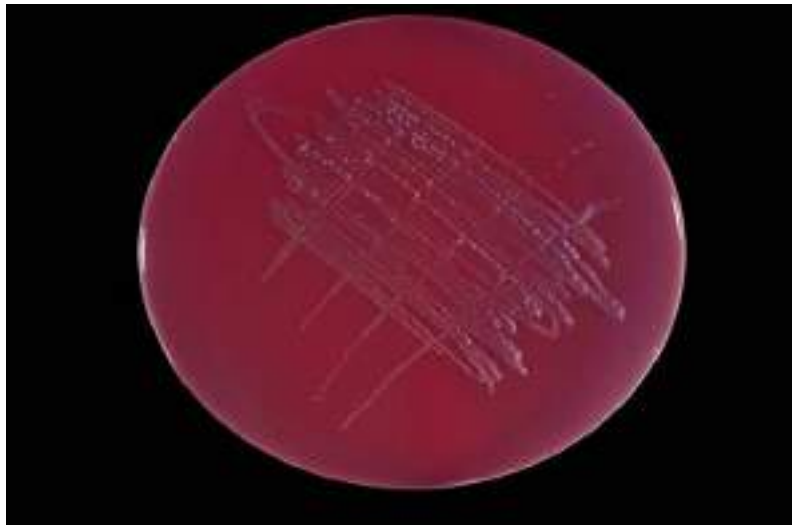
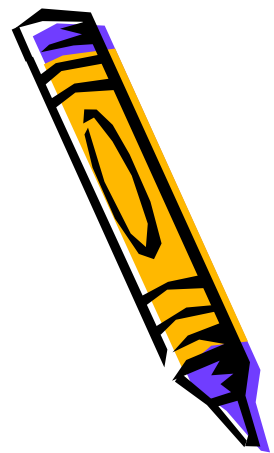
Histoplasma capsulatum

27°C



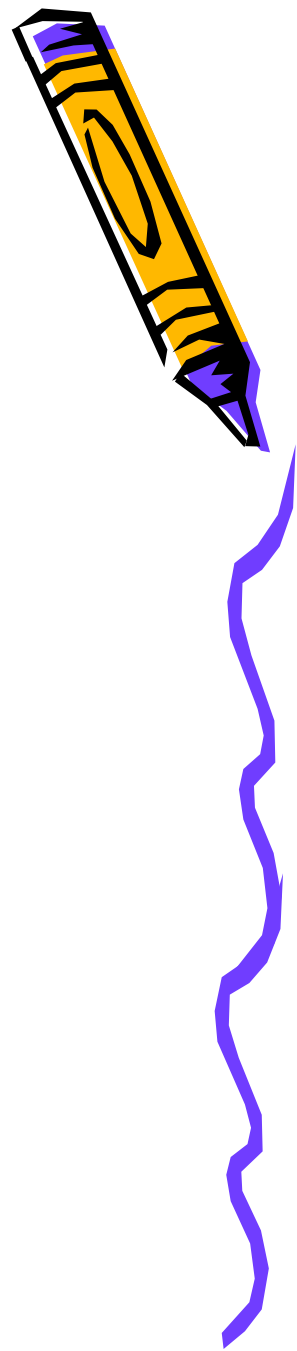
Histoplasma capsulatum

37oc

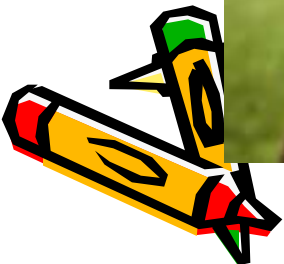
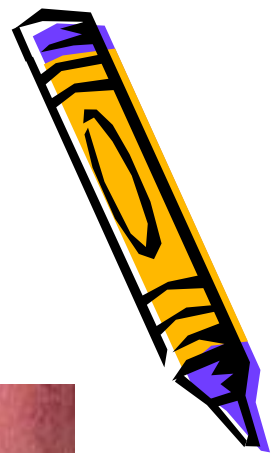


Human fungal infection;

- Superficial
- Subcutaneous
- Systemic



Superficial mycoses



Subcutaneous mycoses

Sporotrichosis



Mycetoma



Chromomycosis



Antimicrobial Chemotherapy

ا.م.د جتین عزالدین علی
ا.د هدیل مزهر یونس

TABLE 12.1

Characteristics of the Ideal Antimicrobial Drug

- Selectively toxic to the microbe but nontoxic to host cells
- Microbicidal rather than microbistatic
- Relatively soluble and functions even when highly diluted in body fluids
- Remains potent long enough to act and is not broken down or excreted prematurely
- Not subject to the development of antimicrobial resistance
- Complements or assists the activities of the host's defenses
- Remains active in tissues and body fluids
- Readily delivered to the site of infection
- Not excessive in cost
- Does not disrupt the host's health by causing allergies or predisposing the host to other infections

Terminology

- **Antimicrobial chemotherapy** : the use of chemotherapeutic drugs to control infection.
- **Antimicrobics** ; any antimicrobial drug, regardless of its origin. (anti-infective agents

- The most important

group of anti-infective agents is the **antibiotics**. These natural substances

are produced by fungi or bacteria (usually Streptomycetes) so :

Antibiotics: Substances produced by the natural metabolic processes of some microorganisms that can inhibit or destroy other microorganisms.

- **Minimum inhibitory concentration (MIC)**- smallest concentration of drug that visibly inhibits growth.
- **minimum bactericidal concentration (MBC)** is the smallest concentration of a substance required to kill 99.9% of the cells in an inoculum.

- Synthetic Antibiotics :
- Semi-synthetic Antibiotics :
- Natural An.
- In 1928 **Alexander Fleming** a professor in bacteriology discover Penicillin . In 1940 **Howard Florey and Ernst chain** performed the first clinical trails of penicillin. In 1943 Penicillin was on market

Efficacy

- The efficacy of an anti-infective agent (kinetics of action) defines **the way it affects a bacterial population.**

Two basic effects are differentiated:

- 1-**bacteriostasis**, i.e., reversible inhibition of growth.
- 2- irreversible **bactericidal** activity .

Many substances can develop both forms of efficacy depending on :

- 1-their concentration,2- the type of organism, and 3-the growth phase.

Interactions: Anti-Infective Agent/Bacterium/Host Organism

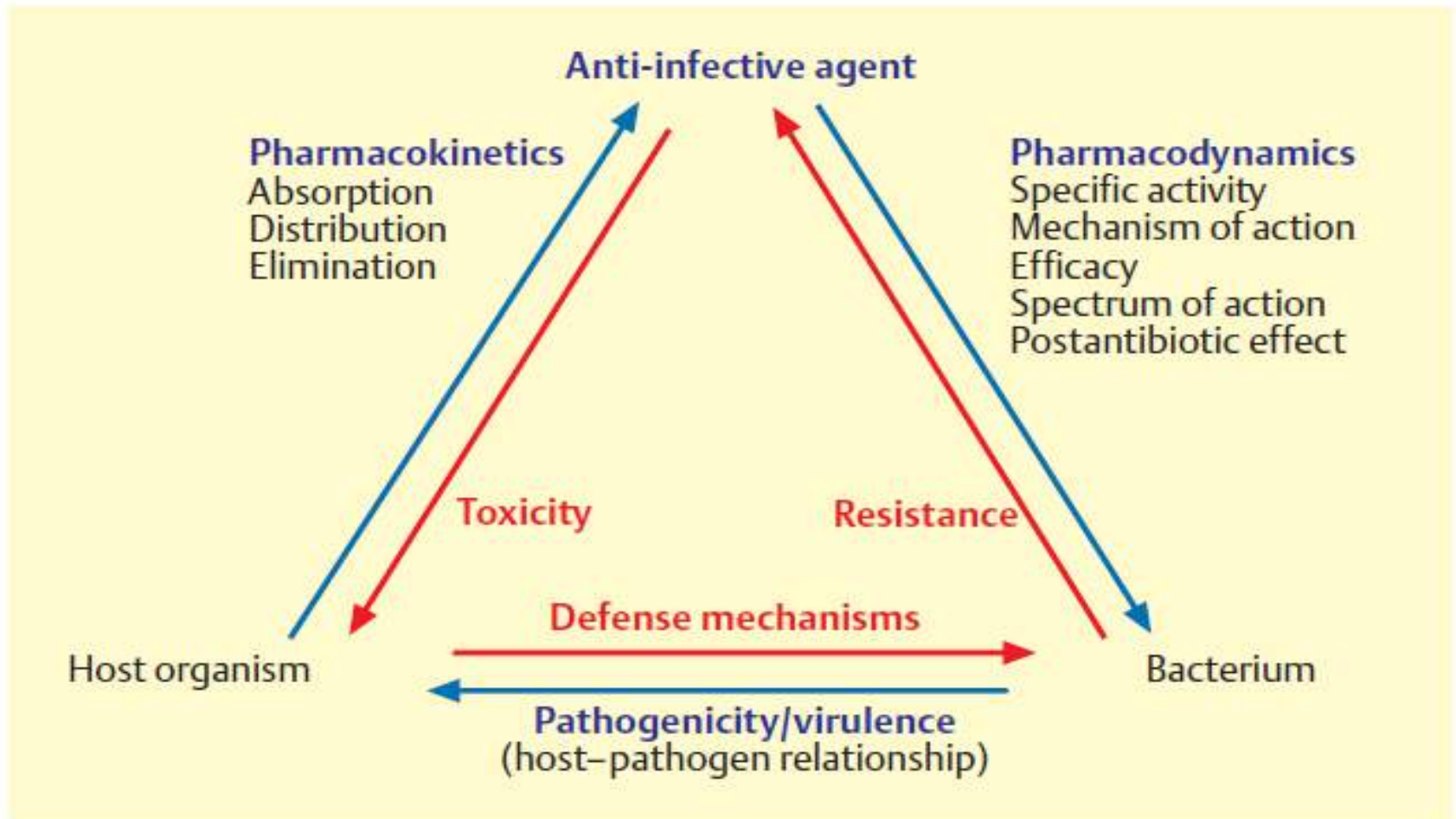


Fig. 3.28 Interactions between the anti-infective agent and host organism are characterized by the terms pharmacokinetics and toxicity; interactions between the anti-infective agent and the bacterial pathogen are characterized in terms of pharmacodynamics and resistance.

Spectrum of Action

- Each anti-infective agent has a certain spectrum of action, which is **a range** of bacterial species showing **natural sensitivity** to the substance.
- Some anti-infective agents have a **narrow spectrum** of action (e.g., vancomycin).
- Most, however, have **broad spectrum** like tetracyclines, which affect different bacterial groups.

Origins of antimicrobial drugs

- Antibiotics are common metabolic products of aerobic spore-forming bacteria & fungi.
 - bacteria in genera *Streptomyces* & *Bacillus*
 - molds in genera *Penicillium* & *Cephalosporium*
- By inhibiting the other microbes in the same habitat, antibiotic producers have less competition for nutrients & space.

Classification of Antibiotics *Based on their sources*

a. Antibiotic from microbes(natural products)

1. Antibiotics from fungi → Penicillin from *P. notatum*,
Cephalothin from *Cephalosporium ssp.*

2. Antibiotics from bacteria

- Polymyxin from *Bacillus polymyxa*
- *Bacitracin* from *Bacillus subtilis*

3. Actinomycetes

- Streptomycin from *Streptomyces griseus*
- Nystatin from *Streptomyces noursei*
- Gentamycin from *Micromonospora purpurea*

b . Antibiotics from algae

c. Antibiotics from higher plants

d. Antibiotics from animals

Selectively toxic

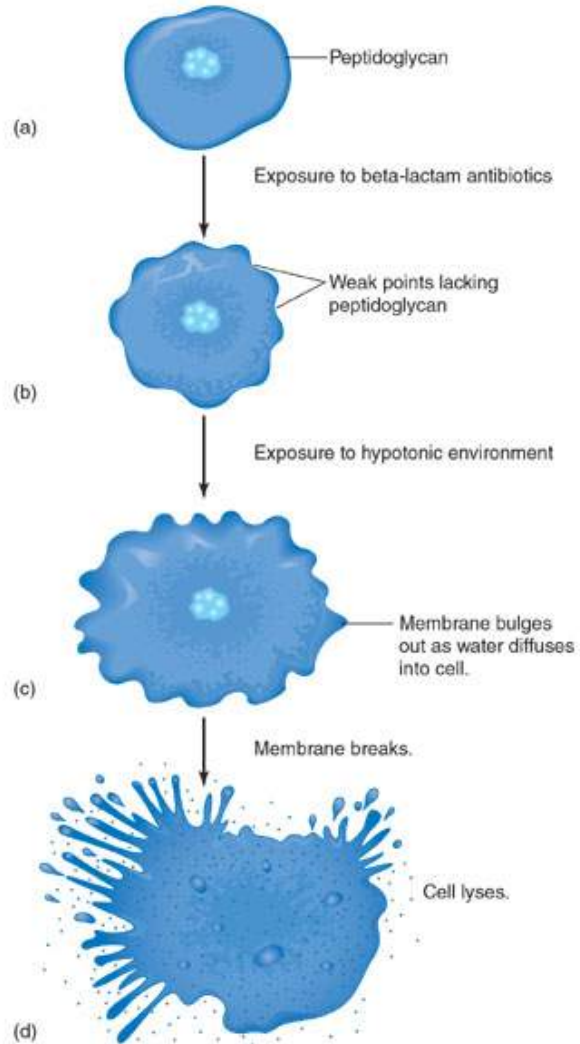
- Drugs should kill or inhibit microbial cells without simultaneously damaging host tissues.
- As the characteristics of the infectious agent become more similar to the vertebrate host cell, complete selective toxicity becomes more difficult to achieve & more side effects are seen.

Targets of antimicrobial drugs

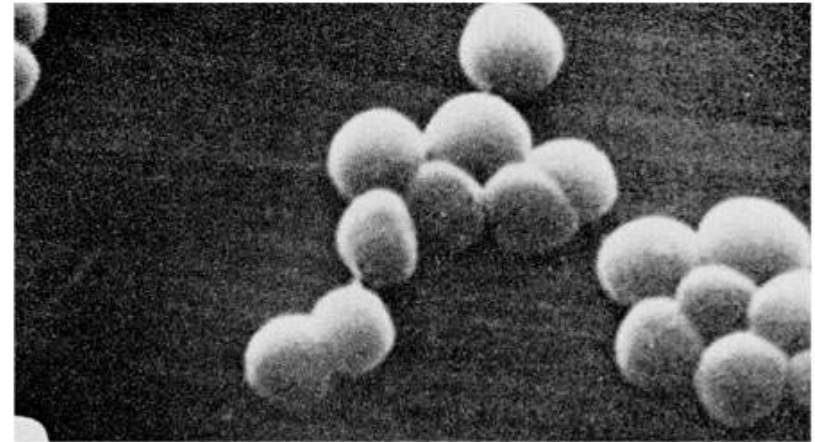
1. Inhibition of cell wall synthesis
2. Inhibition of nucleic acid synthesis, structure or function
3. Inhibition of protein synthesis
4. Disruption of cell membrane structure or function

Targets of antimicrobial drugs

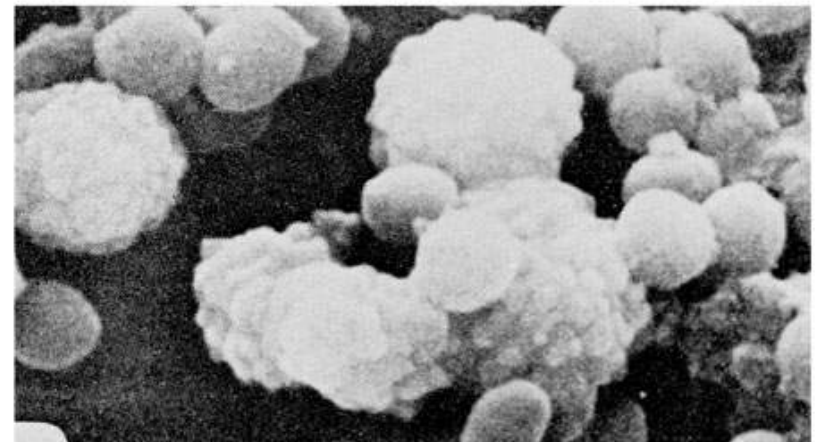
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(e)



(f)

1. Drugs that affect the bacterial cell wall

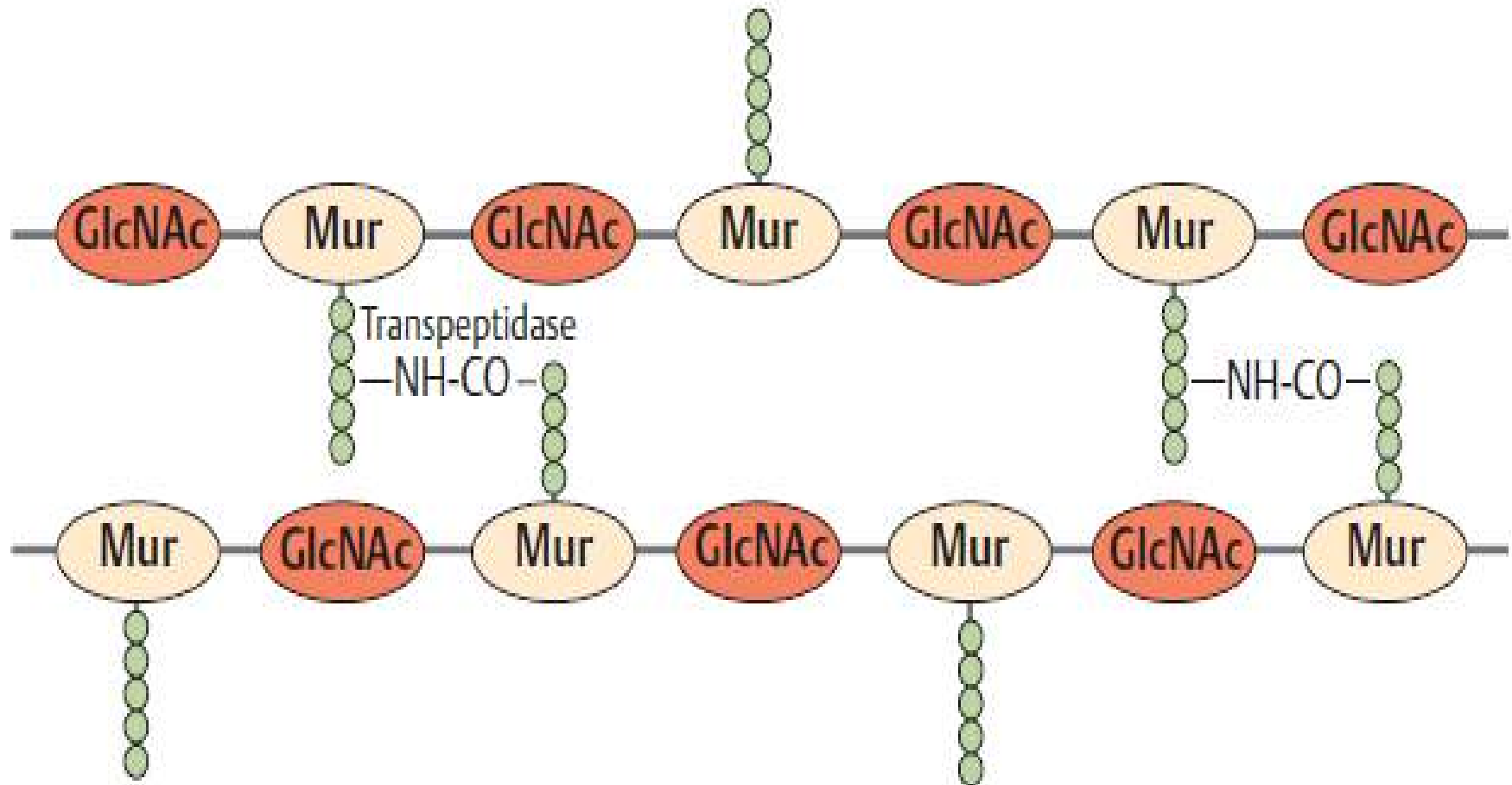
- Most bacterial cell walls contain a rigid girdle of peptidoglycan.
- Penicillin and cephalosporin block synthesis of peptidoglycan, causing the cell wall to lyse.
- Penicillins do not penetrate the outer membrane and are less effective against gram-negative bacteria.
- Broad spectrum penicillins and cephalosporins can cross the cell walls of gram-negative bacteria.

Betalactam antibiotics

Disturbance of murein biosynthesis:

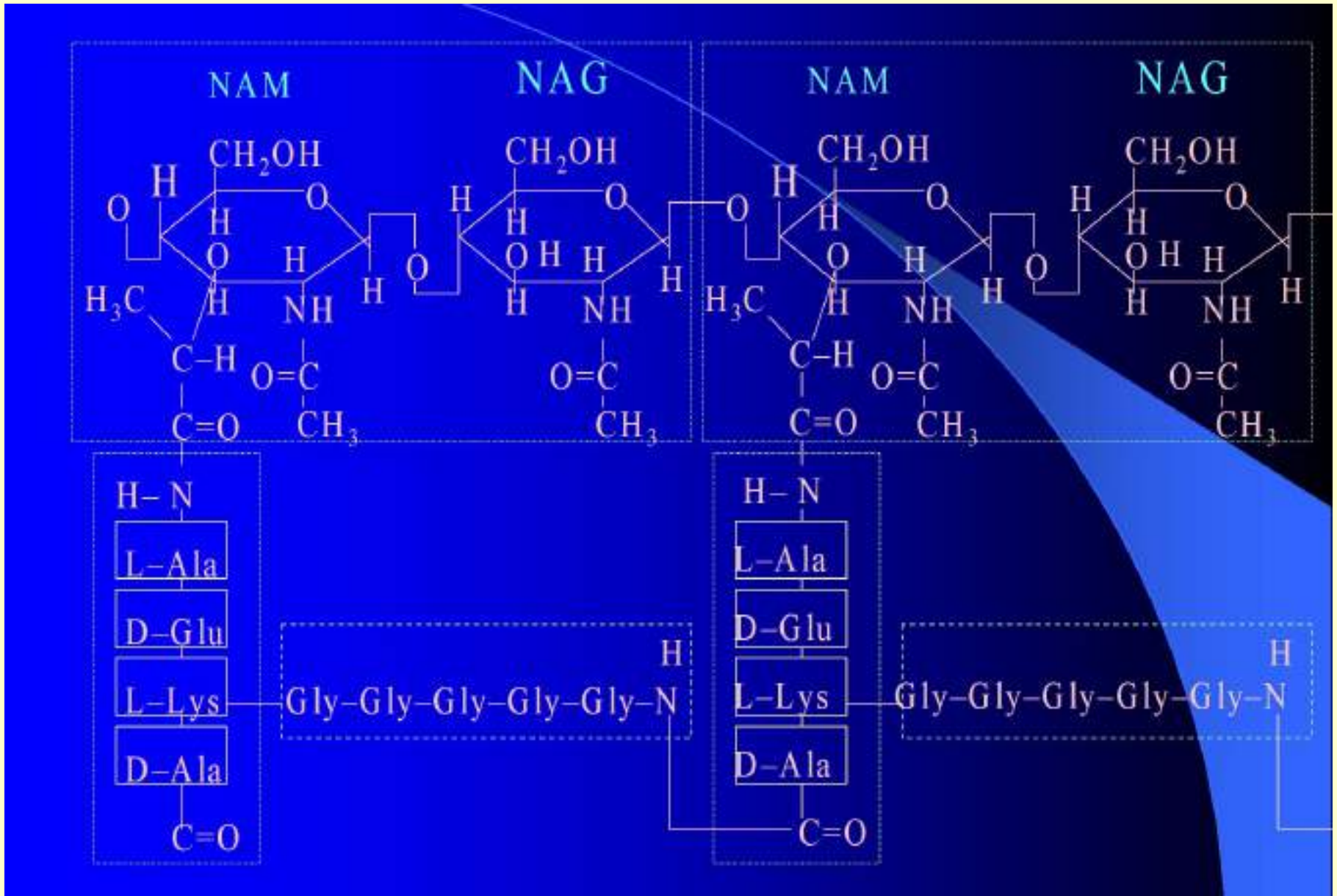
- Irreversible inhibition of DD-transpeptidase, which catalyzes the peptide crosslinkage in murein
- Release of an inhibitor of autolytic murein enzymes
- Enzymatic destruction of murein architecture with autolysins: “wrong place at the wrong time”
- Lysis due to high internal osmotic pressure

The Structure of Murein



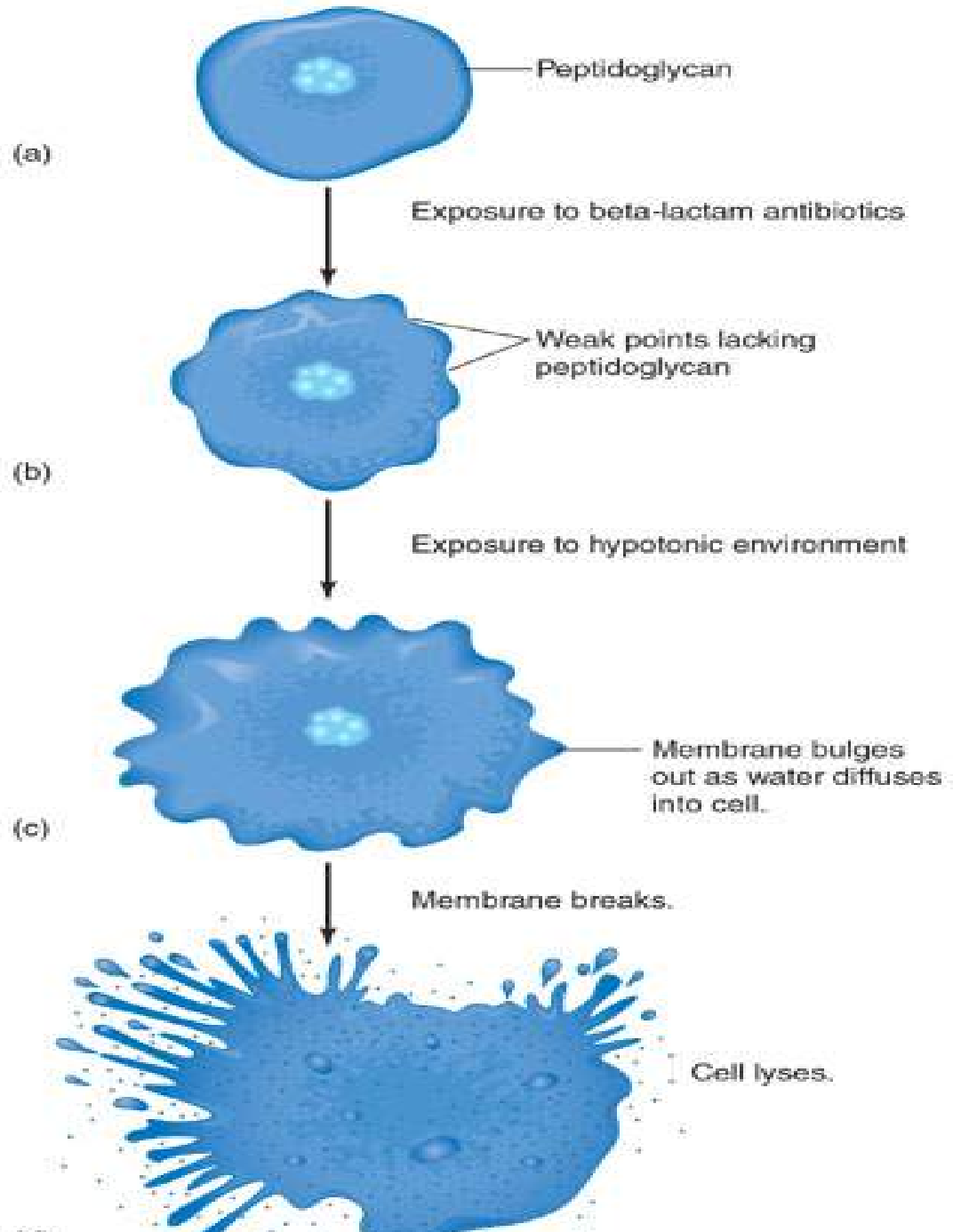
Mur = N-acetyl muramic acid (= 3-O lactyl ether of N-acetyl glucosamine)

GlcNAc = N-acetyl glucosamine ● = Aminosäure



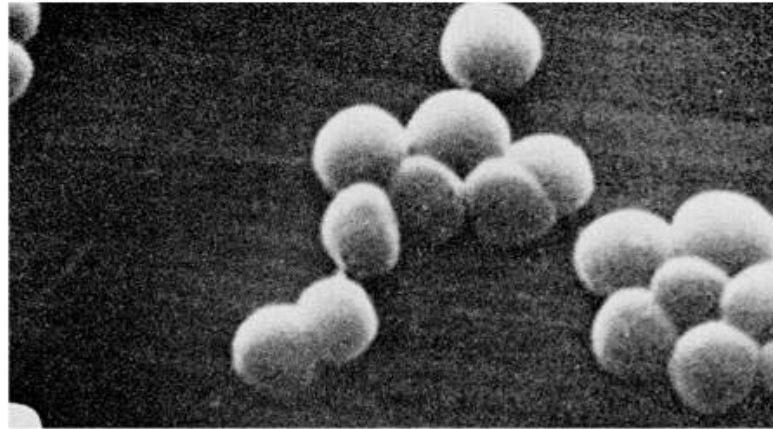
1.

11

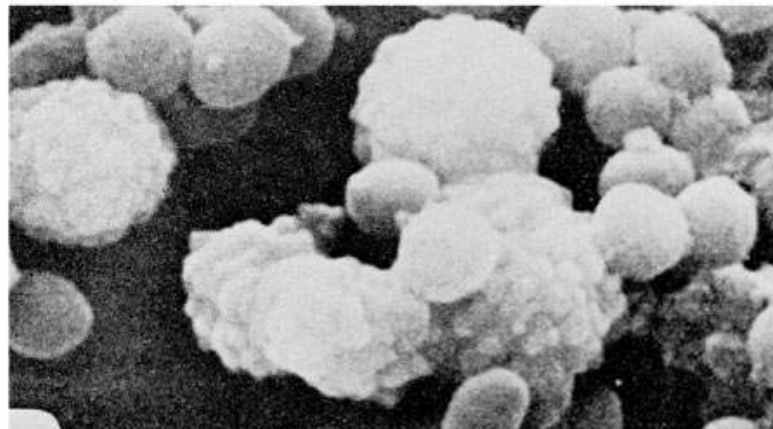


1. Drugs that affect the bacterial cell wall

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2. Drugs that inhibit nucleic acid synthesis

- 1-may block synthesis of nucleotides, inhibit replication, or stop transcription

4-Quinolones

Inhibition of the DNA gyrase and topoisomerase IV resulting in the inhibition of DNA replication

Rifamycin

Transcription:
Blockage of DNA-dependent RNA polymerase

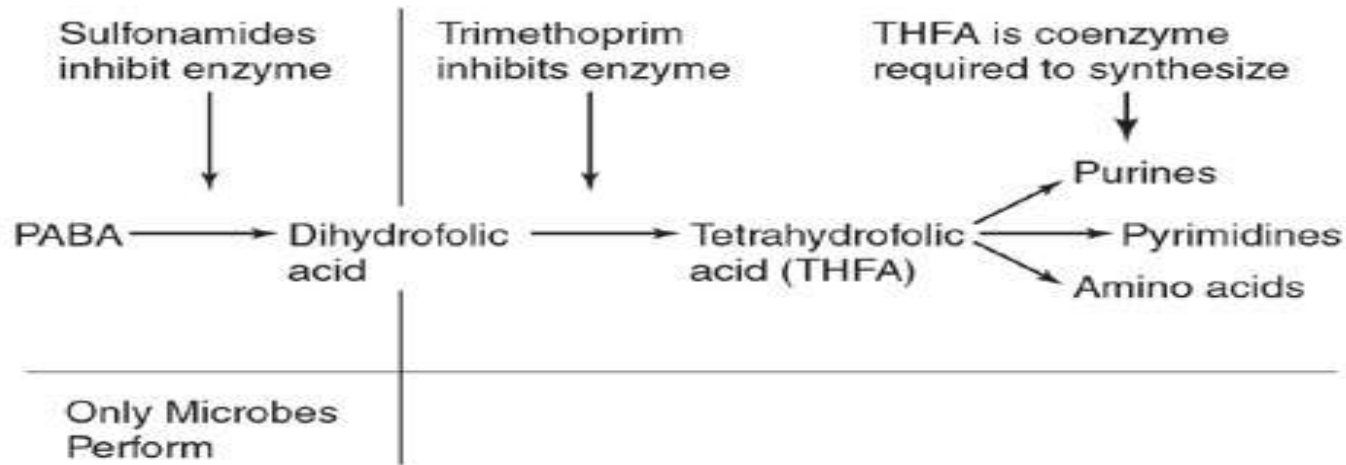
- 2-Sulfonamides and trimethoprim block enzymes required for tetrahydrofolate synthesis needed for DNA & RNA synthesis.

Sulfonamides	Competition with <i>p</i> -aminobenzoic acid as a substrate for dihydropteridic acid synthetase, thus too little tetrahydrofolic acid
Trimethoprim	Inhibition of dihydrofolic acid reductase, thus too little tetrahydrofolic acid

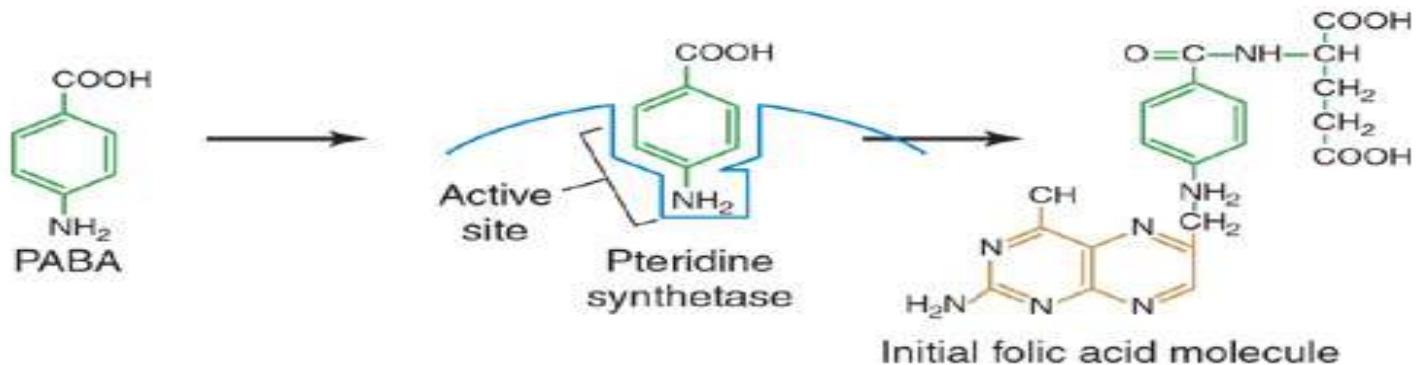
- **competitive inhibition** – drug competes with normal substrate for enzyme's active site

2. Drugs that inhibit nucleic acid synthesis

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(a) Normal metabolic pathway



(b) Normal folic acid synthesis

3. Drugs that block protein synthesis

- Ribosomes of eucaryotes differ in size and structure from procaryotes, so antimicrobics usually have a selective action against procaryotes. But they can also damage the eucaryotic mitochondria.
- Aminoglycosides (streptomycin, gentamicin) insert on sites on the 30S subunit and cause misreading of mRNA.
- Tetracyclines block attachment of tRNA on the A acceptor site and stop further synthesis.

3. Drugs that block protein synthesis

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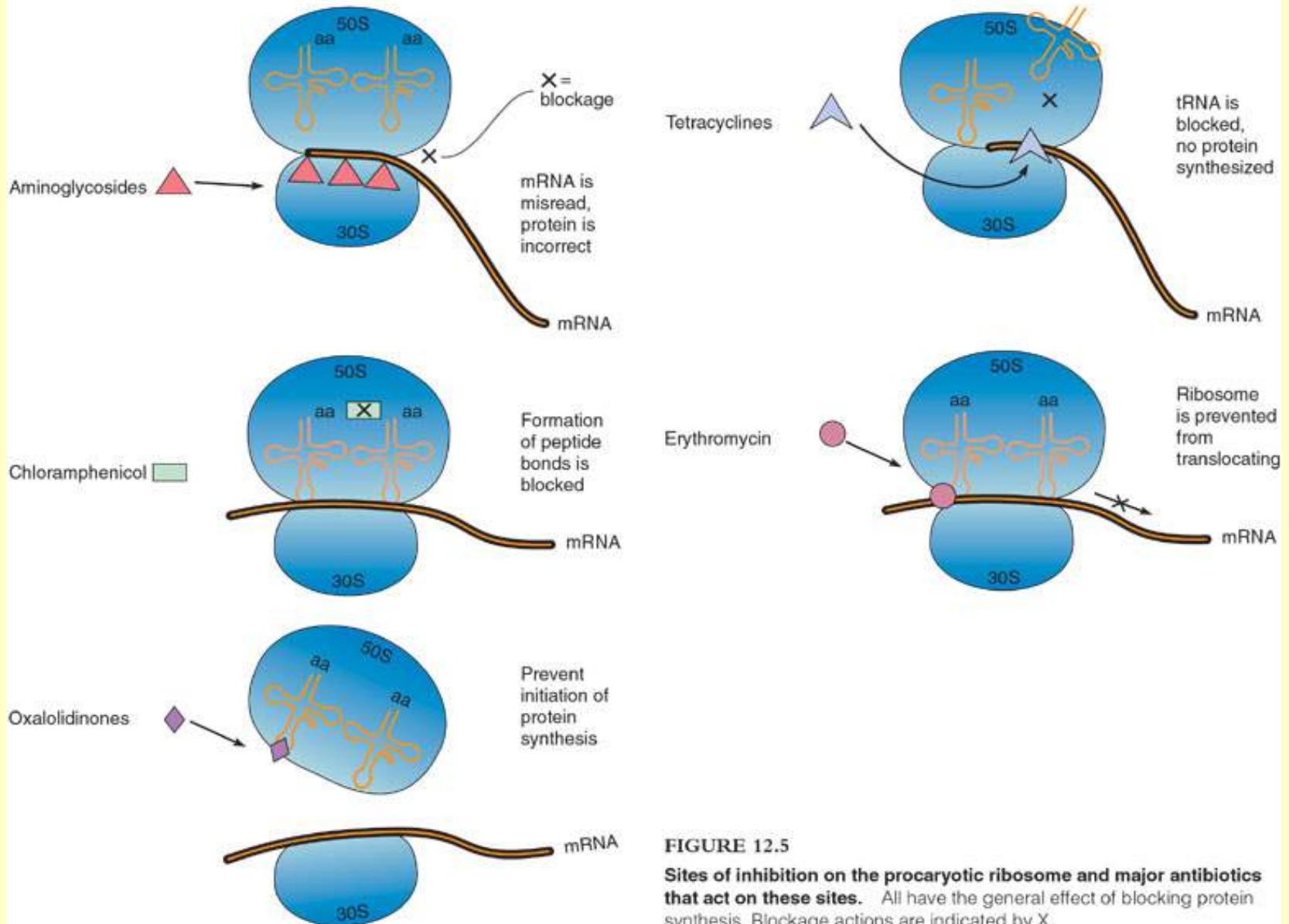
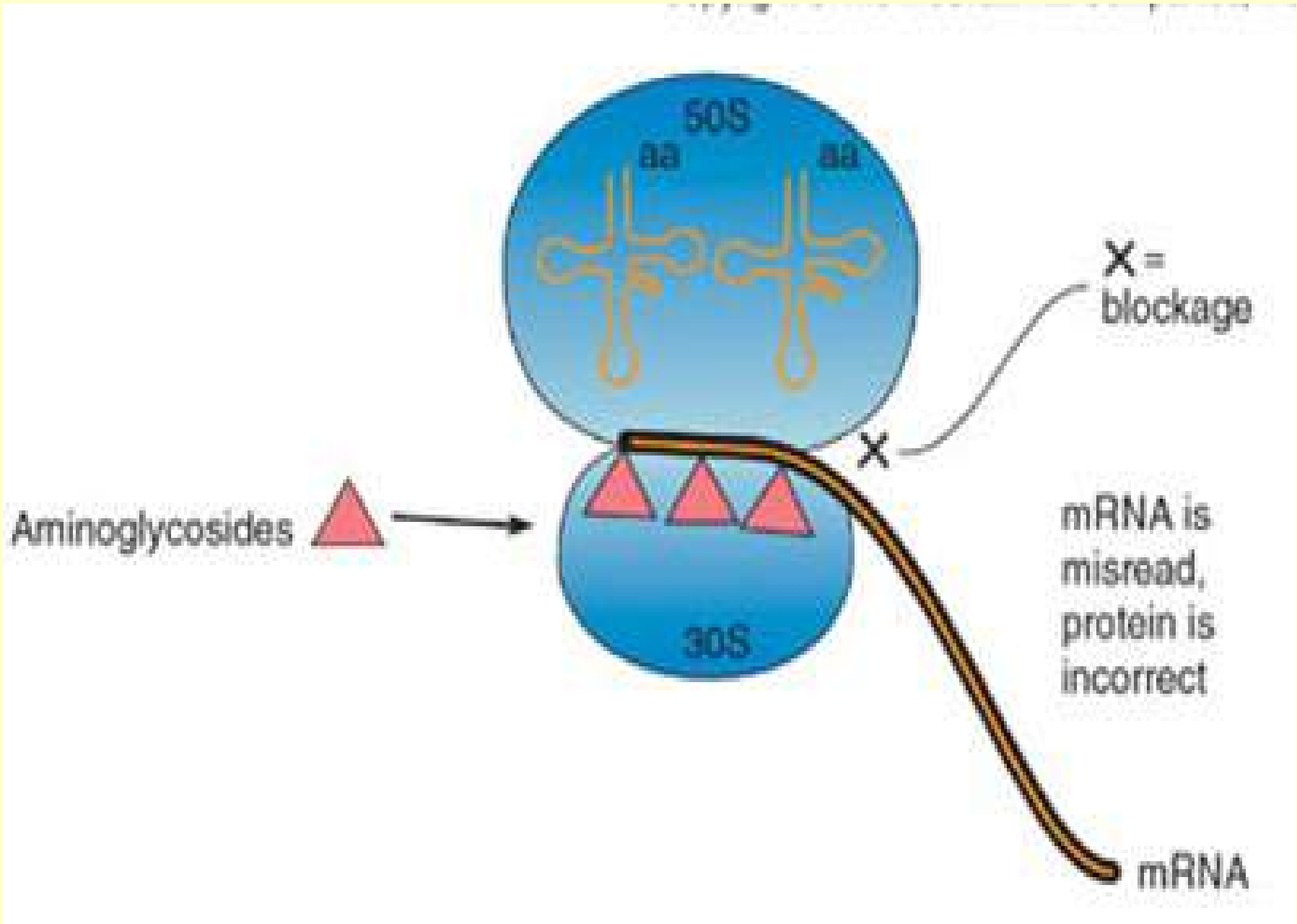
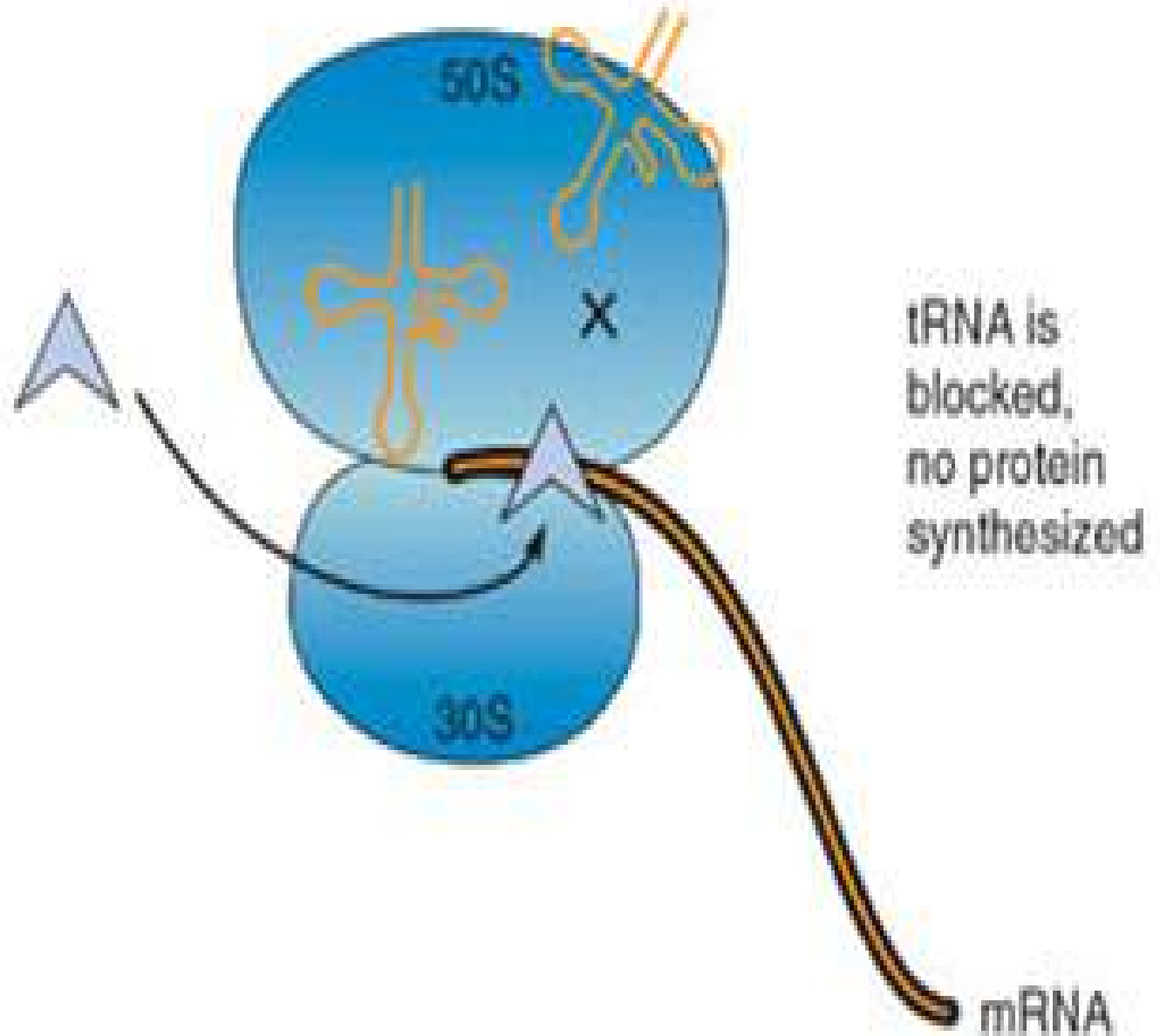


FIGURE 12.5
Sites of inhibition on the prokaryotic ribosome and major antibiotics that act on these sites. All have the general effect of blocking protein synthesis. Blockage actions are indicated by X.



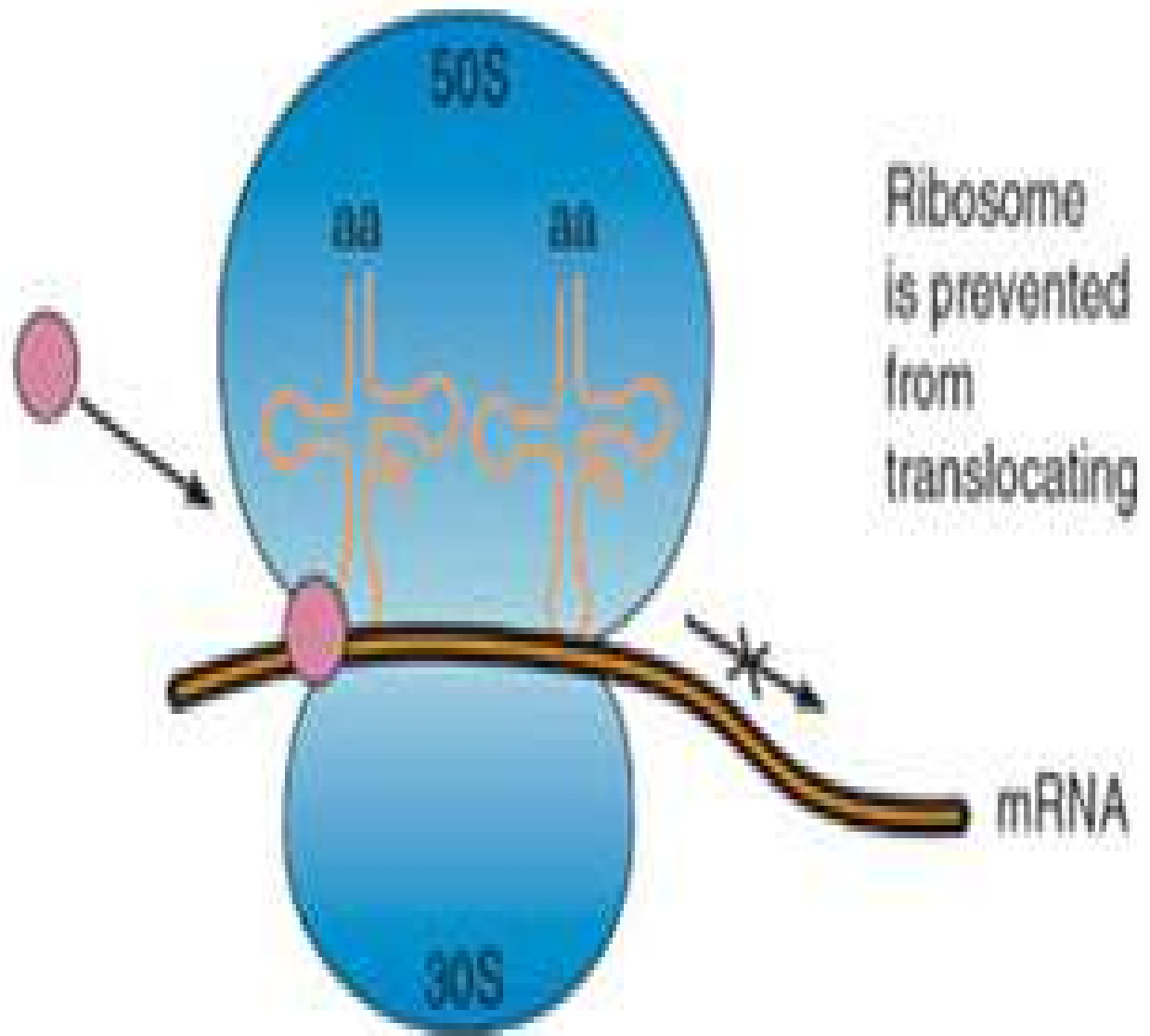
Tetracyclines



tRNA is blocked,
no protein
synthesized

mRNA

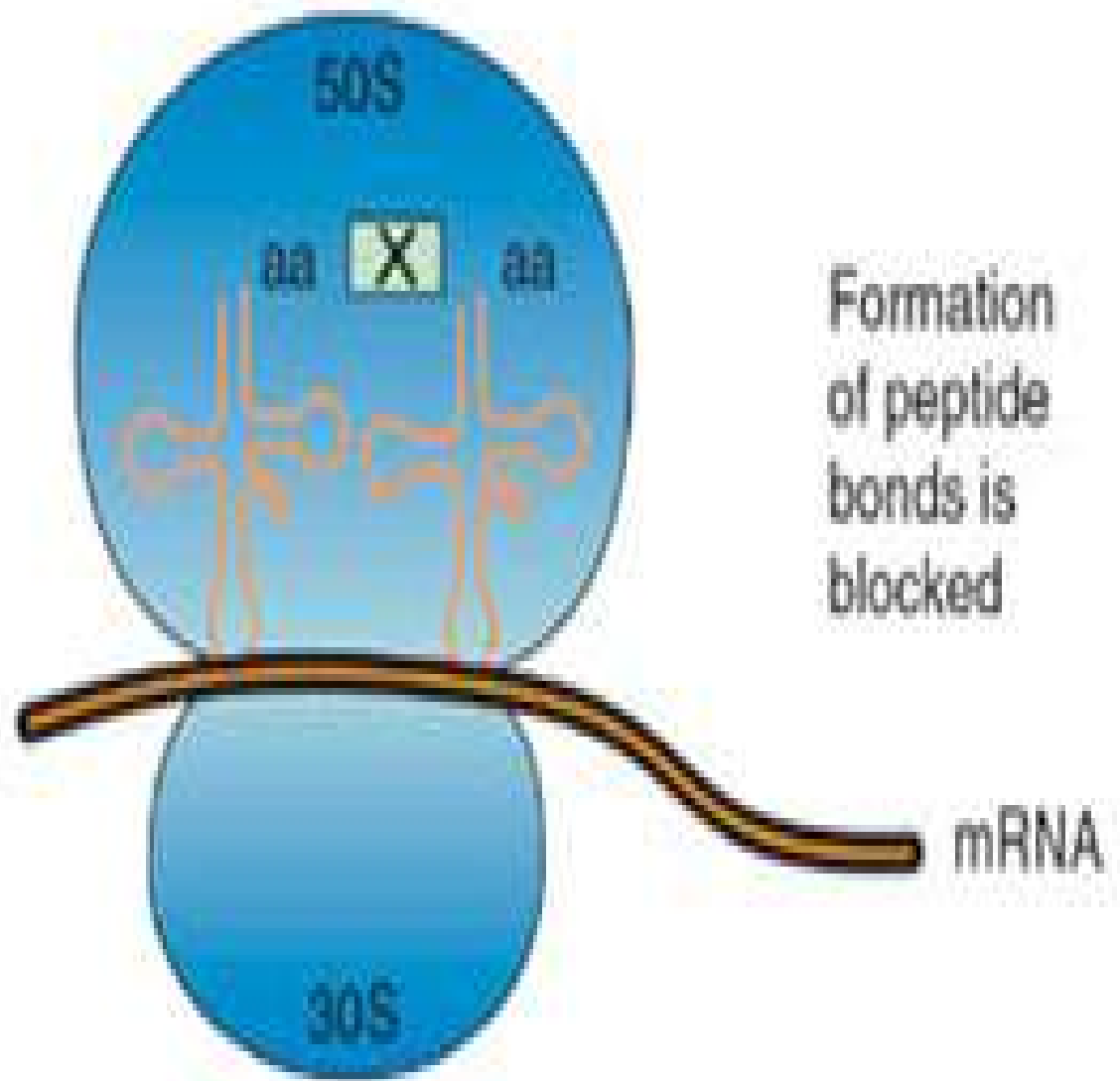
Erythromycin



Ribosome
is prevented
from
translocating

mRNA

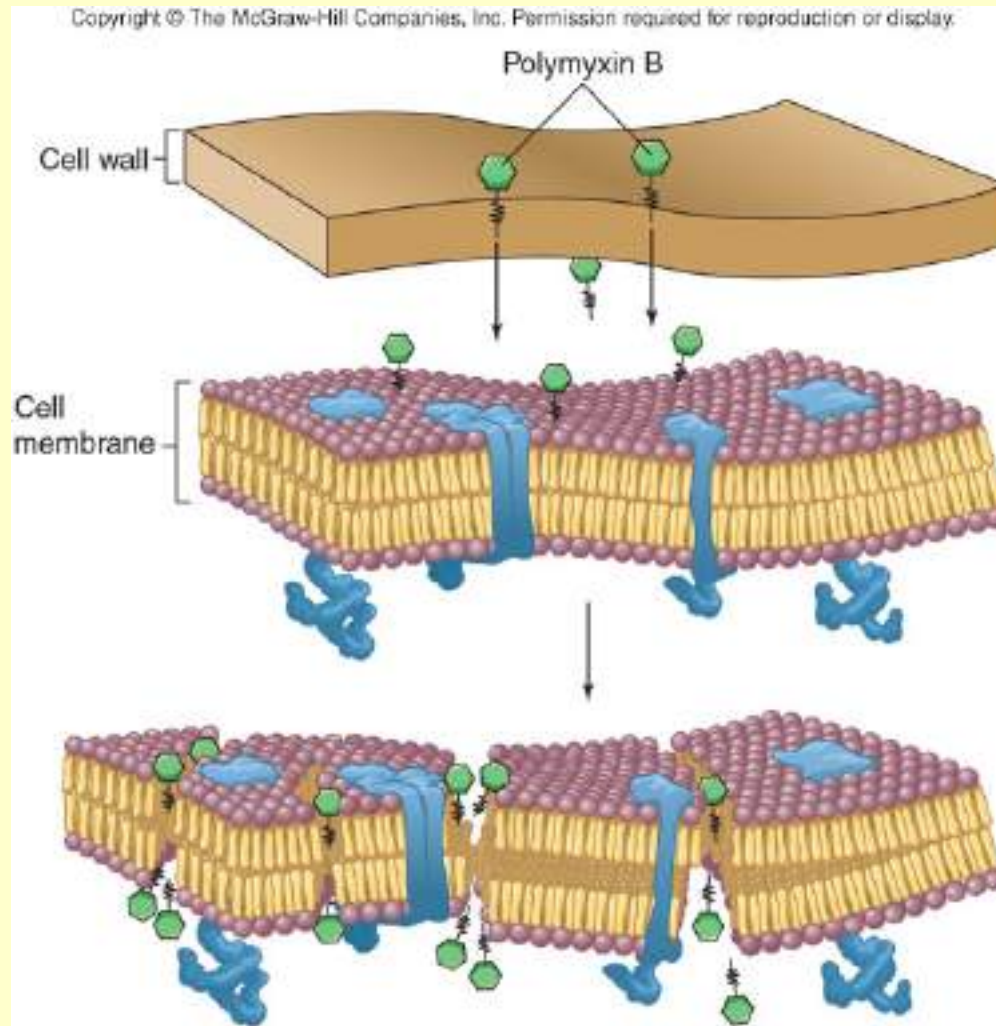
Chloramphenicol 



4. Drugs that disrupt cell membrane function

- A cell with a damaged membrane dies from disruption in metabolism or lysis.
- These drugs have specificity for a particular microbial group, based on differences in types of lipids in their cell membranes.
- Polymyxins interact with phospholipids and cause leakage, particularly in gram-negative bacteria
- Amphotericin B and nystatin form complexes with sterols on fungal membranes which causes leakage.

4. Drugs that disrupt cell membrane function



Survey of major antimicrobial drug groups

- Antibacterial drugs
 - Antibiotics
 - Synthetic drugs
- Antifungal drugs
- Antiparasitic drugs
- Antiviral drugs

About 260 different antimicrobial drugs are classified in 20 drug families.

The Problem of Resistance

Definitions

- **Clinical resistance.** Resistance of bacteria to the concentration of anti-infective agents maintained at the infection site in the macroorganism.
- **Natural resistance.** Resistance characteristic of a bacterial species, genus, or family.
- **Acquired resistance.** sensitive Strains can acquire resistance by way of changes in their genetic material.

Resistance Mechanisms

1-Inactivating enzymes. Hydrolysis or modification of anti-infective agents.

- **A- Betalactamases.** Hydrolyze the betalactam ring of betalactam antibiotics
- . Over 200 different betalactamases are known.
- **B- Aminoglycosidases.** Modify aminoglycosides by means of phosphorylation
- of free hydroxyl groups (phosphotransferases) or acetylation of free amino groups (acetyltransferases).
- **C-Chloramphenicol acetyltransferases.** Modification, by acetylation, of
- chloramphenicol.

2-Resistant target molecules.

- **Gene products with a low affinity to anti-infective agents** are produced based on mutations in natural genes. Example: DNA gyrase subunit A, resistant to 4-quinolones.

3-Permeability mechanisms.

- **Reduced influx.** Reduction of transport of anti-infective agents from outside to inside through membranes; rare.

4-Increased efflux. Active transport of anti-infective agents from inside to outside by means of efflux pumps in the cytoplasmic membrane, making efflux greater than influx; frequent.

Antibacterial antibiotics

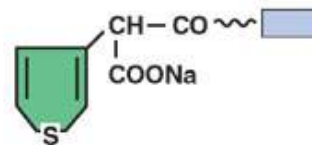
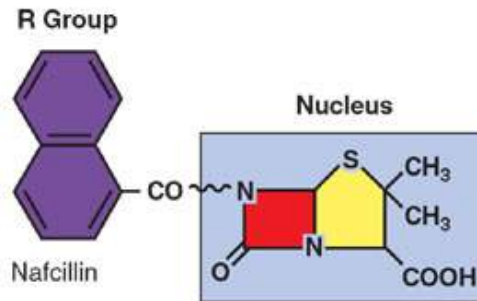
- Penicillins
- Cephalosporins
- Other beta-lactam antibiotics
- Aminoglycosides
- Tetracycline antibiotics
- Chloramphenicol
- Other *Streptomyces* antibiotics
- The *Bacillus* antibiotics
- New classes

Penicillins

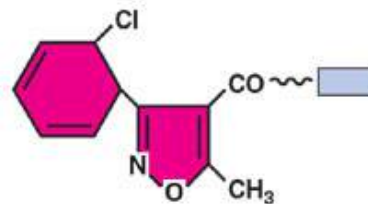
- Large diverse group of compounds
- Could be synthesized in the laboratory
- more economical to obtain natural penicillin through microbial fermentation and modify it to semi-synthetic forms
- *Penicillium chrysogenum* – major source
- All consist of 3 parts
 - thiazolidine ring
 - beta-lactam ring
 - variable side chain dictates microbial activity

Penicillins

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Ticarcillin



Cloxacillin



Carbenicillin

Penicillins


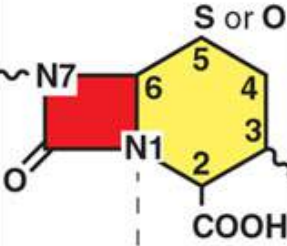
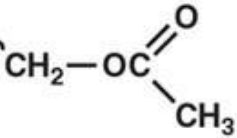
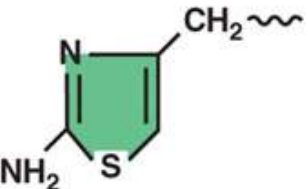

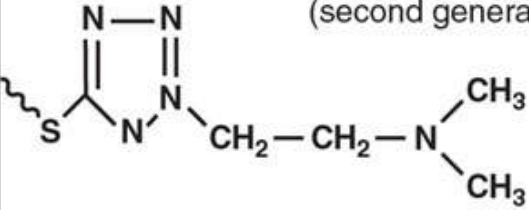
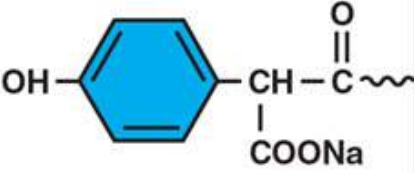

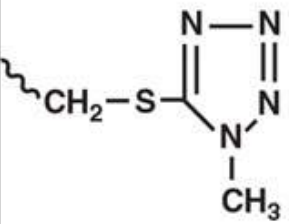
- Penicillins G and V most important natural forms
- Penicillin is the drug of choice for gram-positive cocci (streptococci) and some gram-negative bacteria (meningococci and syphilis spirochete)
- Semisynthetic penicillins – ampicillin, carbenicillin & amoxicillin have broader spectra – gram negative enterics rods
- Penicillinase-resistant – methicillin, nafcillin, cloxacillin
- Primary problems – allergies and resistant strains of bacteria

Cephalosporins

- Account for majority of all antibiotics administered
- Isolated from *Cephalosporium acremonium* mold
- Beta-lactam ring that can be altered
- Relatively broad-spectrum, resistant to most penicillinases, & cause fewer allergic reactions
- Some are given orally, many must be administered parenterally

Cephalosporins

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R Group 1	Basic Nucleus	R Group 2
		<p>Cephalothin (first generation)</p> 
		<p>Cefotiam (second generation)</p> 
		<p>Moxalactam (third generation)</p> 

Cephalosporins

- 3 generations exist
- First generation – cephalothin, cefazolin – most effective against gram-positive cocci
- Second generation – cefaclor, cefonacid – more effective against gram-negative bacteria
- Third generation – cephalexin, cefotaxime – broad-spectrum activity against enteric bacteria with beta-lactamases
- Ceftriaxone – new semisynthetic broad-spectrum drug for treating wide variety of infections

Other beta-lactam antibiotics

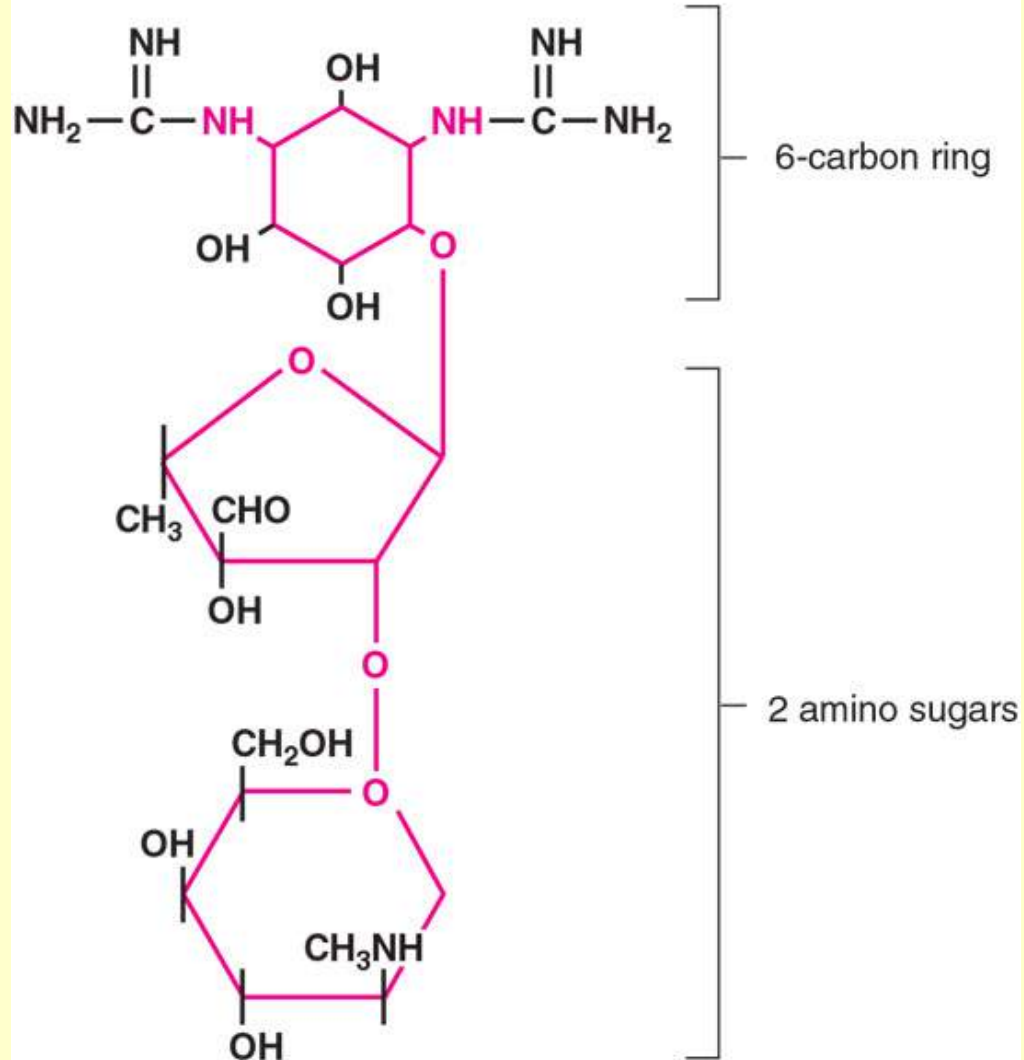
- Imipenem – broad-spectrum drug for infections with aerobic and anaerobic pathogens
- Azeotreonam – isolated from bacteria *Chromobacterium violaceum* – newer narrow-spectrum drug for infections by gram-negative aerobic bacilli. May be used by people allergic to penicillin.

Aminoglycosides

- composed of 2 or more amino sugars and an aminocyclitol (6C) ring
- products of various species of soil actinomycetes in genera *Streptomyces* & *Micromonospora*
- Broad-spectrum, inhibit protein synthesis, especially useful against aerobic gram-negative rods & certain gram-positive bacteria
 - Streptomycin – bubonic plague, tularemia, TB
 - Gentamicin – less toxic, used against gram-negative rods
 - Newer – Tobramycin & amikacin gram-negative bacteria

Aminoglycosides

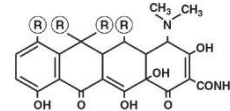
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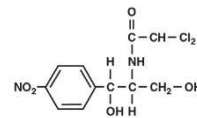
Tetracycline antibiotics

- Broad-spectrum, block protein synthesis
- Doxycycline & minocycline – oral drugs taken for STDs, Rocky Mountain spotted fever, Lyme disease, typhus, acne & protozoa

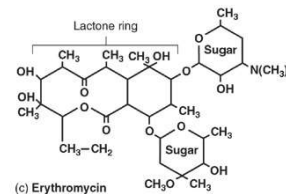
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(a) Tetracyclines



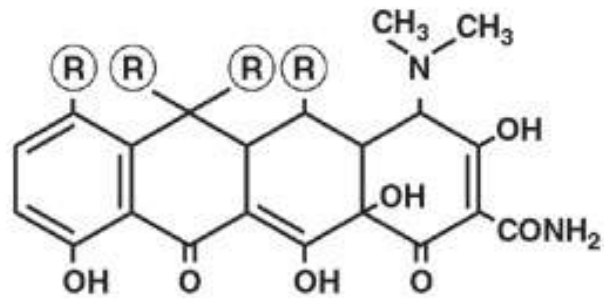
(b) Chloramphenicol



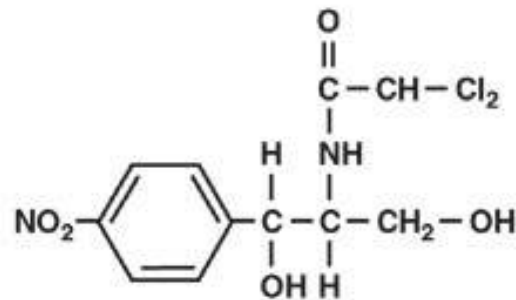
(c) Erythromycin

Chloramphenicol

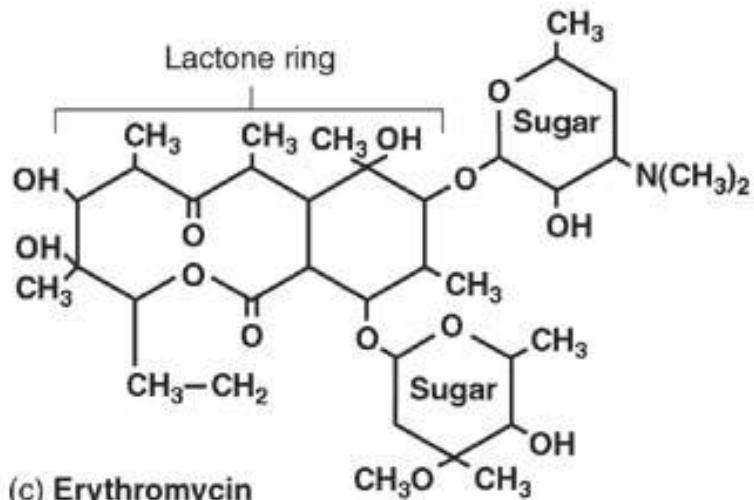
- Isolated from *Streptomyces venezuelae*
- Potent broad-spectrum drug with unique nitrobenzene structure
- Blocks peptide bond formation
- No longer derived from natural source
- Very toxic, restricted uses, can cause irreversible damage to bone marrow
- Typhoid fever, brain abscesses, rickettsial & chlamydial infections



(a) Tetracyclines



(b) Chloramphenicol



(c) Erythromycin

Other *Streptomyces* antibiotics

- Erythromycin – macrolide, large lactone ring with sugars
- Broad-spectrum, fairly low toxicity
- Attaches to ribosome
- Taken orally for Mycoplasma pneumonia, legionellosis, Chlamydia, pertussis, diphtheria and as a prophylactic prior to intestinal surgery
- For penicillin-resistant – gonococci, syphilis, acne
- Newer semi-synthetic macrolides – clarithromycin, azithromycin

Other *Streptomyces* antibiotics

- Clindamycin – broad-spectrum, serious abdominal anaerobic infections
- Vancomycin – narrow-spectrum, effective against penicillin & methicillin resistant staphylococcal infections; very toxic, hard to administer
- Rifampin – limited spectrum, cannot pass through many cell membranes, used to treat gram-positive bacteria, TB, leprosy

The *Bacillus* antibiotics

- Bacitracin- narrow-spectrum peptide produce by *Bacillus subtilis*, major ingredient of neosporin ointment
- Polymyxin - narrow-spectrum peptide with fatty acid component, detergent activity; limited by toxicity to kidney; drug resistant *Pseudomonas aeruginosa* & UTI

New classes of antibiotics

- Fosfomycin trimethamine – a phosphoric acid effective as alternate treatment for UTIs, inhibits cell wall synthesis
- Synercid – effective against *Staphylococcus* & *Enterococcus* that cause endocarditis & surgical infections; inhibits protein synthesis

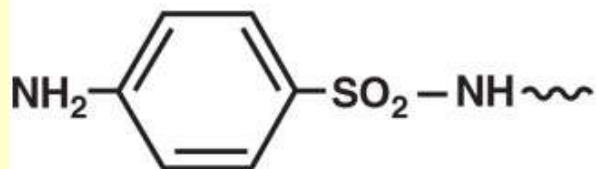
Synthetic antibacterial drugs

- Sulfonamides, sulfa drugs – first antimicrobial drugs
- Sulfisoxazole – shigellosis, UTI, protozoan infections
- Silver sulfadiazine – burns, eye infections
- Trimethoprim – given in combination with sulfamethoxazole – UTI, PCP

Sulfonamides

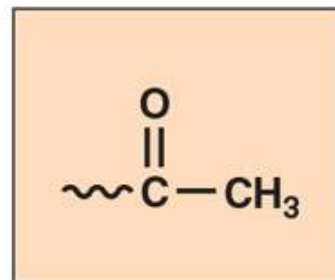
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Nucleus

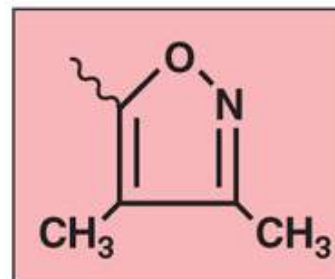


R Group

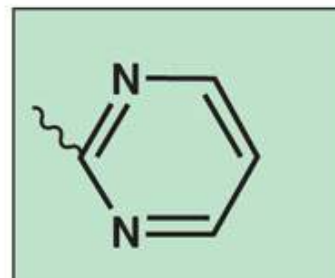
(a)



(b)



(c)



Miscellaneous antibacterial drugs

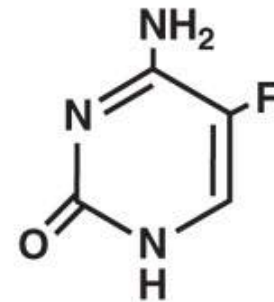
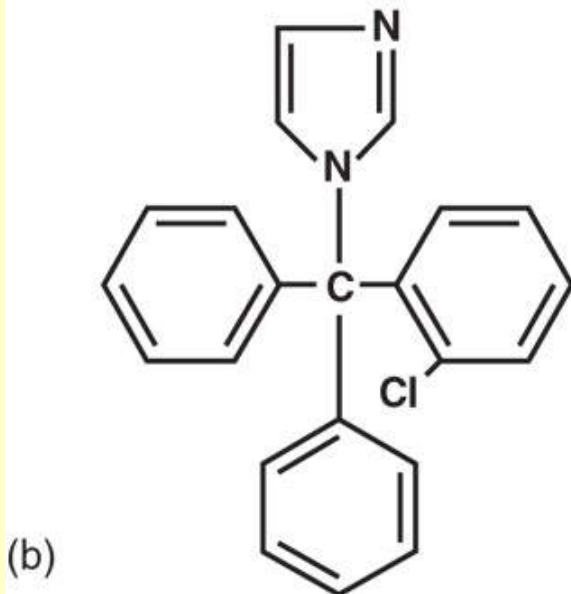
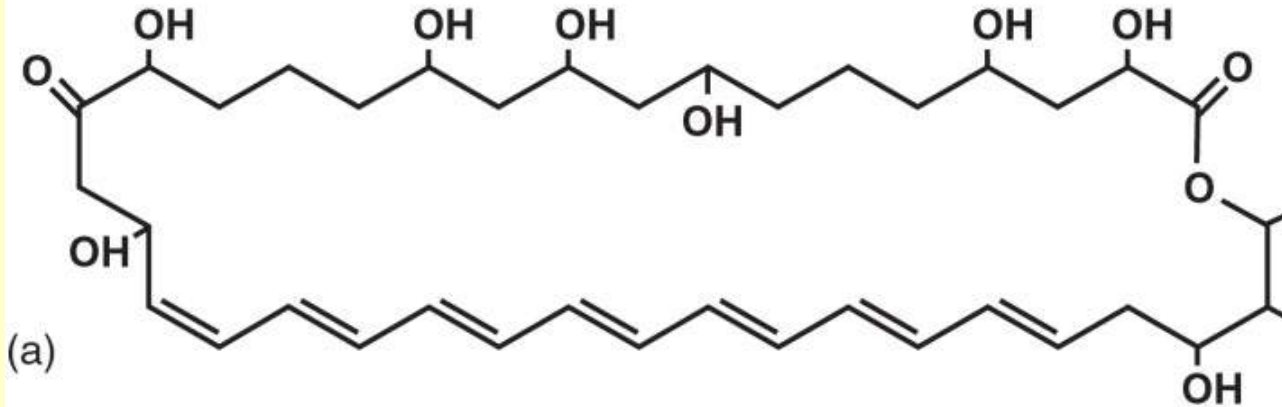
- Isoniazid –used with rifampicin to treat TB
- Oxazolidinones- new class of antibacterial drugs inhibit initiation of protein synthesis
 - Linezolid – MRSA, VRE
- Fluoroquinolones –broad-spectrum, potent
 - norfloxacin, ciprofloxacin – UTI, STD, GI, osteomyelitis, respiratory & soft tissue infections
 - sparofloxacin, levofloxacin – pneumonia, bronchitis, sinusitis

Antifungal drugs

- Macrolide polyene
 - Amphotericin B –mimic lipids, most versatile & effective, topical & systemic treatments
 - Nystatin – topical treatment
- Griseofulvin – stubborn cases of dermatophyte infections, nephrotoxic
- Synthetic azoles – broad-spectrum; ketoconazole, clotrimazole, miconazole
- Flucytosine – analog of cytosine; cutaneous mycoses or in combination with amphotericin B for systemic mycoses

Antifungal drugs

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Antiparasitic drugs

- Antimalarial drugs – quinine, chloroquine, primaquine, mefloquine
- Antiprotozoan drugs - Metronidazole (Flagyl), quinacrine, sulfonamides, tetracyclines
- Anthelmintic drugs – immobilize, disintegrate, or inhibit metabolism
 - mebendazole, thiabendazole- broad-spectrum – inhibit function of microtubules, interferes with glucose utilization & disables them
 - pyrantel, piperazine- paralyze muscles
 - niclosamide – destroys scolex

Antiviral drugs

- Block penetration into host cell
- Block transcription or translation
 - Nucleotide analogs
 - Acyclovir – herpesviruses
 - Ribavirin- a guanine analog – RSV, hemorrhagic fevers
 - AZT – thymine analog - HIV
- Prevent maturation of viral particles
 - Protease inhibitors – HIV
- Interferon - HCV

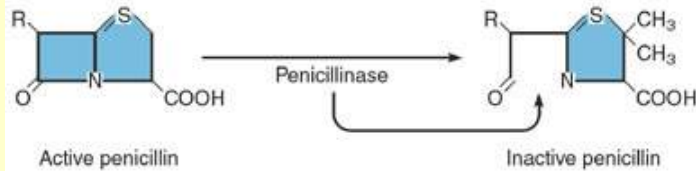
Mechanisms drug resistance

- Drug inactivation – penicillinases
- Decreased permeability to drug or increased elimination of drug from cell
- Change in metabolic patterns
- Change in drug receptors

Mechanisms drug resistance

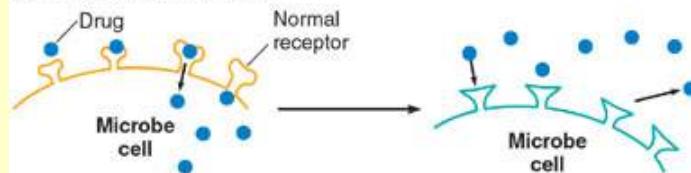
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(a) Drug inactivation



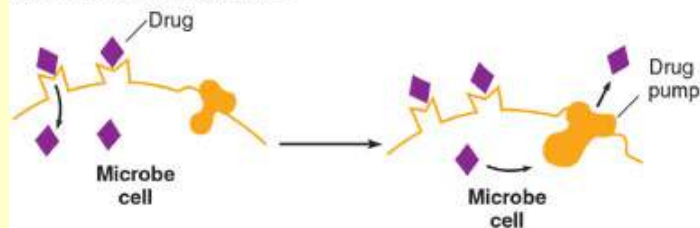
Inactivation of a drug like penicillin by penicillinase, an enzyme that cleaves a portion of the molecule and renders it inactive.

(b) Decreased permeability



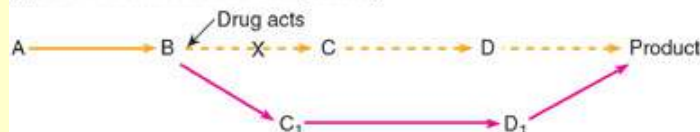
The receptor that transports the drug is altered, so that the drug cannot enter the cell.

(c) Activation of drug pumps



Specialized membrane proteins are activated and continually pump the drug out of the cell.

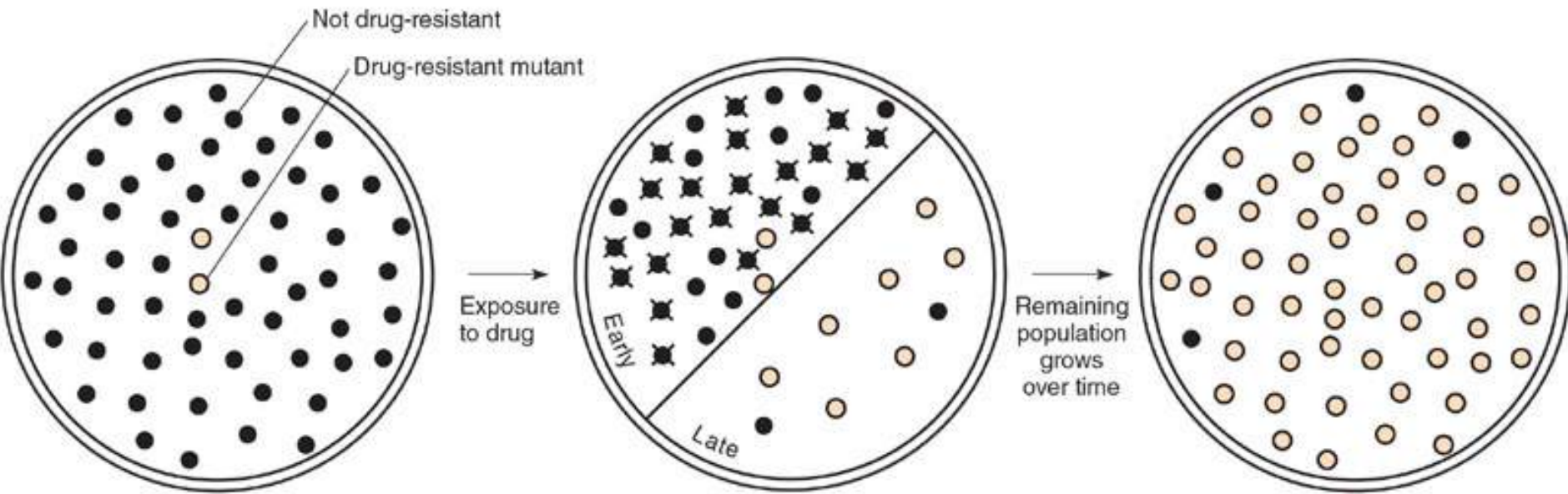
(d) Use of alternate metabolic pathway



The drug has blocked the usual metabolic pathway, so the microbe circumvents it by using an alternate, unblocked pathway that achieves the required outcome.

Selection for drug resistance

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(a) Population of microbial cells

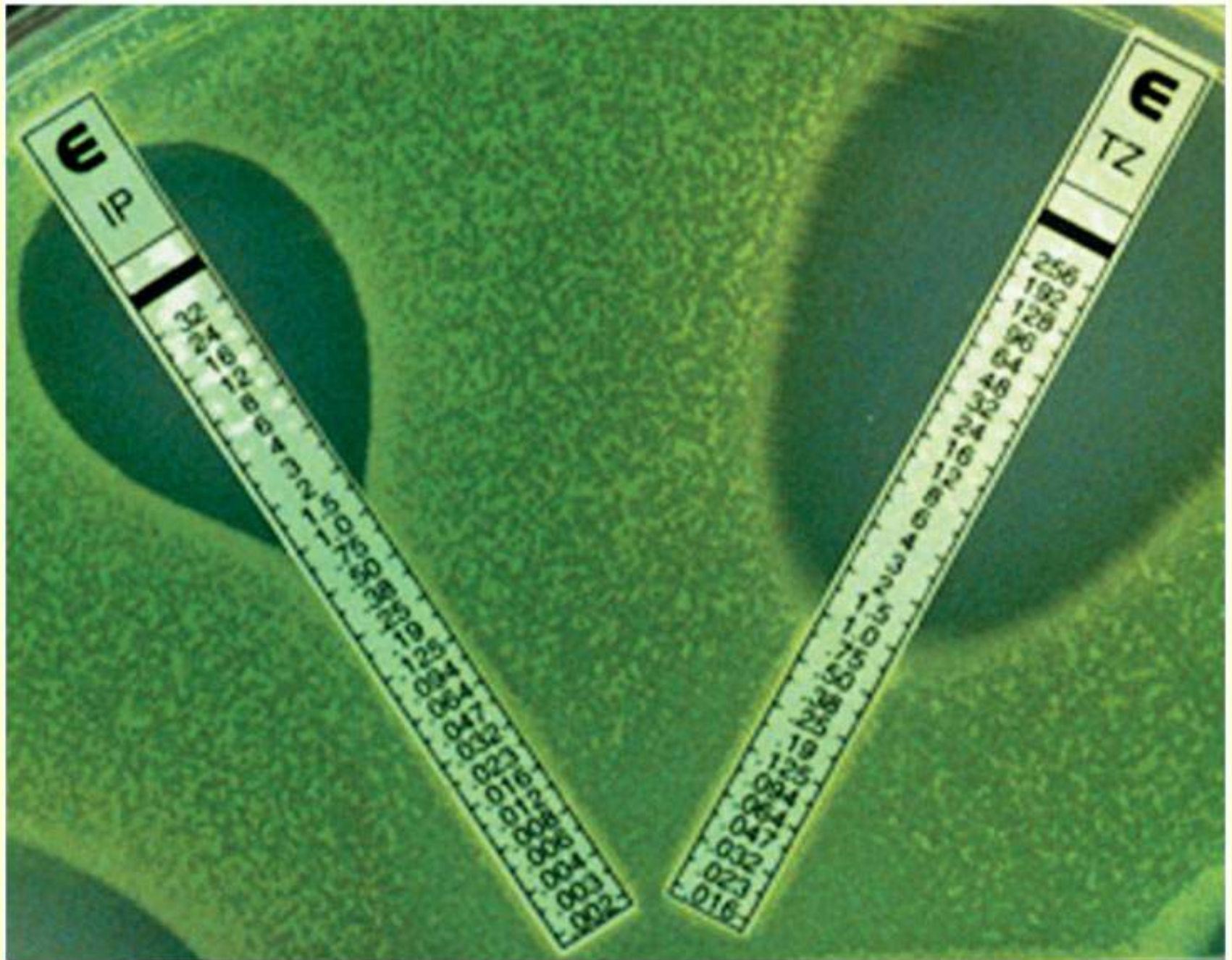
(b) Sensitive cells eliminated by drug ✖; resistant mutants survive

(c) Most cells are now resistant

Side effects of drugs

1. Toxicity to organs
2. Allergic responses
3. Suppression and alteration of microflora





Considerations in selecting an antimicrobial drug

1. nature of microbe causing infection
2. degree of microbe's sensitivity to various drugs
3. overall medical condition of patient

- Minimum inhibitory concentration (MIC)- smallest concentration of drug that visibly inhibits growth
- Therapeutic index – the ratio of the dose of the drug that is toxic to humans as compared to its minimum effective dose

TABLE 12.2

Terminology of Chemotherapy

Chemotherapeutic drug	Any chemical used in the treatment, relief, or prophylaxis of a disease
Prophylaxis*	Use of a drug to prevent imminent infection of a person at risk
Antimicrobial chemotherapy*	The use of chemotherapeutic drugs to control infection
Antimicrobics	All-inclusive term for any antimicrobial drug, regardless of its origin
Antibiotics*	Substances produced by the natural metabolic processes of some microorganisms that can inhibit or destroy other microorganisms
Semisynthetic drugs	Drugs which are chemically modified in the laboratory after being isolated from natural sources
Synthetic drugs	The use of chemical reactions to synthesize antimicrobial compounds in the laboratory
Narrow spectrum (limited spectrum)	Antimicrobics effective against a limited array of microbial types—for example, a drug effective mainly on gram-positive bacteria
Broad spectrum (extended spectrum)	Antimicrobics effective against a wide variety of microbial types—for example, a drug effective against both gram-positive and gram-negative bacteria

Antimicrobial combinations are used widely, although most infections in patients with normal defenses can be treated with a single antimicrobial agent. Few reasons justify the use of antimicrobial combinations:

- (1) Broad-spectrum coverage for the initial therapy of severely infected patients;
- (2) Polymicrobial infections;
- (3) Prevention of selection of resistant microorganisms when a high mutation rate of the causal organism exists to the antibiotic indicated;
- (4) Reduction of dose-related toxicity ; related to the use of sulfonamides
- (5) Antimicrobial synergistic activity. It is appealing to use combinations and treat two types of infections— infections resulting from resistant or relatively resistant organisms and infections requiring a bacterial eradication (high bactericidal effect), considering the site of infection and the host defenses

- **synergistic effect** – an additive effect, achieved by multiple drugs working together, requiring a lower dose of each

Other Typical Antimicrobial Agents

- Many systemic antibiotics were not developed to treat oral bacteria or are not specific to treat oral diseases. The application of systemic antibiotics has gradually reduced during recent decades, with other antimicrobial agents having been developed to target oral bacteria that cause oral diseases, such as fluoride, chlorhexidine, quaternary ammonium salts, and antimicrobial peptides (AMPs).
- **Fluoride:** is a successful cavity prevention agents and dental caries, incorporated in mouthwashes, toothpastes, and oral supplements in small quantities. Its mechanism is that fluoride ions contact the mineral of the tooth surface and increase remineralization to prevent the acid-induced demineralization caused by cariogenic bacteria as mutans streptococci and *Lactobacillus acidophilus*. It inhibits enolase and result in the growth inhibition and reduced acid production of *S. mutans*. However, the development of fluoride-resistant oral bacteria, has led to a reconsideration of the administration of fluoride.

- **Chlorhexidine**

- Is one of the first antiseptic agents proposed for dental caries and has proved to be the most effective and the “gold standard” of antiplaque agents. Chlorhexidine is active against gram-positive and gram-negative bacteria, facultative anaerobes, aerobes, and yeasts by damaging the inner cytoplasmic membrane, it can block the acidic groups of glycoproteins present in saliva to reduce plaque adhesion also can reduce the binding of bacteria to tooth surfaces. However, chlorhexidine causes genotoxicity by inducing DNA damage in leukocytes, kidney cells and oral mucosal cells, and it can also induce cellular apoptosis.

- **Quaternary Ammonium Salts**

- Are widely used as antimicrobial agents, and were first incorporated into mouth rinses to inhibit oral plaque, used as additives in dental materials to give them antimicrobial abilities, they promote the bacterial lysis by binding to bacterial membranes. Their side effects include gastrointestinal symptoms, coma, convulsions, hypotension, and death

- **Antimicrobial Peptides (AMPs)**

- Are host-defence molecules that exert potent antimicrobial activities against a broad spectrum of microorganisms. In the oral cavity, there are many natural AMP molecules, such as hBD-1,2,3 (human β -defensin-1,2,3), LL-37 (a cathelicidin), nisin and histatins, which possess antimicrobial activities against oral pathogenic bacteria and biofilms. Their antimicrobial mechanism is cell permeabilization followed by membrane disruption, which depends on their relatively strong electrostatic attraction to negatively charged bacterial cells.

- **Remineralizing Agents**

- Many of these agents are being used clinically to treat dental caries. In addition to fluorides, calcium phosphate materials, nanoparticles (such as nanoHAP particles, ACP nanoparticles, and nanobioactive glass materials), polydopamine, oligopeptides and many others are used for remineralization and teeth repair and to restore the presence of minerals to the hydroxyapatite (HAP) crystal lattice in ionic forms.

Staphylococcus

Classification

Family: **Micrococcaceae**

Genus : *Micrococcus and Staphylococcus*

Species : . *S. aureus*

S. saprophyticus

S. epidermidis

FAMILY: Micrococcaceae (**catalase positive**)

-**Coagulase-positive** *Staphylococcus aureus*

-**Coag.-neg.** *Staphylococcus epidermidis*, *S. saprophyticus*

Morphology

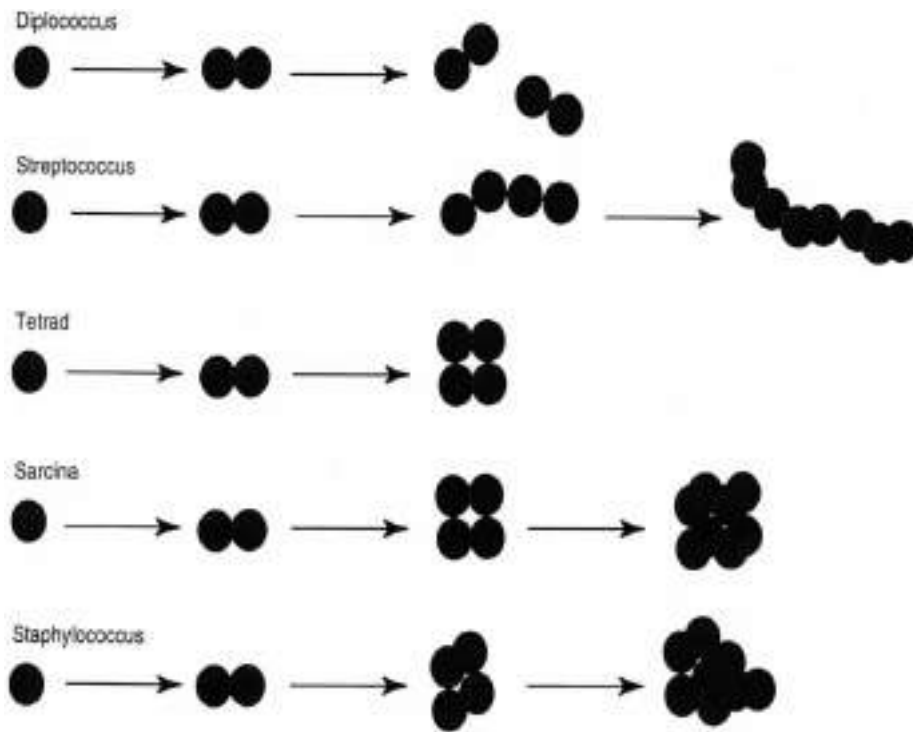
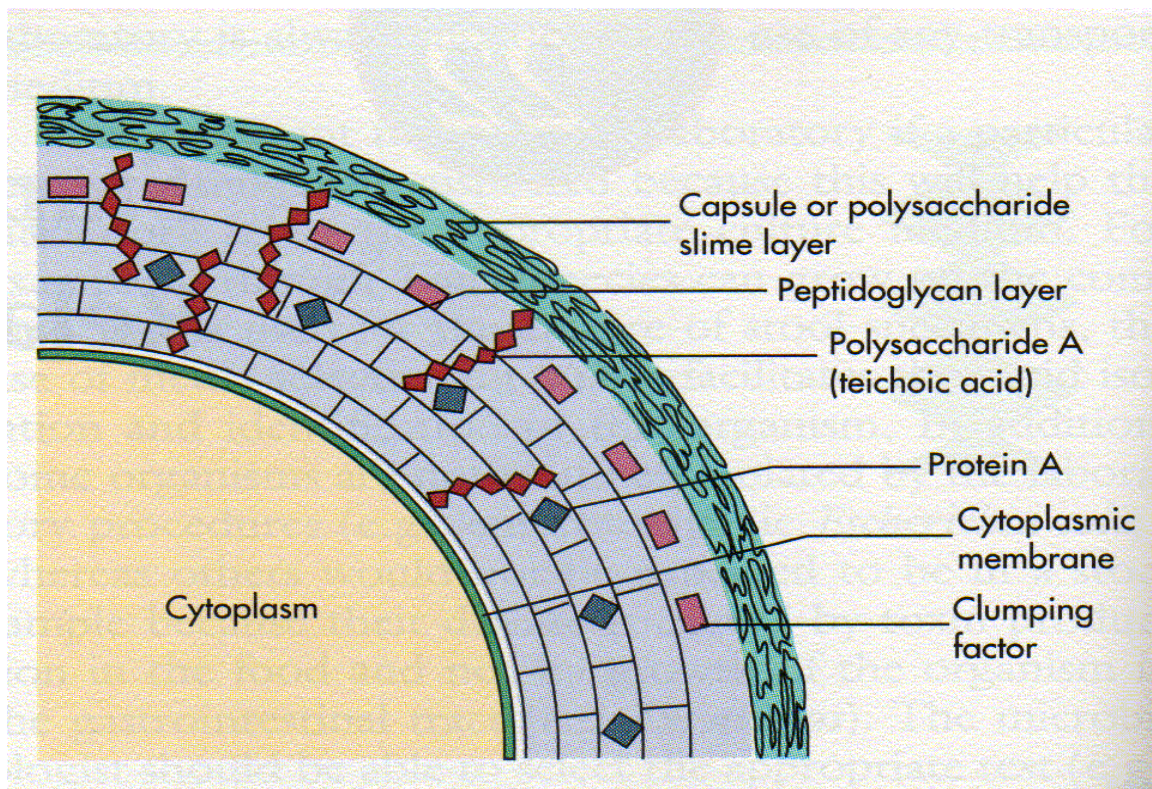


TABLE 22-2. *Staphylococcus, Micrococcus, Stomatococcus, and Alloisococcus* and Their Diseases

Organism	Diseases
<i>Staphylococcus aureus</i>	Toxin-mediated (food poisoning, toxic shock syndrome ^{SSSS}); cutaneous (impetigo, folliculitis, furuncles, carbuncles, wound infections); other (bacteremia, endocarditis, pneumonia, empyema, osteomyelitis, septic arthritis)
<i>Staphylococcus epidermidis</i>	Bacteremia; endocarditis; surgical wounds; urinary tract infections; opportunistic infections of catheters, shunts, prosthetic devices, and peritoneal dialysates
<i>Staphylococcus saprophyticus</i>	Urinary tract infections, opportunistic infections
<i>Staphylococcus copris</i>	Bacteremia, endocarditis, urinary tract infections, wound infections, pneumonia, bone and joint infections, opportunistic infections
<i>Staphylococcus aureolyticus</i>	Bacteremia, endocarditis, urinary tract infections, wound infections, and opportunistic infections
<i>Micrococcus</i> spp.	Opportunistic infections
<i>Stomatococcus mucilaginosus</i>	Bacteremia, endocarditis, opportunistic infections
<i>Alloisococcus otitidis</i>	Chronic middle ear infections

Staph. Antigenic Structure:



Peptidoglycan :

important in pathogenesis of infection

- 1- elicit production of IL-1 (endogenous pyrogen)
- 2- elicit production of opsonic Abs.
- 3- It can be a chemoattractant for PMNs
- 4- have endotoxin-like activity
- 5- Activates complement

Protein A : is a cell wall component of many *S. aureus* strains that binds to the Fc portion of IgG except IgG3.

Cell-Associated Virulence Factors

- 1- Capsule or slime layer (**glycocalyx**)
- 2- **Peptidoglycan** (PG)

3- Teichoic acid is covalently linked to PG and is species specific:

S. aureus ribitol teichoic acid
(polysaccharide A)

S. epidermidis glycerol teichoic acid
(polysaccharide B)

4- Protein A is covalently linked to PG

5- Clumping factor (bound coagulase)

Virulence Factors Extracellular Enzymes

- 1- **Coagulases** (bound or free)

Antigenic

2- Hyaluronidase

“spreading factor” of *S. aureus*

3- Nuclease

Cleaves DNA and RNA in *S. aureus*

4- Protease

Staphylokinase (fibrinolysin)

5- Lipases

6- Esterases

Virulence Factors: Exotoxins

Cytolytic (cytotoxins; cytolysins) :

1- Alpha toxin - hemolysin

Reacts with RBCs

2- Beta toxin

Sphingomyelinase

3- Gamma toxin

Hemolytic activity

4- Delta toxin

a- Cytopathic for:

RBCs, Macrophages, Lymphocytes, Neutrophils, Platelets

b- Enterotoxic activity

5- Leukocidin

Important Exotoxins

1- **Exfoliative toxin (epidermolytic toxin)** causing SSSS (Staphylococcal scalded skin syndrome) it is superantigen.

2- **Toxic Shock Syndrome Toxin : TSST-1**, prototypical superantigen

3- **Enterotoxin: causing food poisoning**

4- **Pyrogenic exotoxins**

Virulence Factors	Biologic Effects
Structural Components	
Capsule	Inhibits chemotaxis and phagocytosis; inhibits proliferation of mononuclear cells; facilitates adherence to foreign bodies
Peptidoglycan	Provides osmotic stability; stimulates production of endogenous pyrogen (endotoxin-like activity); leukocyte chemoattractant (abscess formation); inhibits phagocytosis
Teichoic acid	Regulates cationic concentration at cell membrane; binds to fibronectin
Protein A	Inhibits antibody-mediated clearance by binding IgG ₁ , IgG ₂ , and IgG ₄ Fc receptors; leukocyte chemoattractant; anticomplementary
Cytoplasmic membrane	Osmotic barrier; regulates transport into and out of cell; site of biosynthetic and respiratory enzymes
Toxins	
Cytotoxins (α , β , δ , γ , P-V leukocidin)	Toxic for many cells, including leukocytes, erythrocytes, macrophages, platelets, and fibroblasts
Exfoliative toxins (ETA, ETB)	Serine proteases that split the intercellular bridges in the stratum granulosum epidermis
Enterotoxins (A-E, G-I)	Superantigens (stimulates proliferation of T cells and release of cytokines); stimulates release of inflammatory mediators in mast cells, increasing intestinal peristalsis and fluid loss, as well as nausea and vomiting
Toxic Shock Syndrome Toxin-1	Superantigen (stimulates proliferation of T cells and release of cytokines); produces leakage or cellular destruction of endothelial cells
Enzymes	
Coagulase	Converts fibrinogen to fibrin
Catalase	Catalyzes removal of hydrogen peroxide
Hyaluronidase	Hydrolyzes hyaluronic acids in connective tissue, promoting the spread of staphylococci in tissue
Fibrinolysin	Dissolves fibrin clots
Lipases	Hydrolyzes lipids
Nucleases	Hydrolyzes DNA
Penicillinase	Hydrolyzes penicillins

Pathogenesis

-Pass skin – first line of defense produce :

1- Benign infection

Phagocytosis

Antibody

Inflammatory response

2-Chronic infections

Delayed hypersensitivity

Clinical Manifestations/Disease

SKIN infection

- 1- folliculitis
- 2- boils (furuncles)
- 3- carbuncles
- 4- **impetigo** (bullous & pustular)
- 5- **scalded skin syndrome**
Neonates and children under 4 years

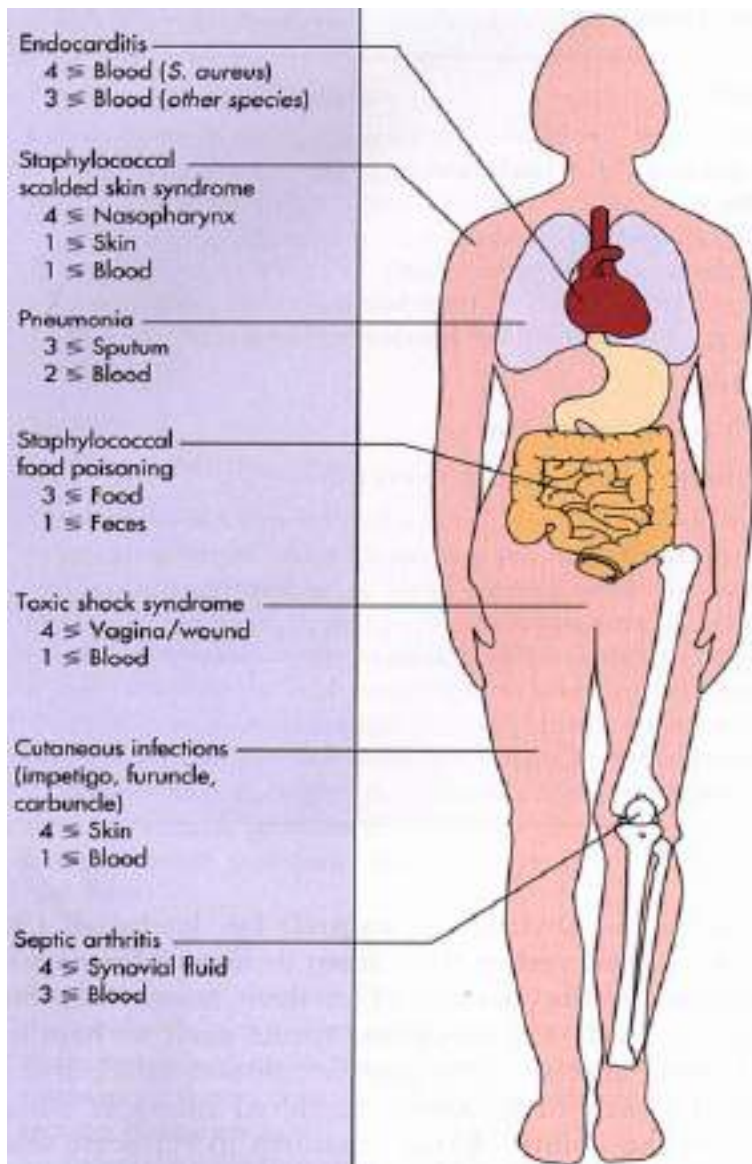
Clinical Manifestations/Disease

Other infections

- 1- Primary staphylococcal pneumonia
- 2- Food poisoning vs. foodborne disease
- 3- Toxic shock syndrome

Metastatic Infections

- **Bacteremia**
- **Osteomyelitis**
- Pulmonary and cardiovascular infection



Coagulase-Negative Staphylococci

1- *Staphylococcus epidermidis*

2- *S. saprophyticus*

Staphylococcal Lab ID & Diagnostic Tests

Microscopic: cluster G+ cocci

Differential Characteristics

- 1- Coagulase positive *S. aureus* (Fibrinogen → Fibrin)

2- Catalase



Streptococci *negative* vs. Staphylococci *positive*

Treatment

1-Drain infected area

2-Deep/metastatic infections

semi-synthetic penicillins

cephalosporins

erythromycin

clindamycin

3-Endocarditis

semi-synthetic penicillin + an aminoglycoside

Prevention

1-Carrier status prevents complete control

2-Proper hygiene, segregation of carrier from highly susceptible individuals

3-Good aseptic techniques when handling surgical instruments

4-Control of nosocomial infections

Lactobacilli

Lactobacilli are saprophytes in vegetable and animal material (e.g. milk). Some species are common animal and human commensals inhabiting the oral cavity and other parts of the body. They have the ability to tolerate acidic environments and hence are believed to be associated with the carious process.

The taxonomy of lactobacilli is complex. They are characterized into two main groups: **homofermenters**, which produce mainly lactic acid (65%) from glucose fermentation (e.g. *Lactobacillus casei*), and **heterofermenters**, which produce lactic acid as well as acetate, ethanol and carbon dioxide (e.g. *Lactobacillus fermentum*). *L. casei* and *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and the newly described species, *Lactobacillus oris*, are common in the oral cavity

- **Habitat and transmission**

Lactobacilli are found in the oral cavity, gastrointestinal tract and female genital tract. In the oral cavity, they constitute less than 1% of the total flora.

- **Characteristics**

Gram-positive coccobacillary forms (mostly bacillary), α - or non-haemolytic, facultative anaerobes non spore forming. These organisms ferment carbohydrates to form acids (i.e. they are **acidogenic**) and can survive well in acidic milieu (they are **aciduric**); they may be homofermentative or heterofermentative. The question as to whether they are present in carious lesions because they prefer the acidic environment, or whether they generate an acidic milieu and destroy the tooth enamel.

- .

Lactobacilli are also major constituents of the vaginal flora and help maintain its low pH equilibrium. Recently, the beneficial role of lactobacilli is maintaining the homoeostasis of the intestinal flora.

- **Culture and identification**
- Lactobacilli grow under microaerophilic conditions in the presence of carbon dioxide and at acidic pH (6.0). Media enriched with glucose or blood promote growth. A special selective medium, tomato juice agar (pH 5.0), Rogosa agar promote the growth of lactobacilli while suppressing other bacteria. Identification is by biochemical reactions.

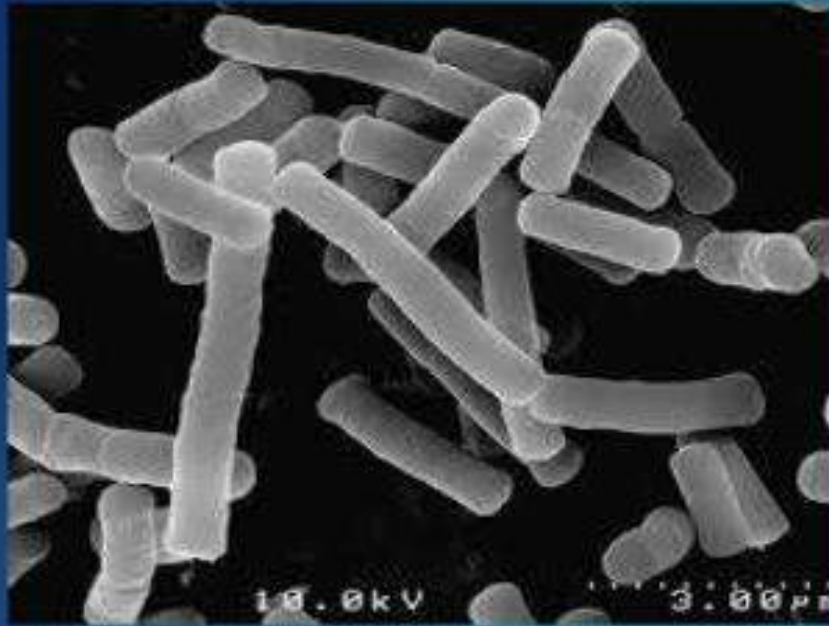
- **Pathogenicity**
- **Lactobacilli cause**
- **Dental caries**
- **Bacterial vaginosis**
- **Lactobacilli and carious lesions**
- Lactobacilli are frequently isolated from deep carious lesions where the pH tends to be acidic. Indeed, early workers believed that lactobacilli were the main cariogenic agent (a theory that has been disproved), so much so that the number of lactobacilli in saliva (the **lactobacillus count**) was taken as an indication of an individual's caries activity (Caries test). Although this test is not very reliable, it is useful for monitoring the dietary profile of a patient because the level of lactobacilli correlates well with the intake of dietary carbohydrate.
- **TREATMENT**
- Lactobacilli are sensitive to penicillin clindamycin and erythromycin

PrOBIOTICS

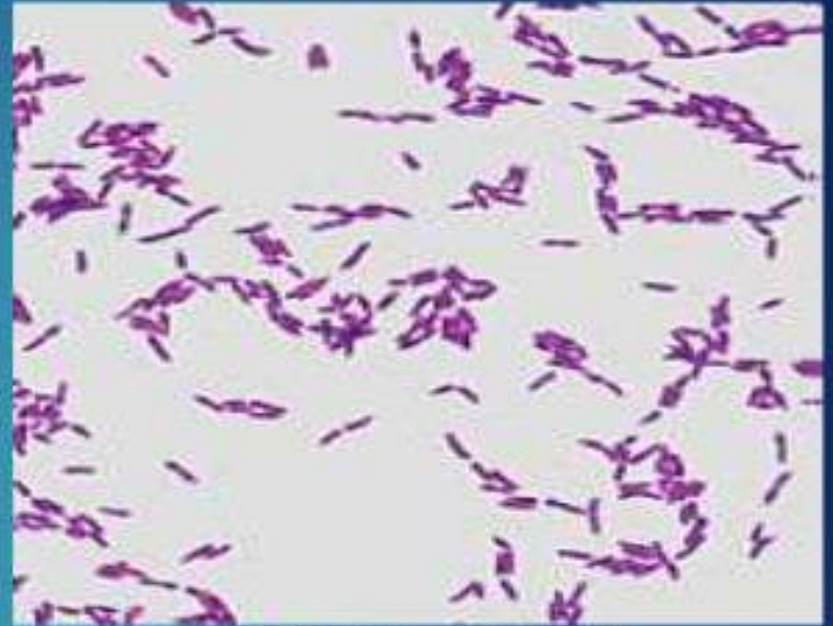
Commercial preparations of lactobacilli are used as probiotics to restore normal flora after the imbalance created by [antibiotic](#) therapy.

- Lactobacillus acidophilus has been used in alternative medicine as a likely effective aid in treating diarrhea in children with rotavirus.
- Lactobacillus acidophilus has been used in alternative medicine as a possibly effective aid (in children or adults) in preventing diarrhea caused by antibiotics, travel, chemotherapy, or hospitalization. Lactobacillus acidophilus is also possibly effective in treating irritable bowel syndrome, bacterial vaginal infection, colic in babies, lung infections in children, skin problems in children who are allergic to milk, and other conditions.
- Lactobacillus acidophilus has also been used to treat lactose intolerance, Crohn's disease, overgrowth of bacteria in the intestines, or vaginal yeast infections caused by antibiotics.

LACTOBACILLI



Electronic microscope show a Lactobacilli rods



light microscope show a lactobacilli rods