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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.

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

☐Macciavello staining
☐Gimenez staining
☐Direct immunoflourescence
☐Indirect immunoflourescence
☐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 pneumonia, 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.

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. pneumonia.*

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.

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 :

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.

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,

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.

☐***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.

☐***Immunofluoresence:*** 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-gonoccocal urethritis.

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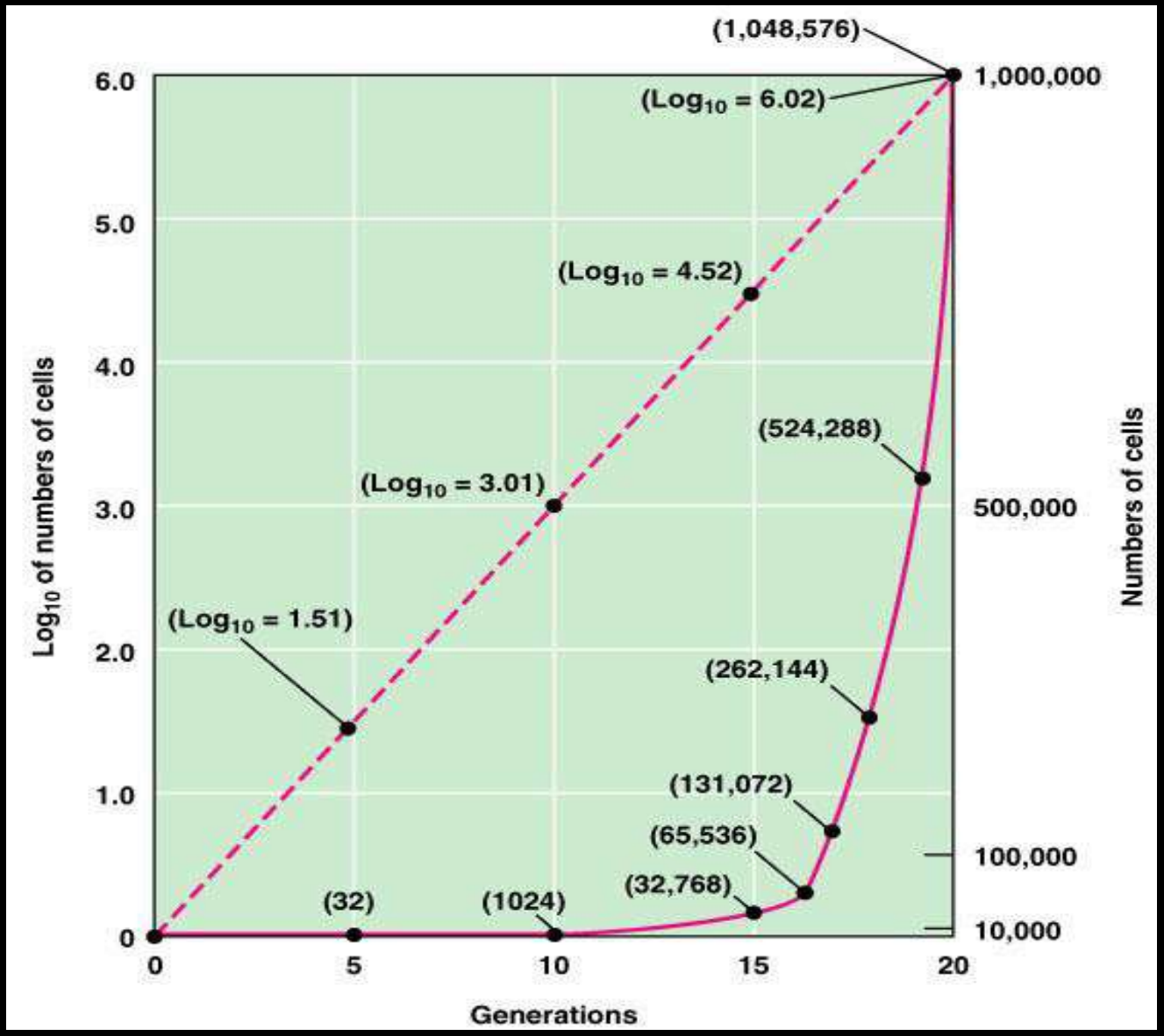
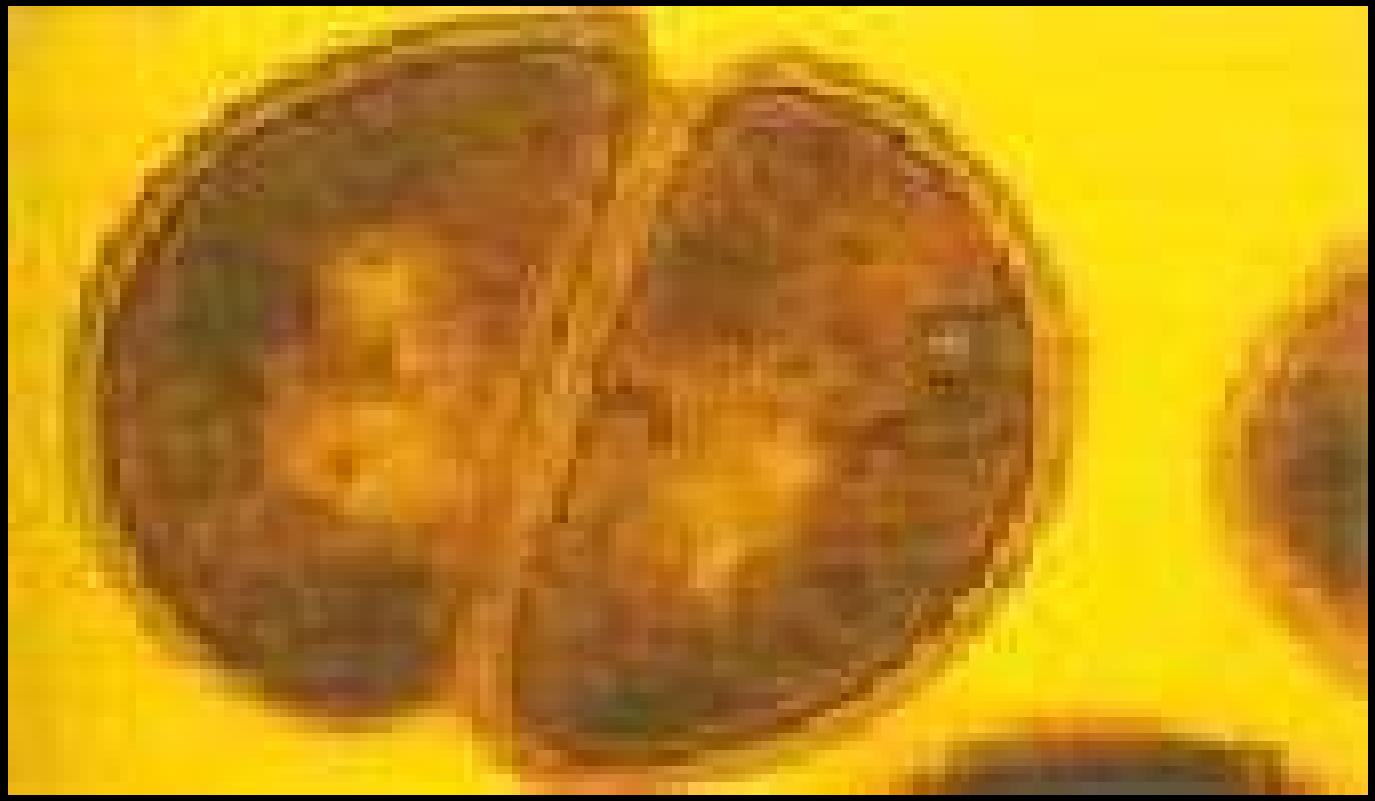
Bacterial growth and metabolism

Growth of Microbes

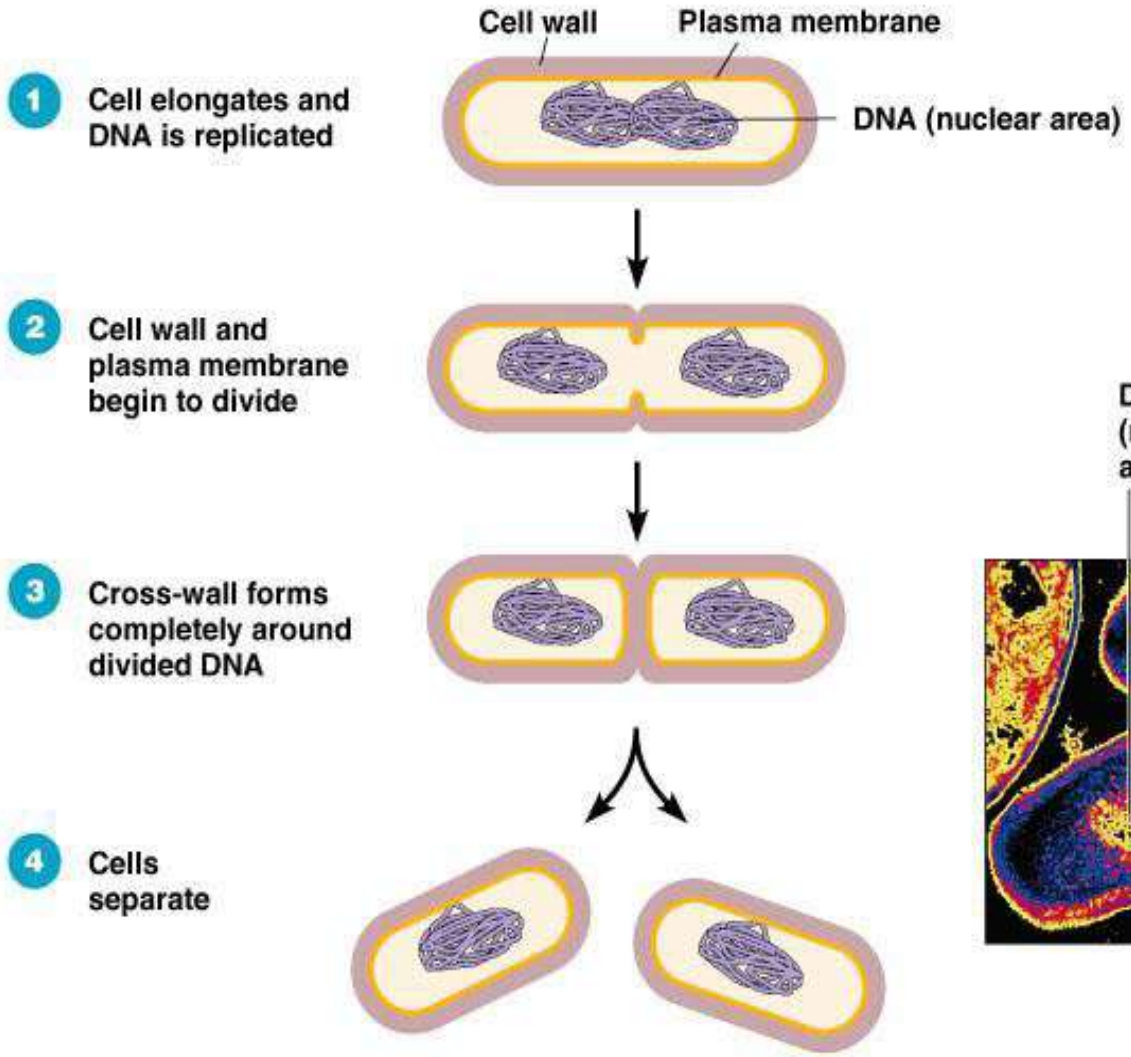
- Microbial growth: Microbes grow via binary fission, resulting in exponential increases in numbers
- Bacterial "growth" means an increase in the number of cells, not an increase in cell size
- One cell becomes colony of millions of cells


(1,048,576)
1,000,000
(Log10 = 6.02)
6.0
5.0
(Log10 = 4.52)
4.0
(524,288)
(Log10 = 3.01)
3.0
500,000
(Log10 = 1.51)
2.0
(262,144)
1.0
(131,072)
(65,536)
100,000
(32,768)
(32)
(1024)
0
10,000
0
5
10
15
20
Generations
Log10 of numbers of cells
Numbers of cells

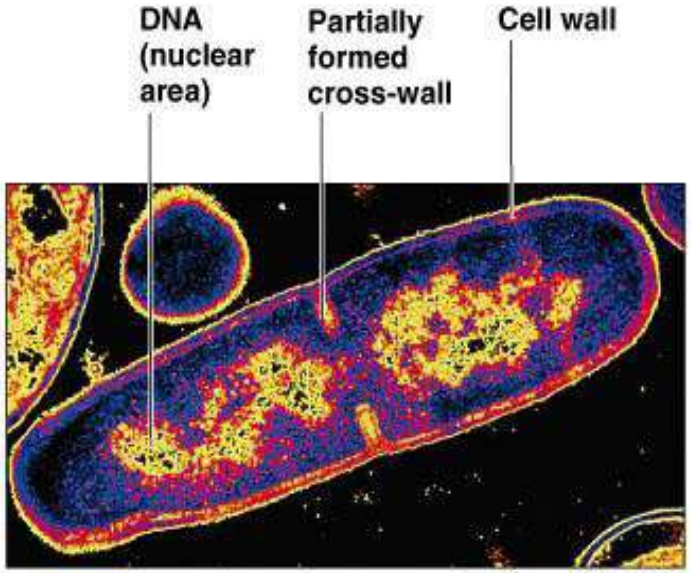
- Bacteria grow by binary fission to produce identical offspring, which cannot be distinguished as a parent or offspring



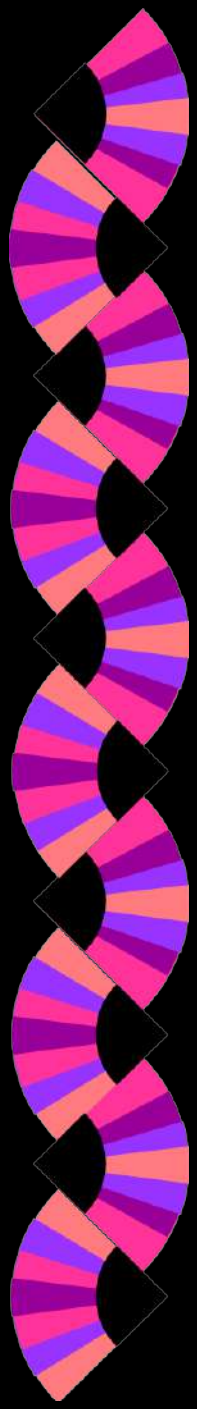
Binary Fission



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

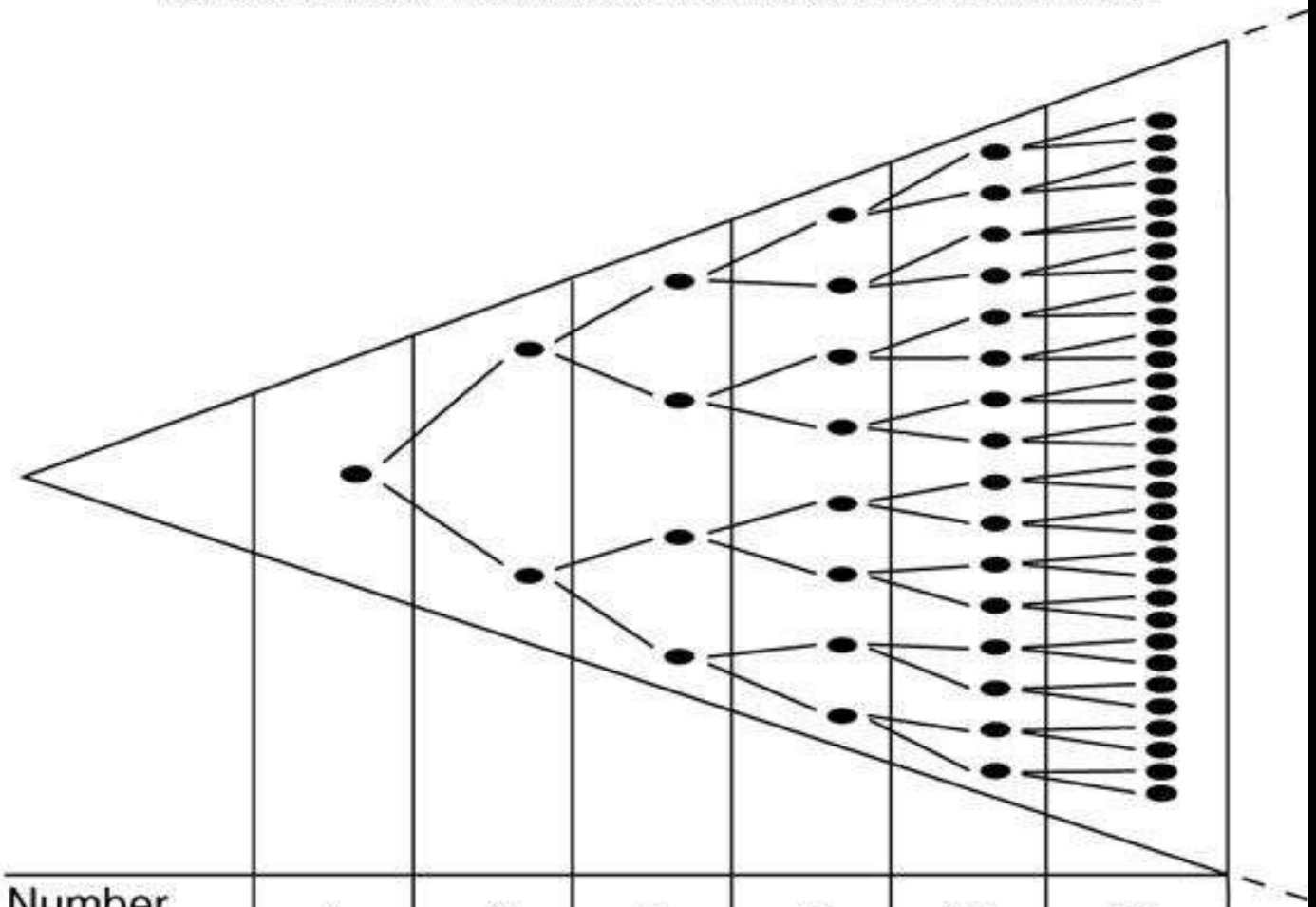


(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.



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

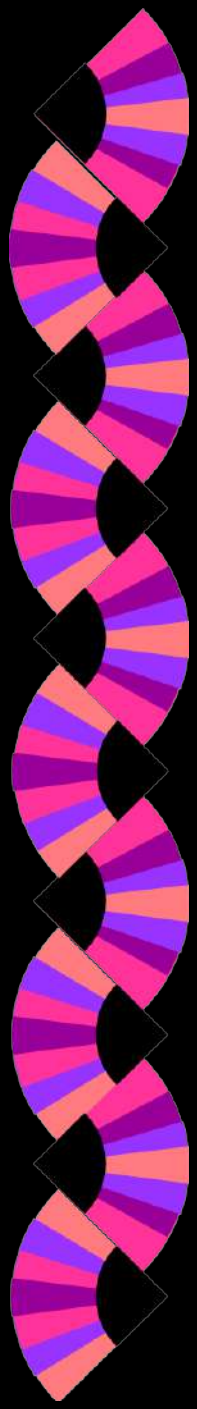
Four main growth phases

-Lag phase

-Exponential (Log) phase

-Stationary phase (Post-exponential)

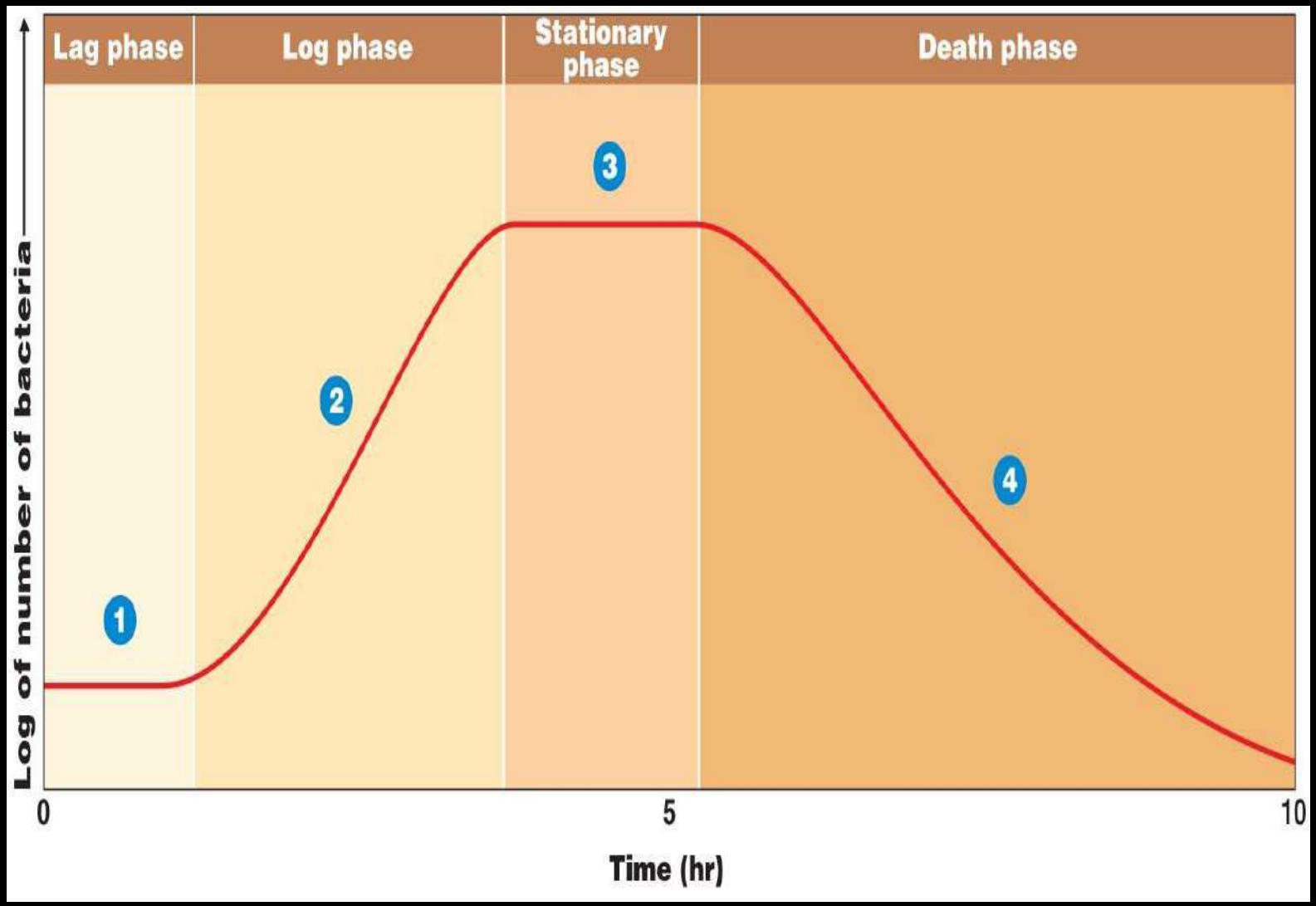
-Decline phase



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.

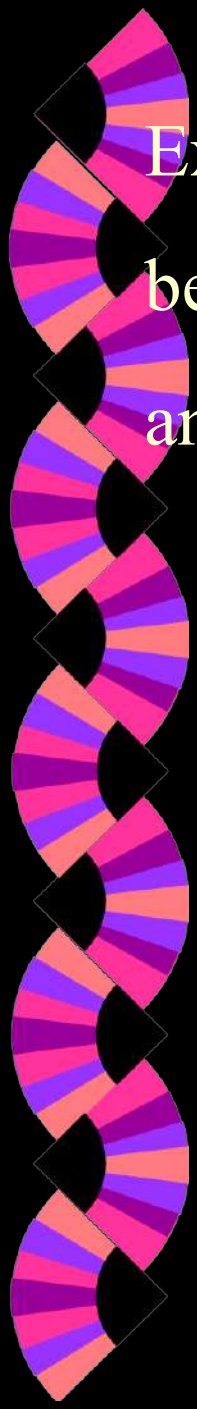
During the lag phase synthesis of RNA, enzymes and other molecules occurs.

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.


Lag phase
Log phase
Stationary phase
Death phase
1
2
3
4
Log of number of bacteria
0
5
10
Time (hr)

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.

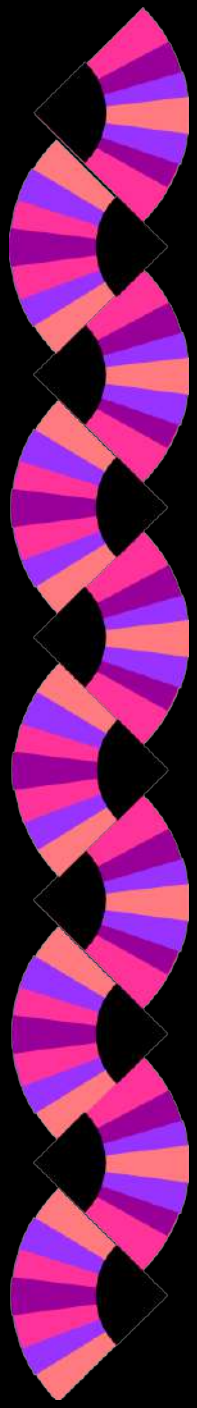
The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid.

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.

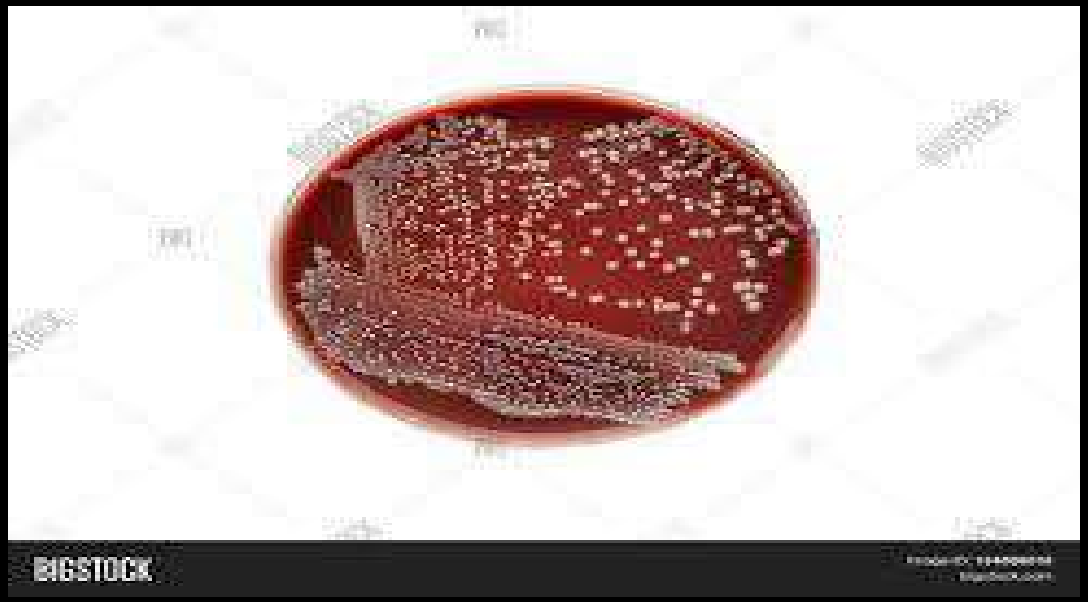


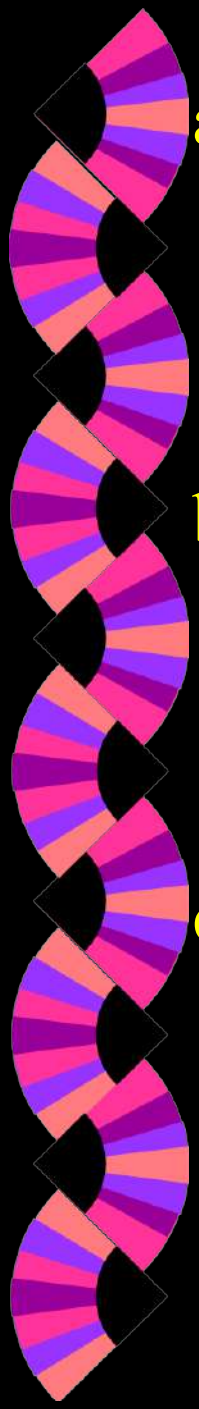
- **Media for bacterial growth**

•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


BIGSTOCK



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**

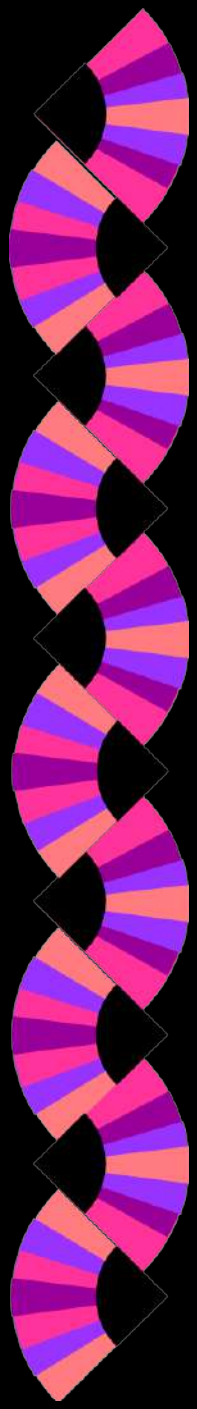
c. Selective media

- 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.

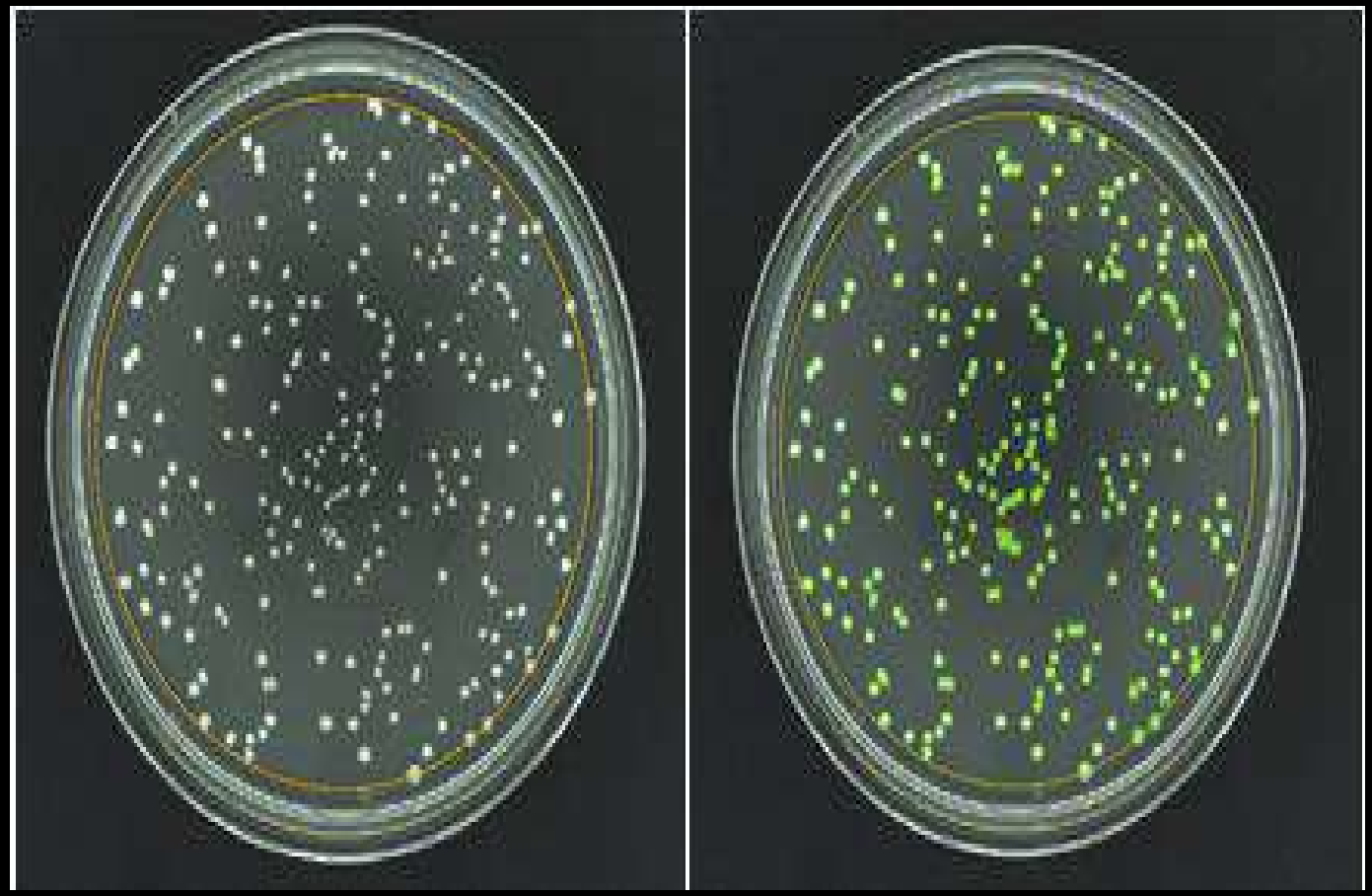
d. Differential media

- 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



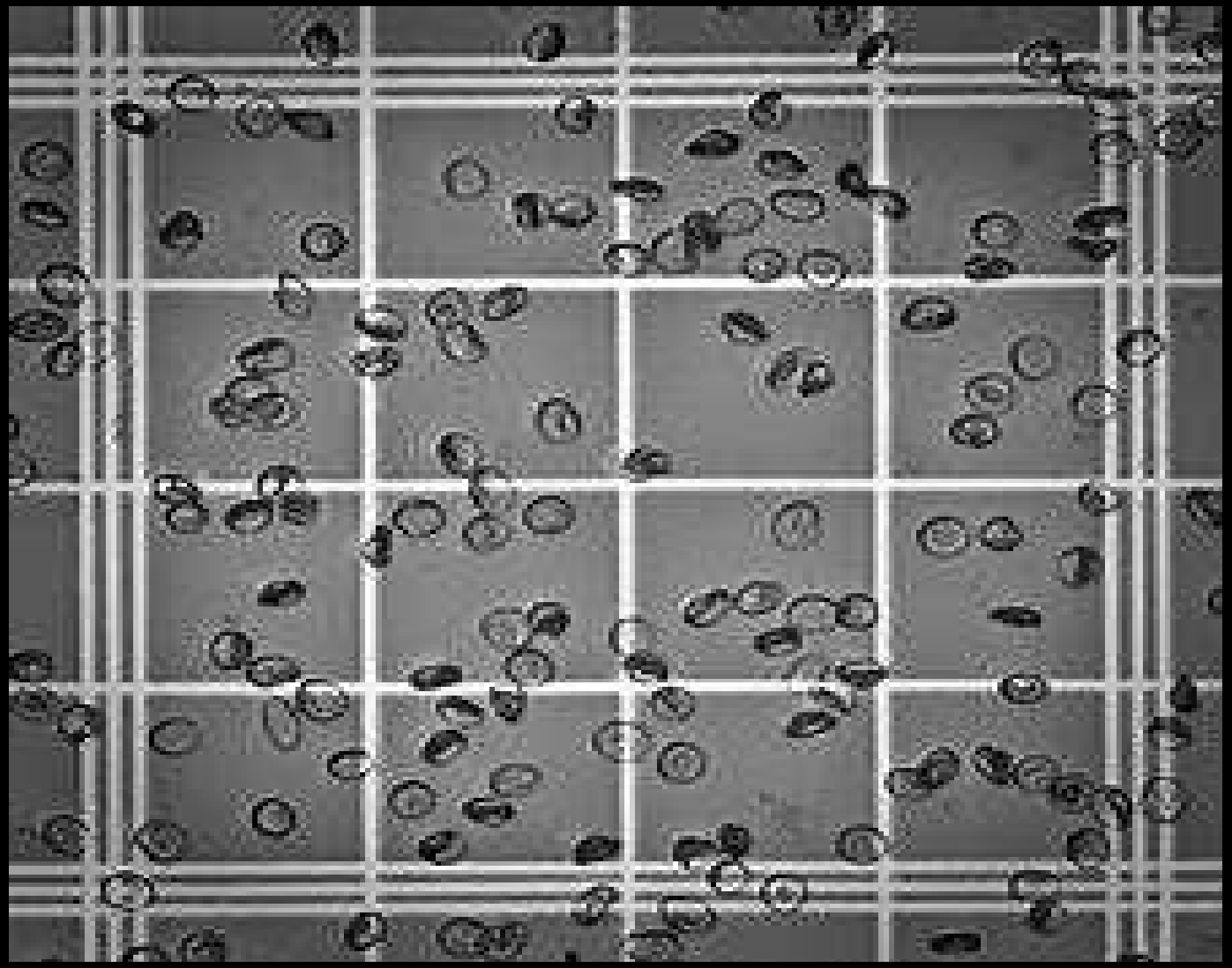
Methods used to measure microbial growth

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

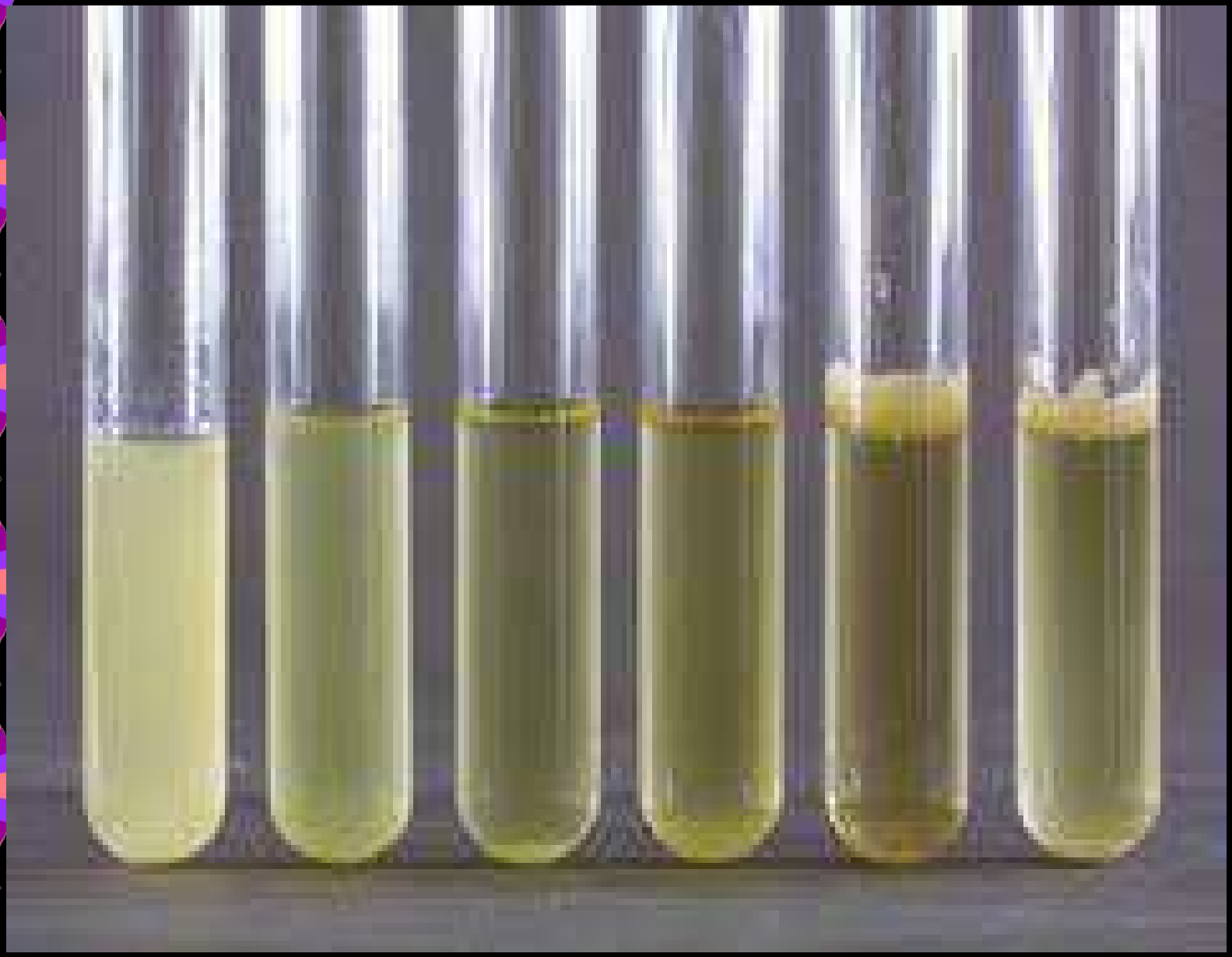


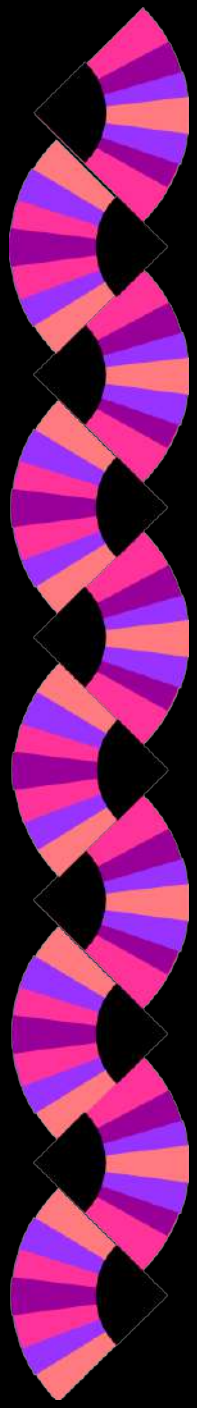
- Count colonies on plate (counts live cells)

- Microscopic counts



- Turbidity





Requirements for Growth

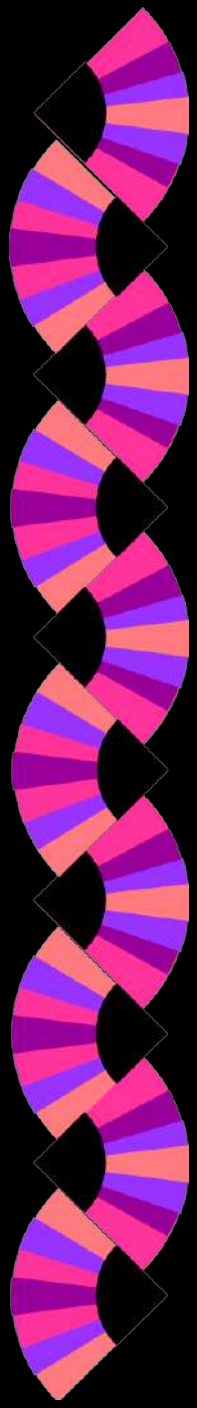
- Bacteria must obtain or synthesize Amino acids, Carbohydrates & Lipids => build up the cell.

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 (CO_2)
- **heterotrophic** – carbon is obtained from organic compounds
- **mixotrophic** – carbon is obtained from both organic compounds and CO_2

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. eque. from inorganic compounds and carbon from CO_2 . e.g.: Knallgas-bacteria

photolithoautotrophs obtain energy from light, reducing equivalents from inorganic compounds and carbon from CO_2. e.g.: Cyanobacteria

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

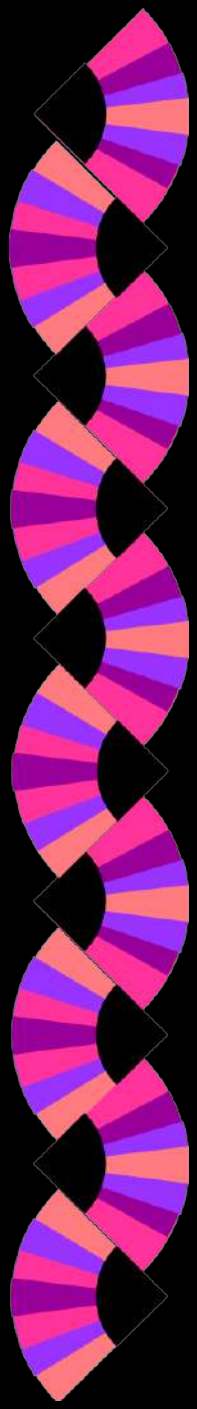
chemoorganoheterotrophs obtain energy, carbon, and reducing equivalents from organic compounds. e.g.: most bacteria, e. g. *Escherichia coli*

2.Temperature

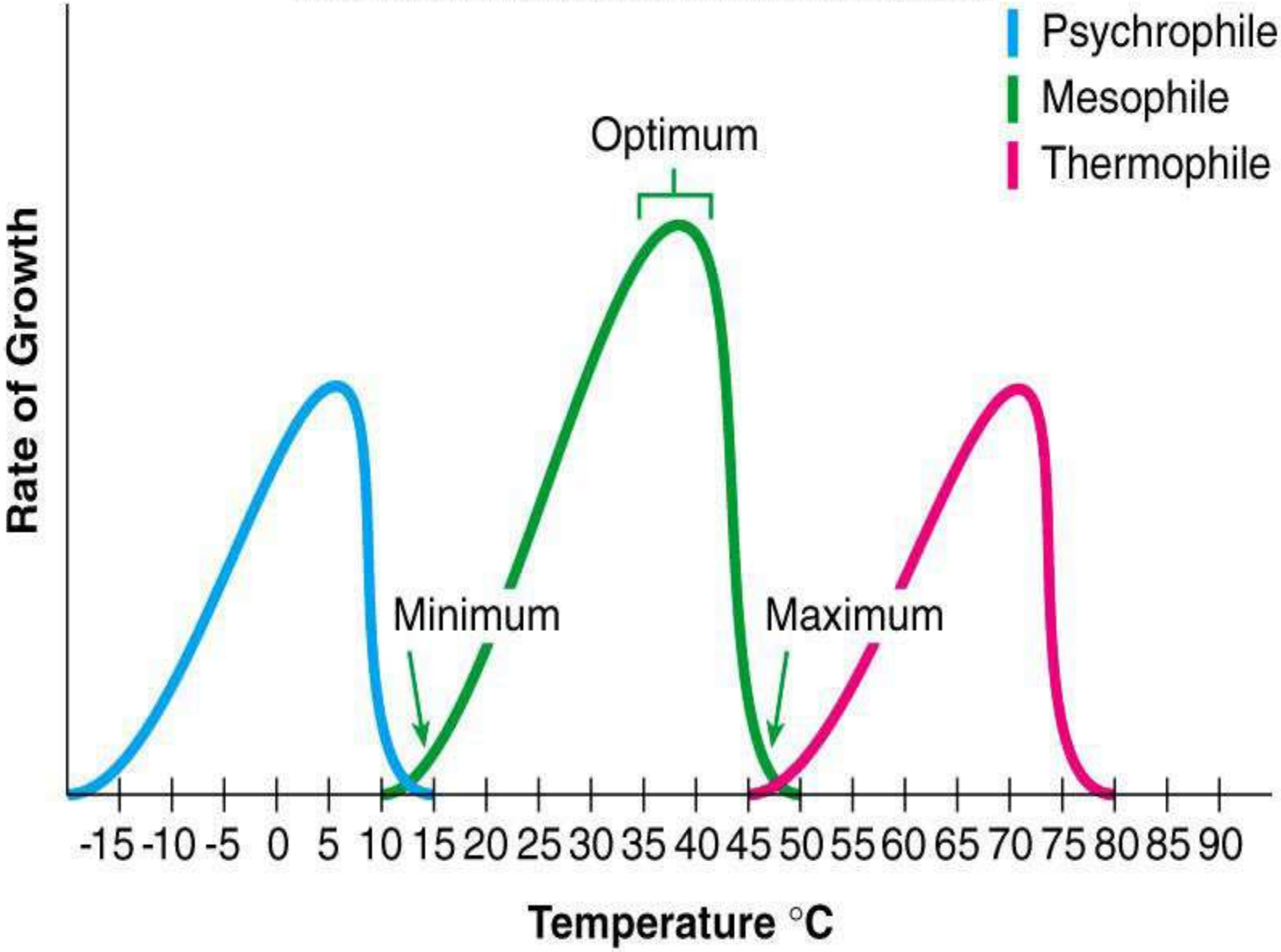
- Psychrophiles: cold-loving, can grow at 0 C.
- Mesophiles: moderate temperature-loving (Most bacteria)

- 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.



3.Oxygen

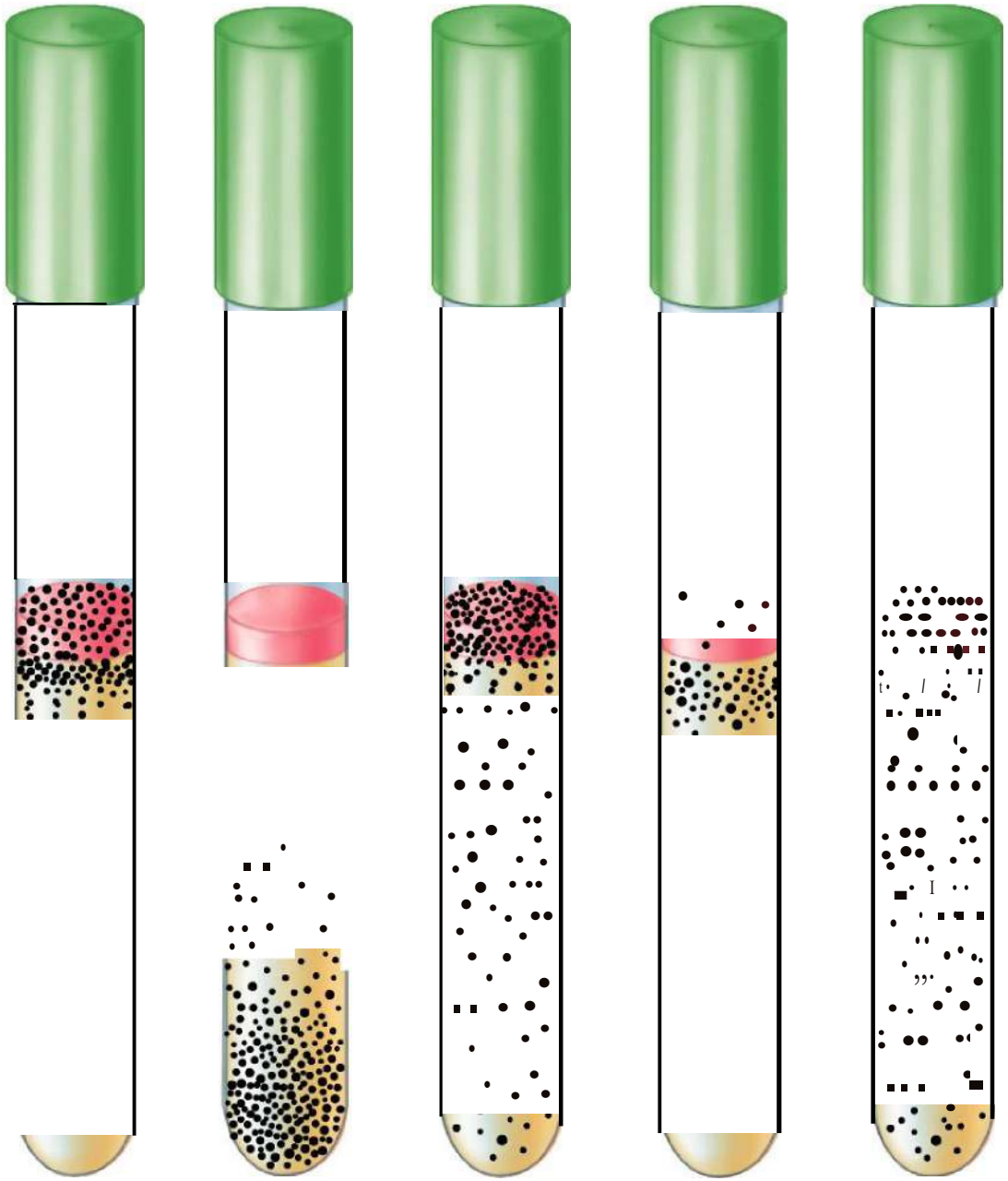
(a) Obligate aerobes – require O_2

(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.

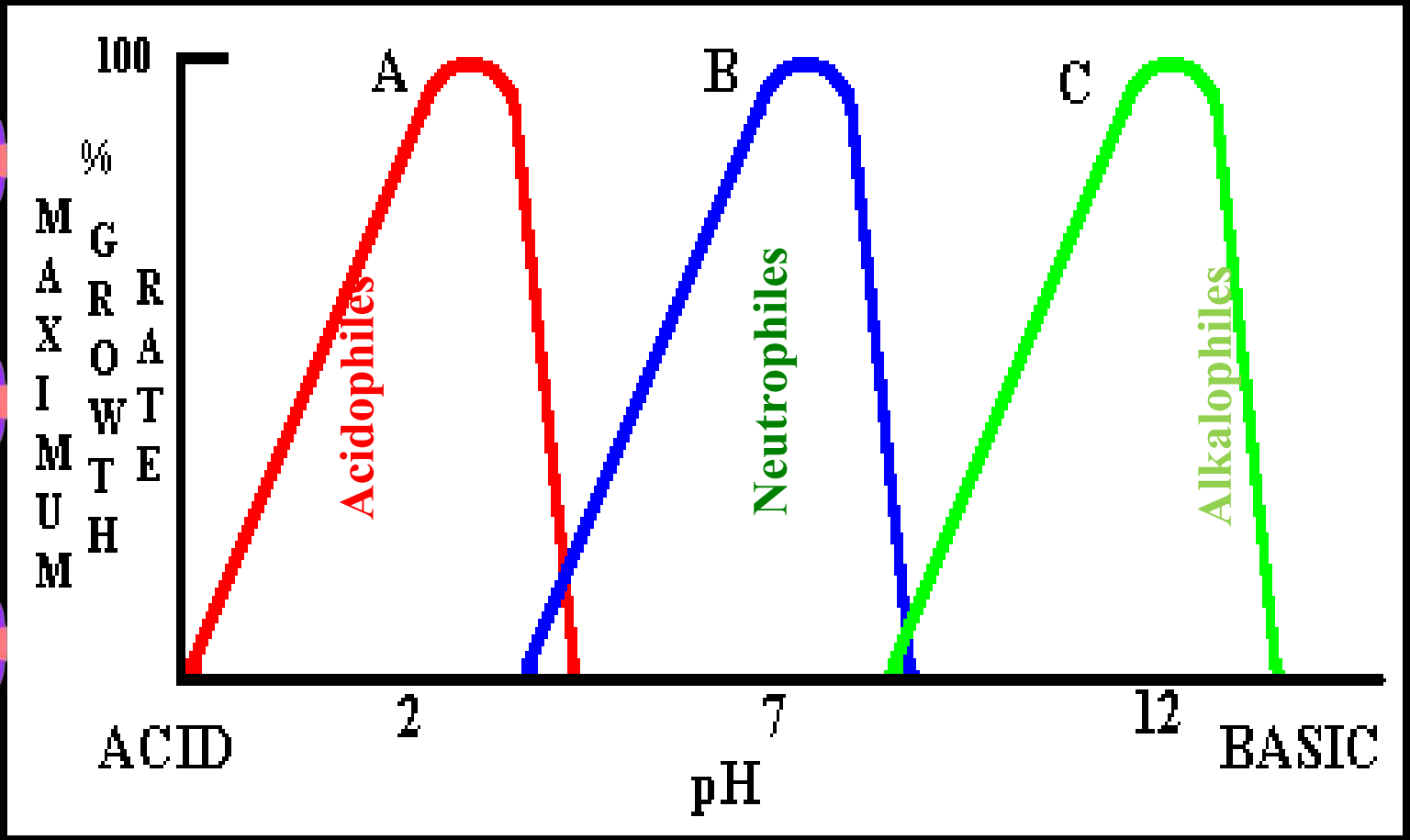
- **<u>Alkaliphiles</u>: “Alkali loving”.**

- 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

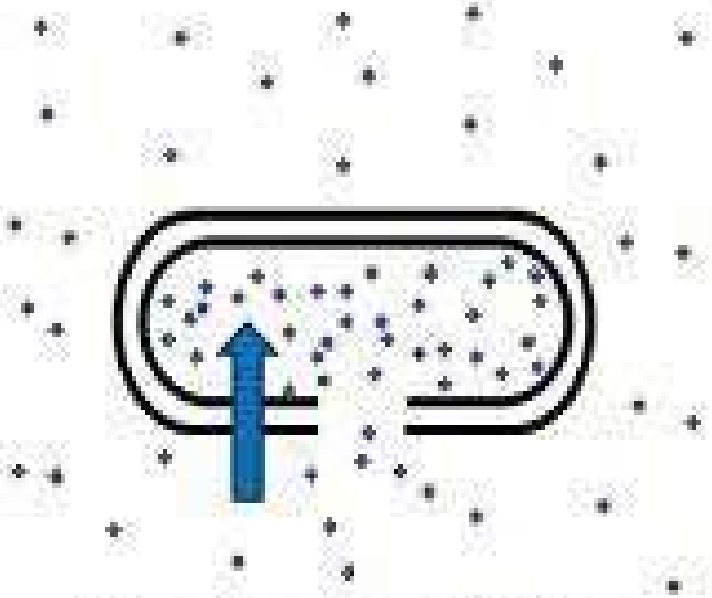


5. Osmotic Pressure

- 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.

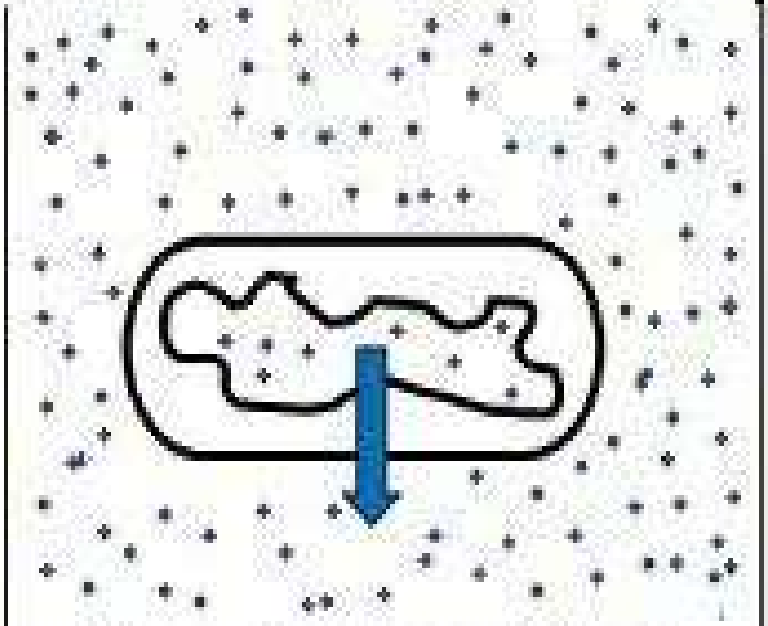
- Osmophilic bacteria requires high osmotic pressure. When the bacterial cell is subjected to the **hypertonic solution**, it may cause osmotic removal of water, resulting in **plasmolysis** or osmotic shrinkage of the protoplasm.
- In contrast, when the bacterial cell is subjected to the hypotonic solution,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

EFFECT OF OSMOTIC PRESSURE TO BACTERIAL CELL



Hypotonic solution

Causes osmotic cell lysis



Hypertonic solution

Causes osmotic plasmolysis

Metabolism

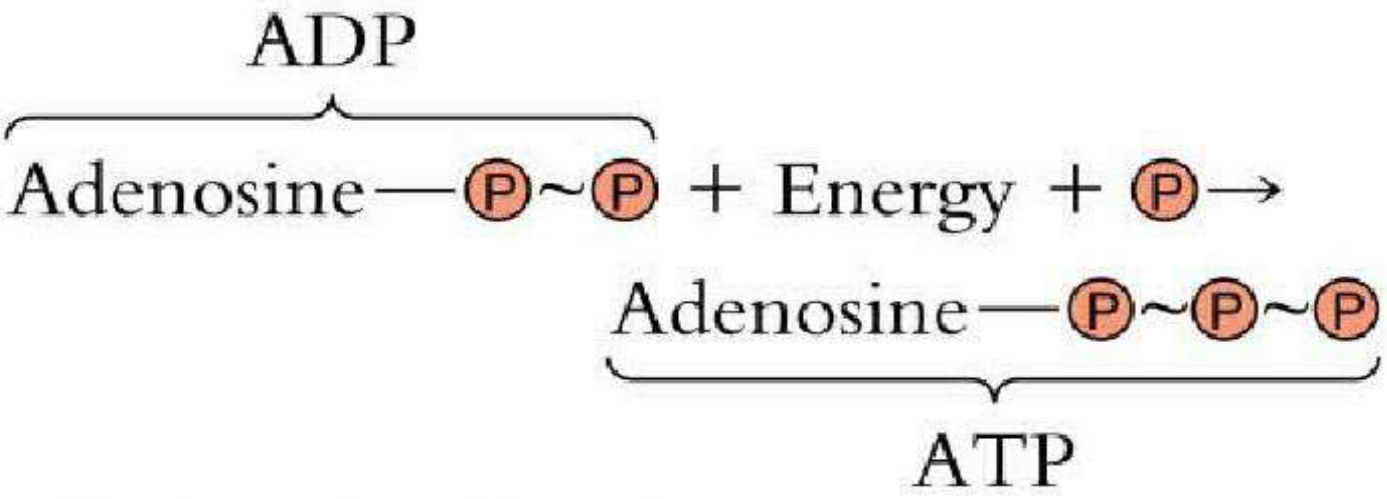
- Sum up all the chemical processes that occur within a cell

1. Anabolism: Synthesis of more complex compounds and use of energy
2. Catabolism: Break down a substrate and capture energy for growth and maintenance.

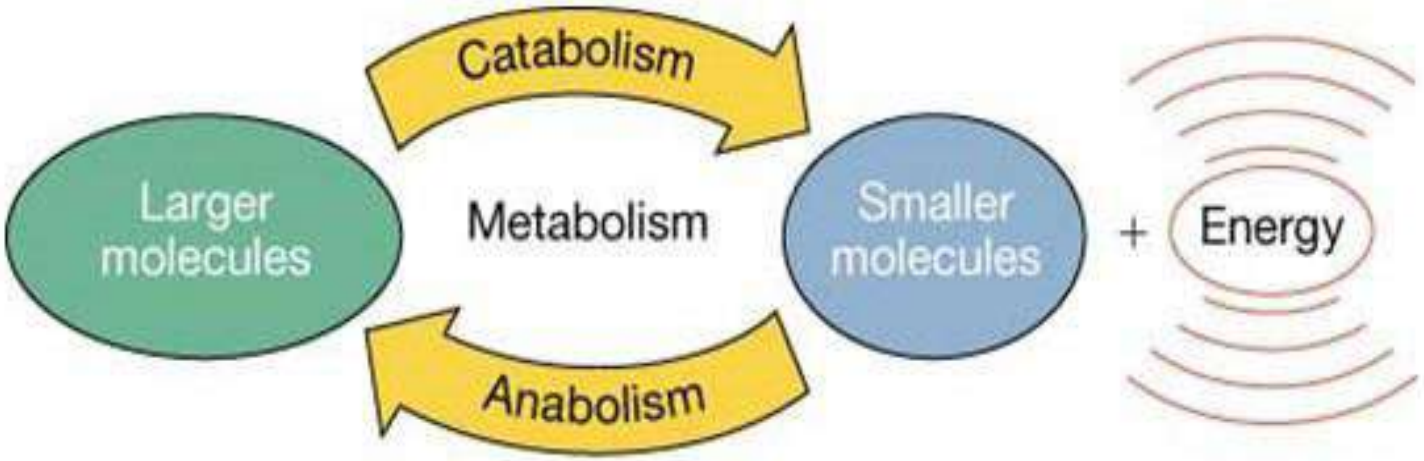
- All cells require the energy supply to survive. The common energy form => ATP (Adenosine Tri-Phosphate)

ATP

is generated by the phosphorylation of ADP



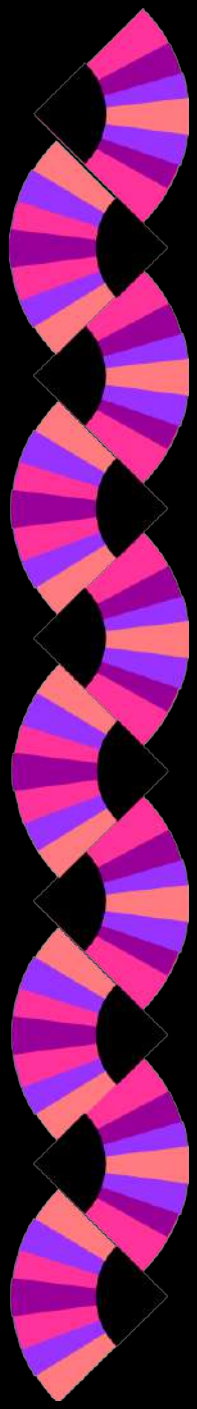
Metabolism Relationships



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.



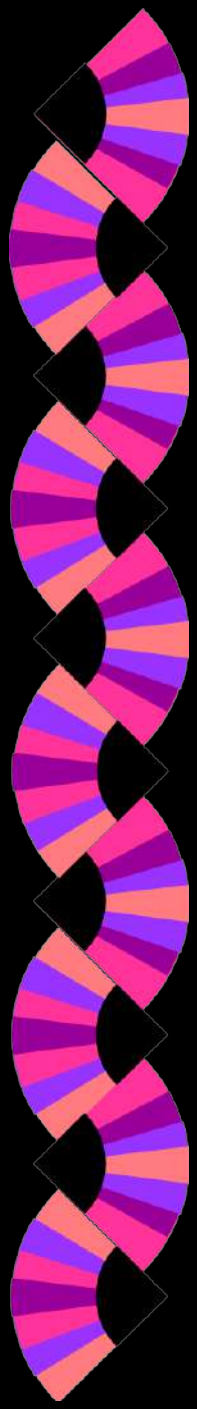
Metabolism of Glucose

- 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

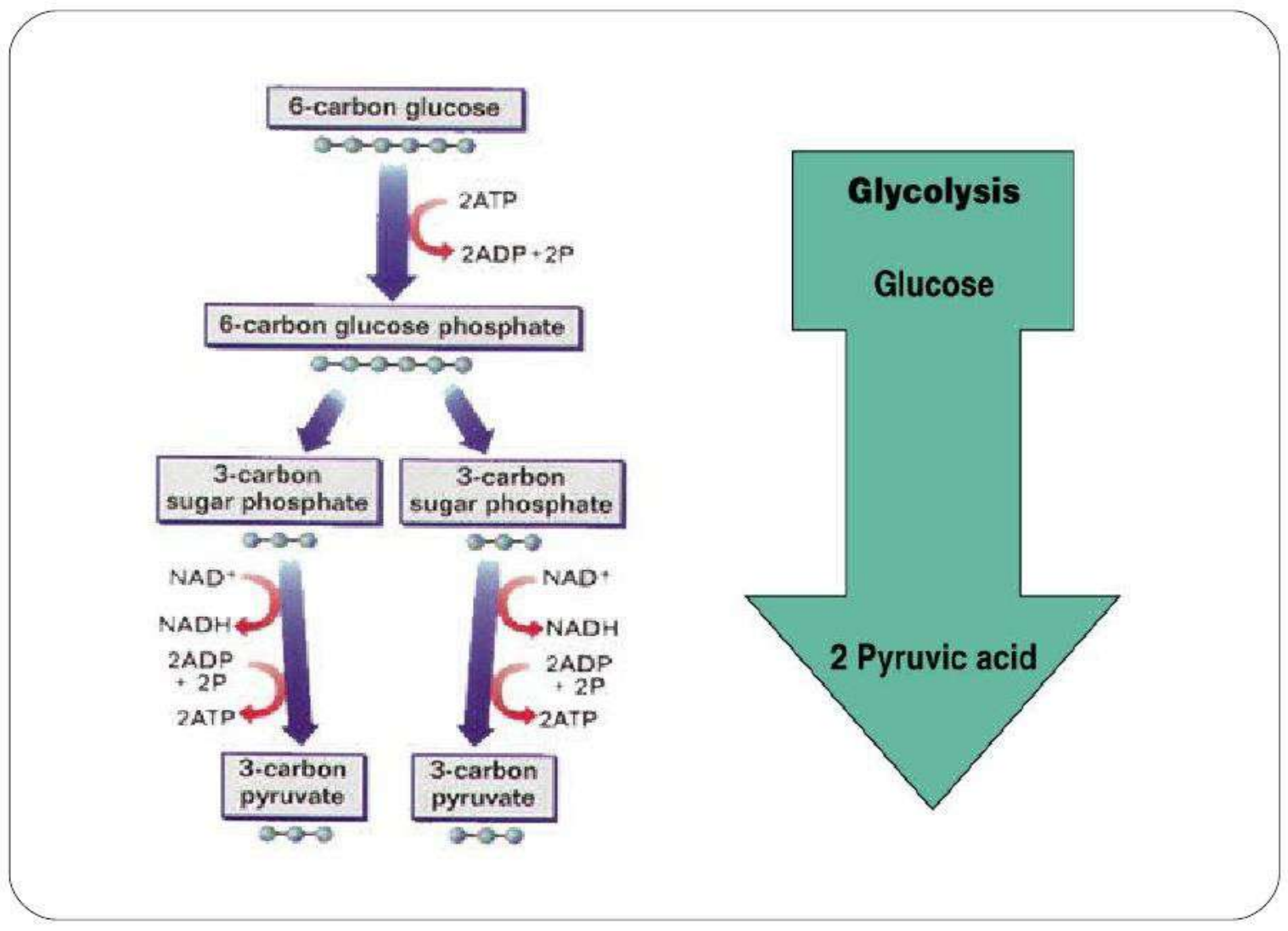
- Glycolytic pathway, the Embden-Meyerhof-Parnas pathway.

- 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 prokaryptic and eukaryotic cells.



Metabolism of Glucose

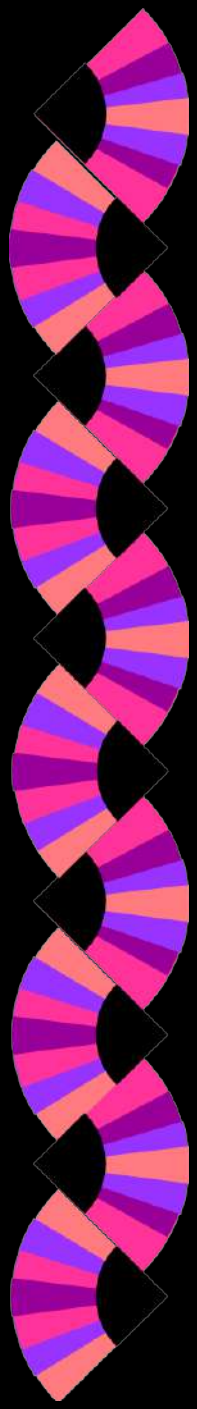
$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 38$ ATP

-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

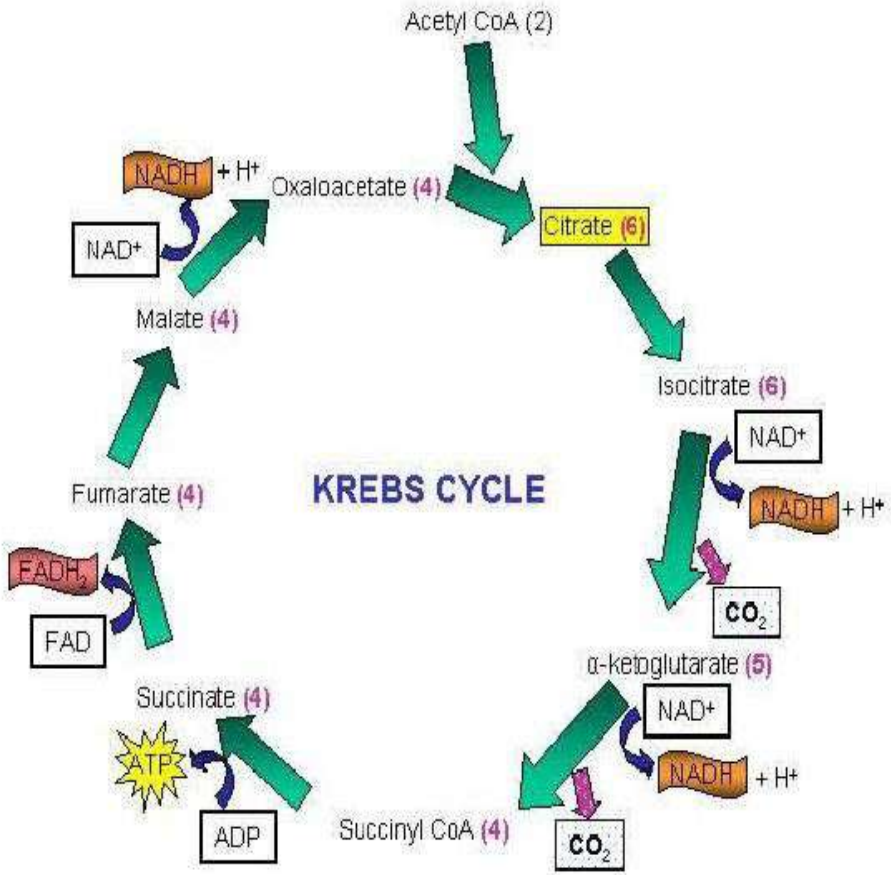
- The pyruvic acid produced during glycolysis are converted into acetyl-CoA.

-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, FADH 2 and H ions

Mitochondria (eukaryotes); cell membrane (prokaryotes).


Acetyl CoA (2)
Citrate (6)
Isocitrate (6)
NAD+
NADH + H+
CO2
α-ketoglutarate (5)
NAD+
NADH + H+
CO2
Succinyl CoA (4)
Succinate (4)
ATP
ADP
Fumarate (4)
FADH2
FAD
Malate (4)
NADH + H+
NAD+
Oxaloacetate (4)
KREBS CYCLE

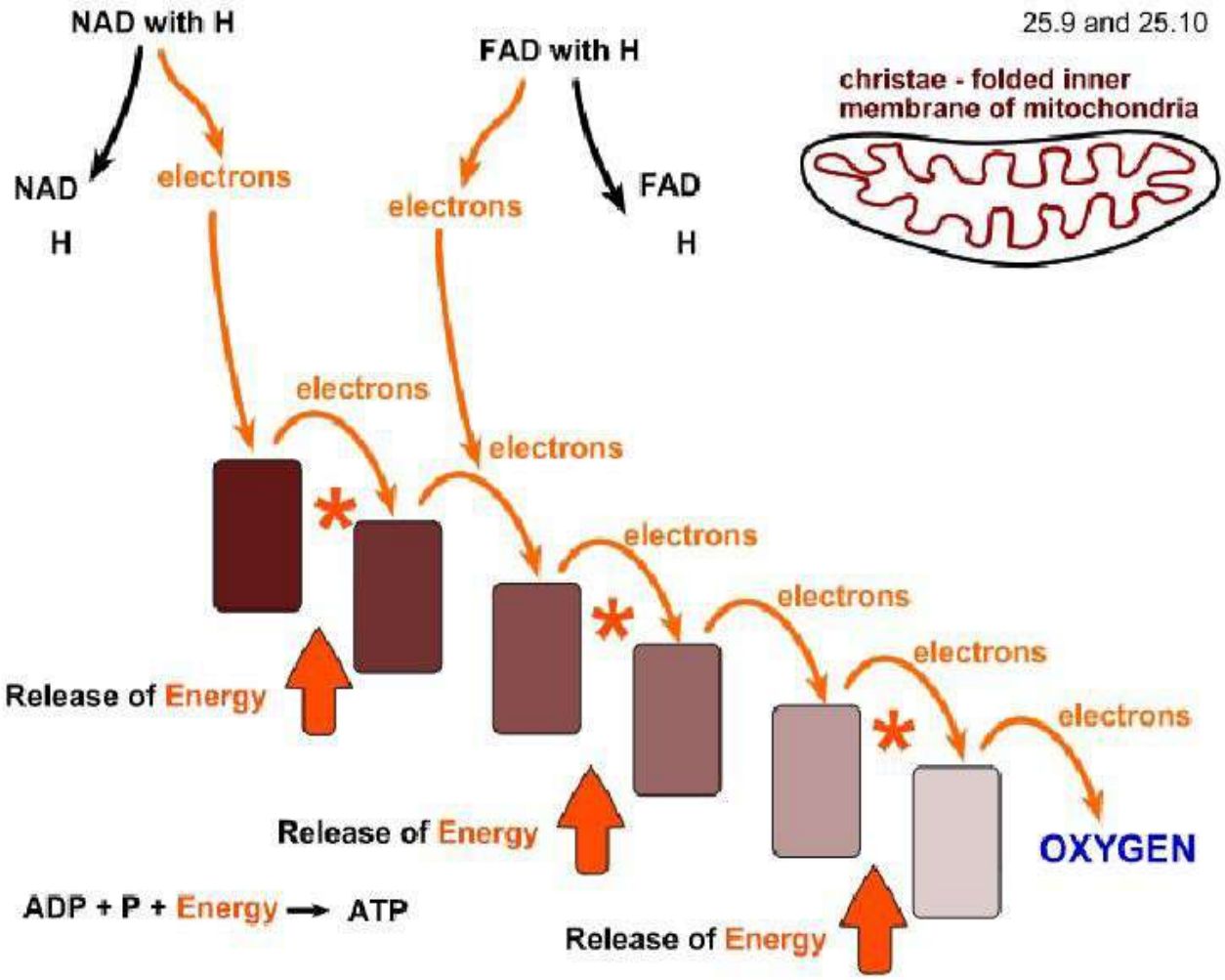
Electron Transport Chain

- 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.


NAD with H
FAD with H
25.9 and 25.10
christae - folded inner membrane of mitochondria
NAD
H
electrons
electrons
FAD
H
electrons
electrons
electrons
electrons
electrons
electrons
Release of Energy
Release of Energy
Release of Energy
OXYGEN
ADP + P + Energy → ATP

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

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

Oral Microbiology

ام د جتين عزالدين علي

ا.د هديل مزهر يونس

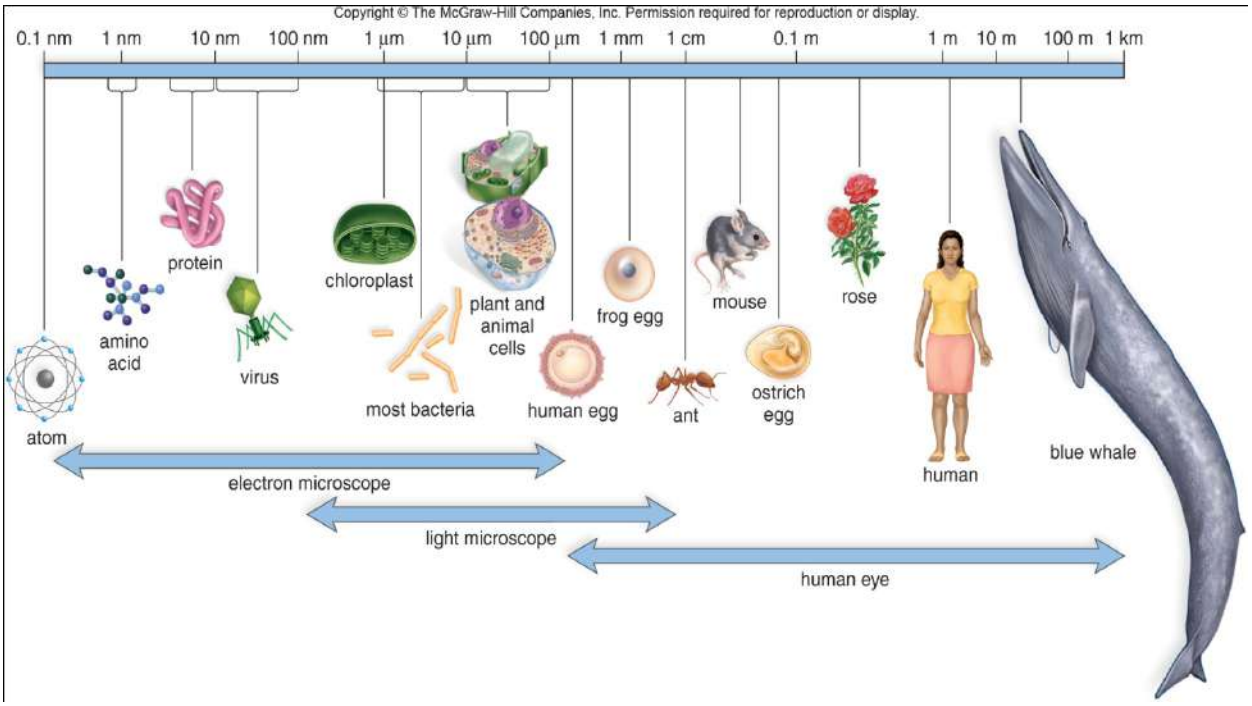
Introduction to microbiology

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 lm 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.
- Thy 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.

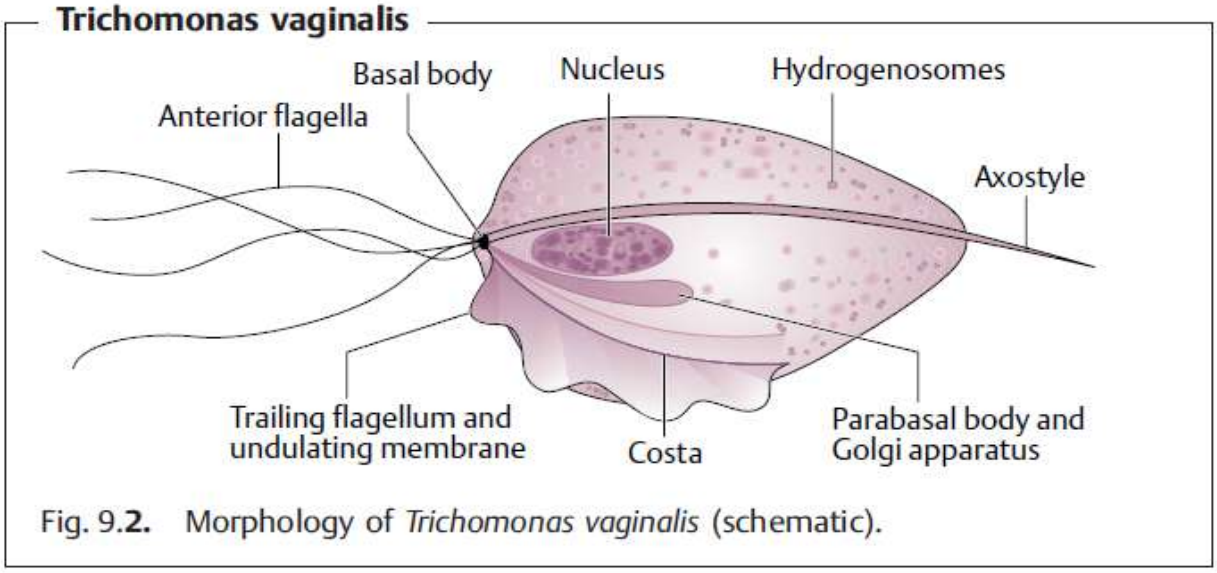
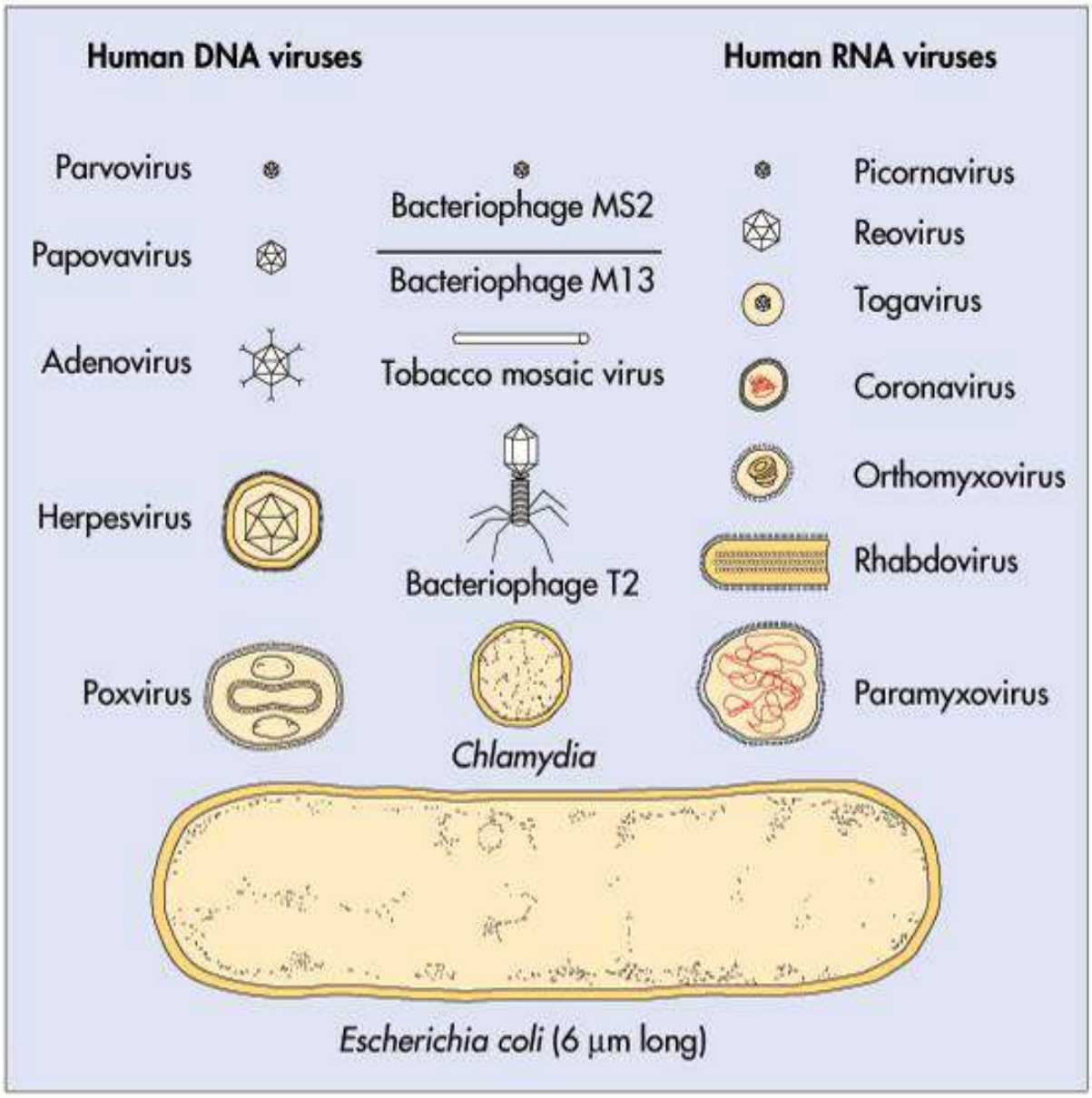
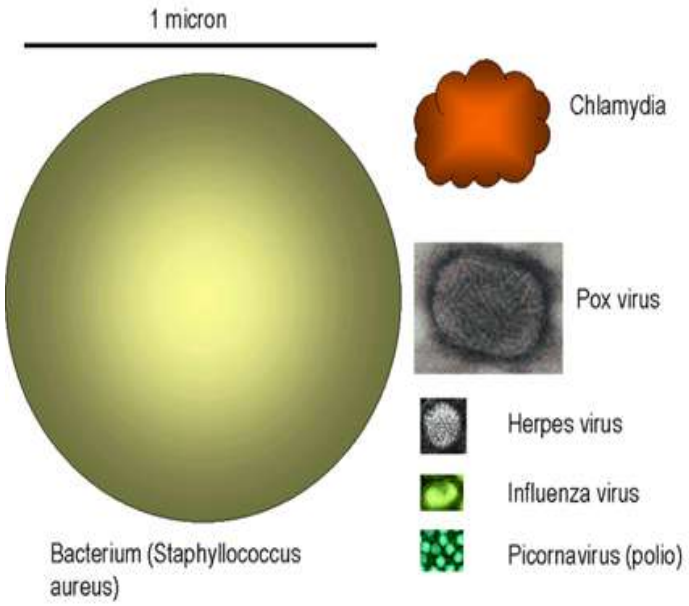


Fig. 9.2. Morphology of *Trichomonas vaginalis* (schematic).

- 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.

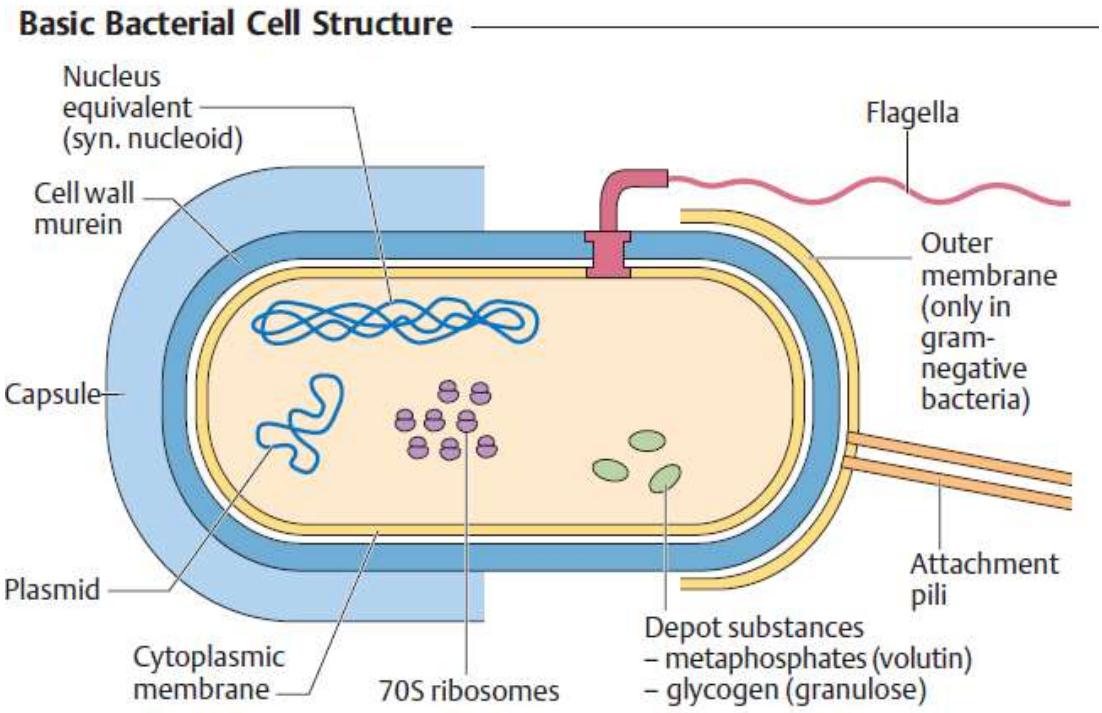
Viruses : are obligate intracellular parasites


Human DNA viruses
Human RNA viruses
Parvovirus
Papovavirus
Adenovirus
Herpesvirus
Poxvirus
Bacteriophage MS2
Bacteriophage M13
Tobacco mosaic virus
Bacteriophage T2
Chlamydia
Picornavirus
Reovirus
Togavirus
Coronavirus
Orthomyxovirus
Rhabdovirus
Paramyxovirus
Escherichia coli (6 μm long)



Bacterial Structure

Essential and Particular bacterial structures



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

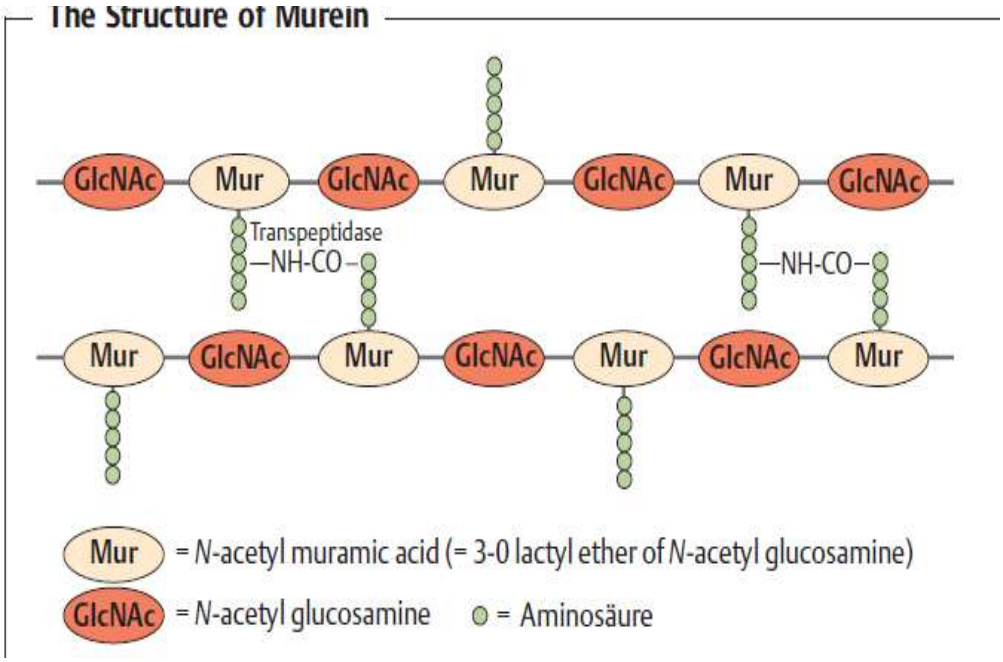
4- to facilitate communication with its surroundings.

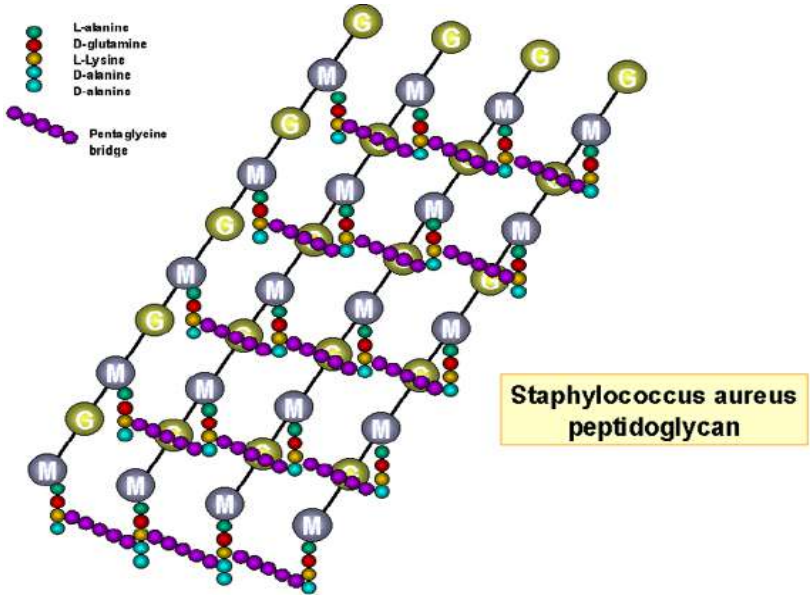
peptidoglycan

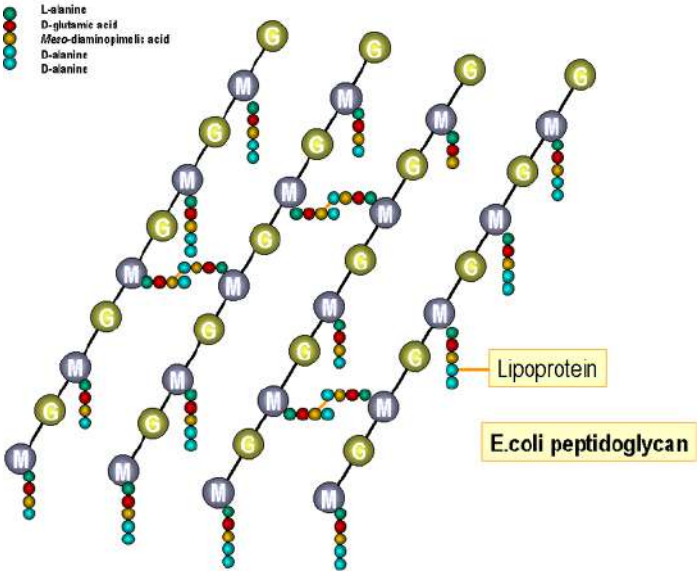
Murein (syn. peptidoglycan). The most important structural element of the wall is murein, a netlike polymer material surrounding the entire cell . It is made up of polysaccharide chains crosslinked by peptides

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-acetyl-muramic 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



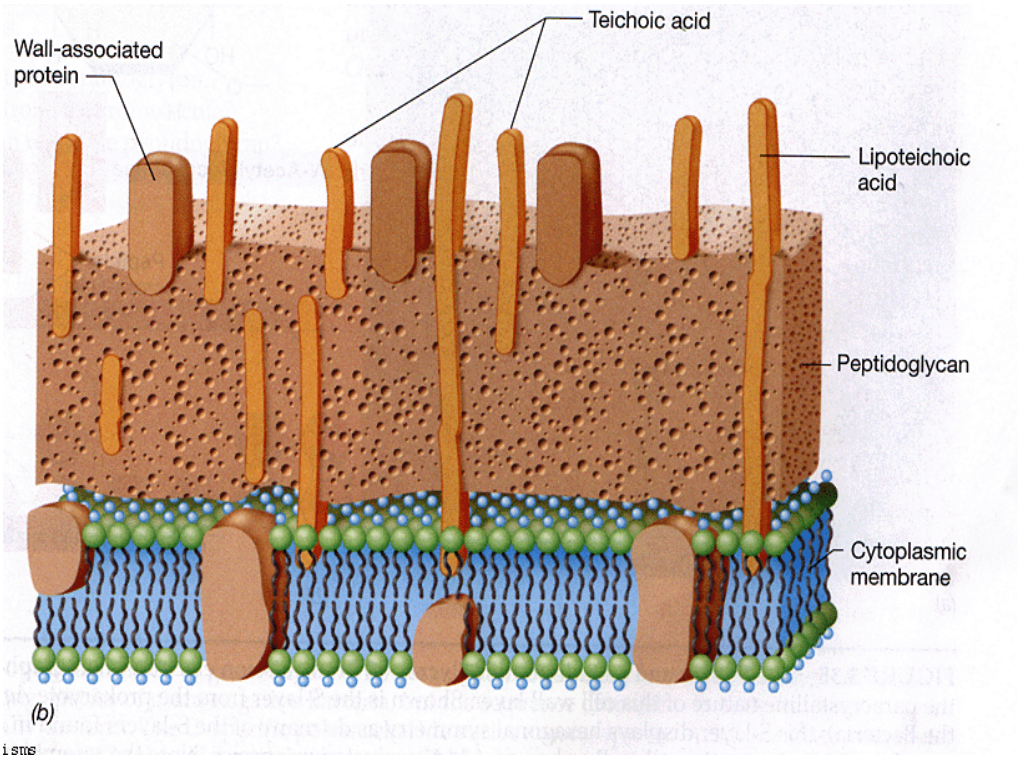




Special components of Gram positive cell wall

Teichoic acid

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



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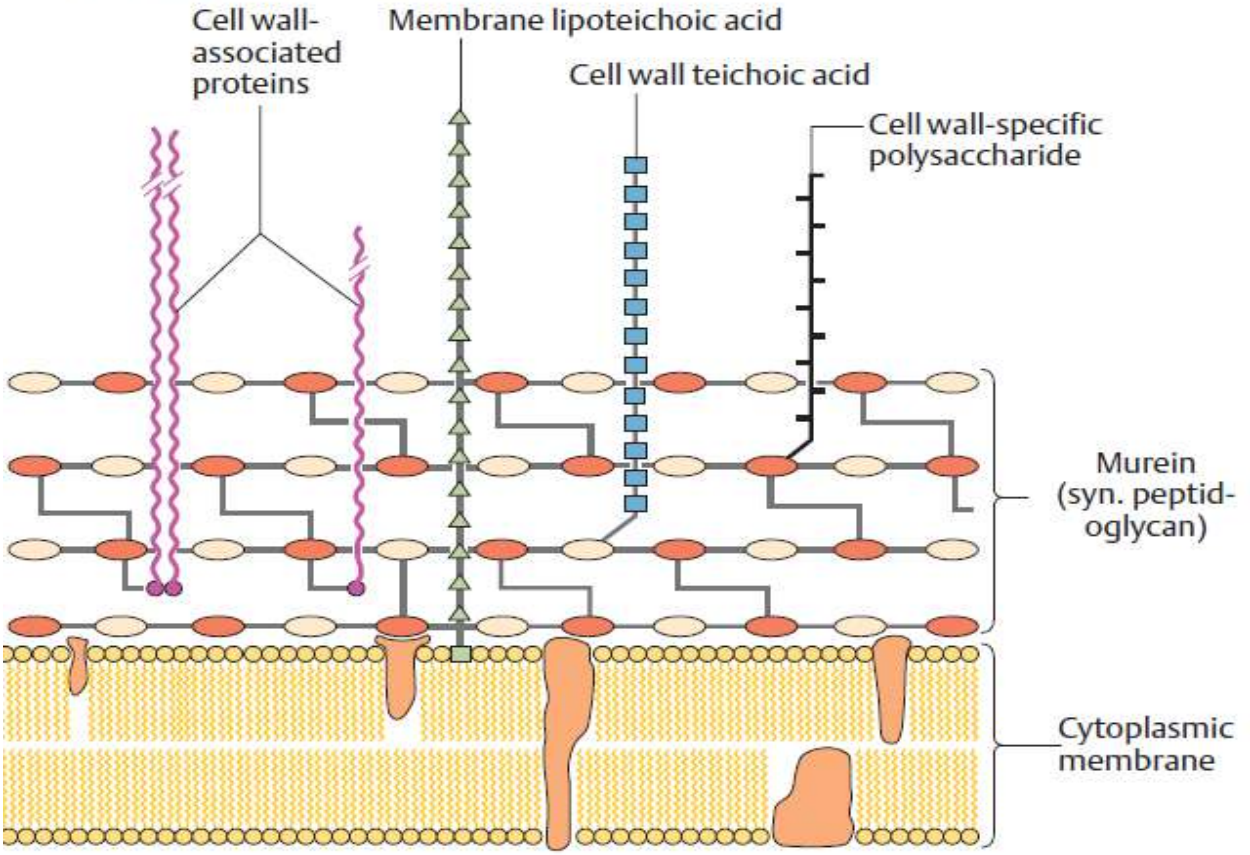
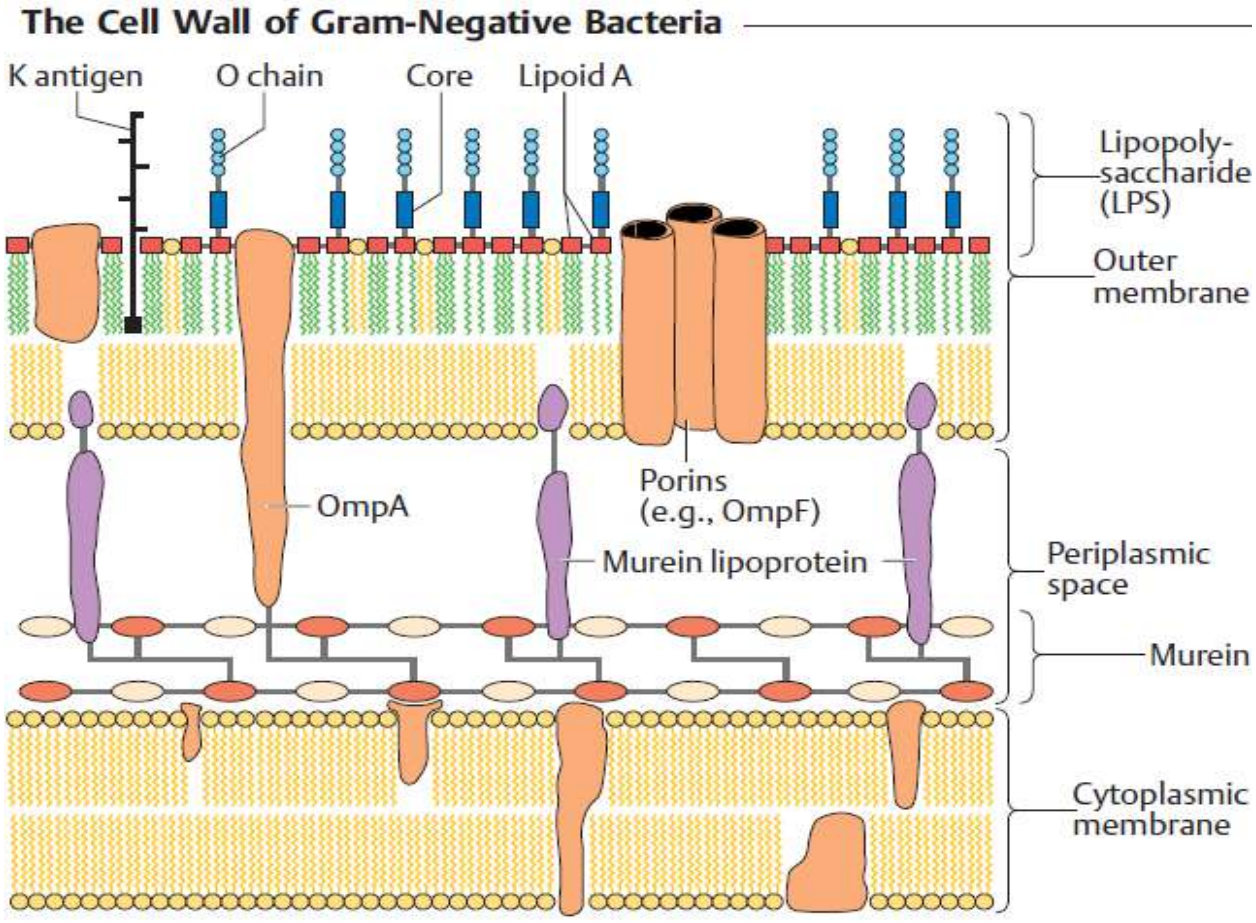
The Cell Wall of Gram-Positive Bacteria



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)



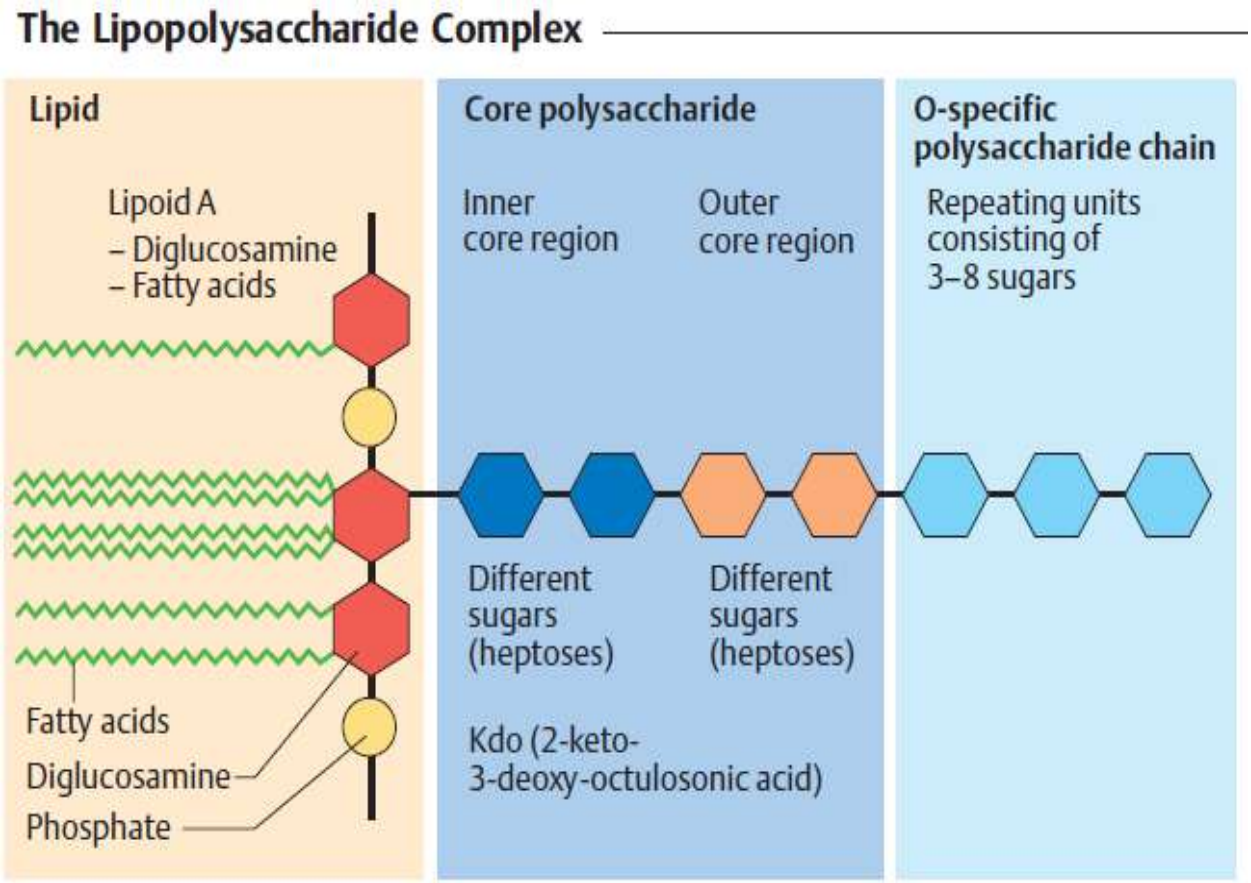
Special components of Gram negative cell wall

- Lipopolysaccharide (LPS).
- This molecular complex, also known as endotoxin,is comprised of the

  1- lipoid A
  2- the core polysaccharide

3- and the O-specific polysaccharide chain

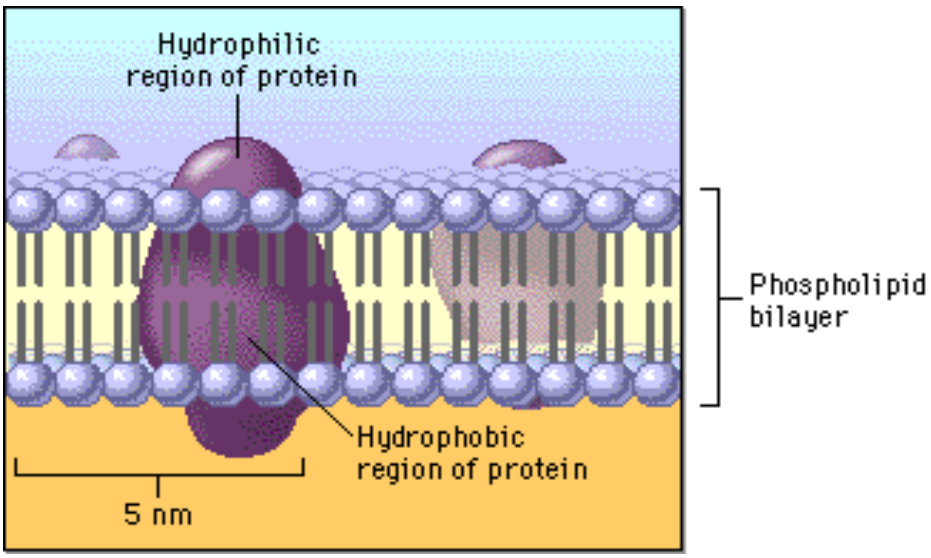
Lipid A is responsible for toxic effect

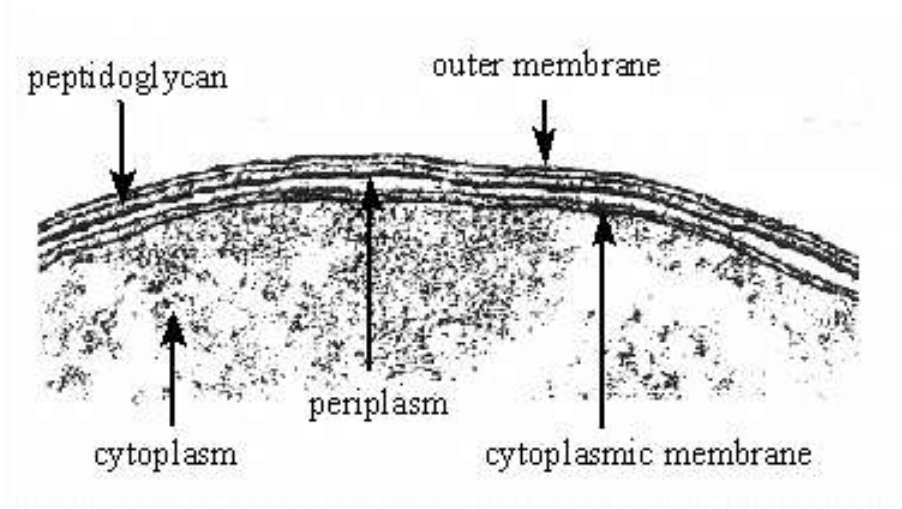


Cell membrane

Is a phospholipid bilayer locates beneath the cell wall . The function are :

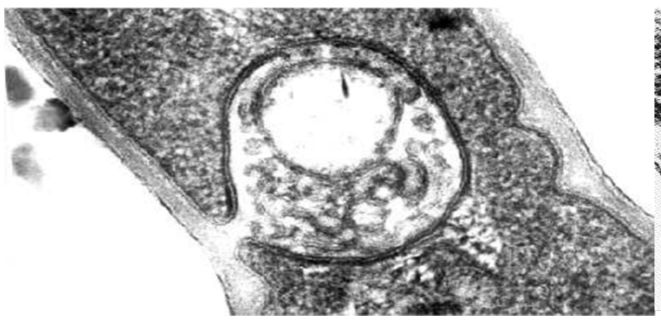
- Site of biosynthesis of DNA, cell wall polymers and membrane lipids.
- Site of Selective permeability and transport of solutes into cells
- Electron transport and oxidative phosphorylation
- Excretion of hydrolytic exoenzymes

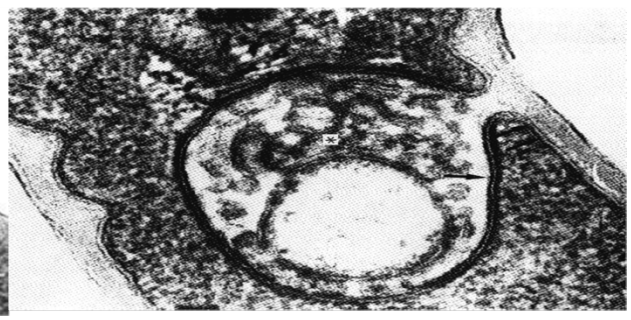




Mesosomes

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



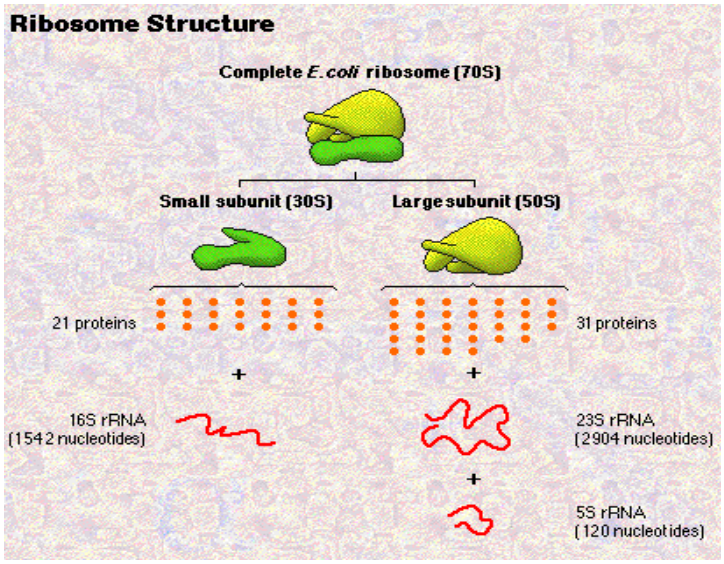


Cytoplasm

- It is the fluid fase 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

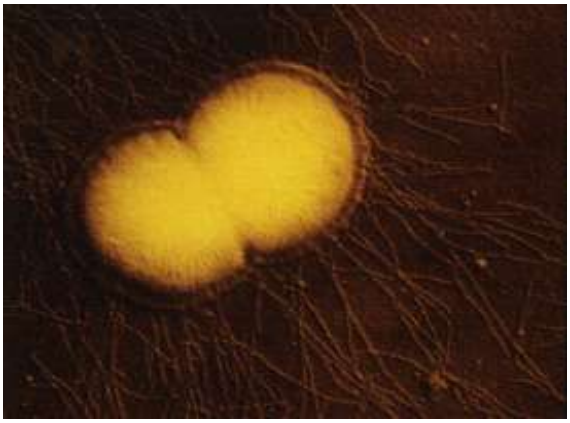
Pili

- 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 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. aquatics, a bacteria that live in hot springs)

3-deoxyribonuleoside 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 94^0c (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 ^{0}c)
4- The DNA polymerase enzyme (Taq polymerase and the nucleotide are added to the primed template DNA and incubated at 72 ^{0} c for synthesis of new complementary strands or amplicons (synthesis step)
5- The mixture is again heated to 94 0c 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.

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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 phenylanine, 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 cholorea)

 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:

 Gonorrhea(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 caused 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-Oppertunistic 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 pathogincity (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 cholerea 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(invassivenss)

 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 Membranebrane--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, gonorrhea)

 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/typus 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 aeroginosa ,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 thrue 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 aeroginosa .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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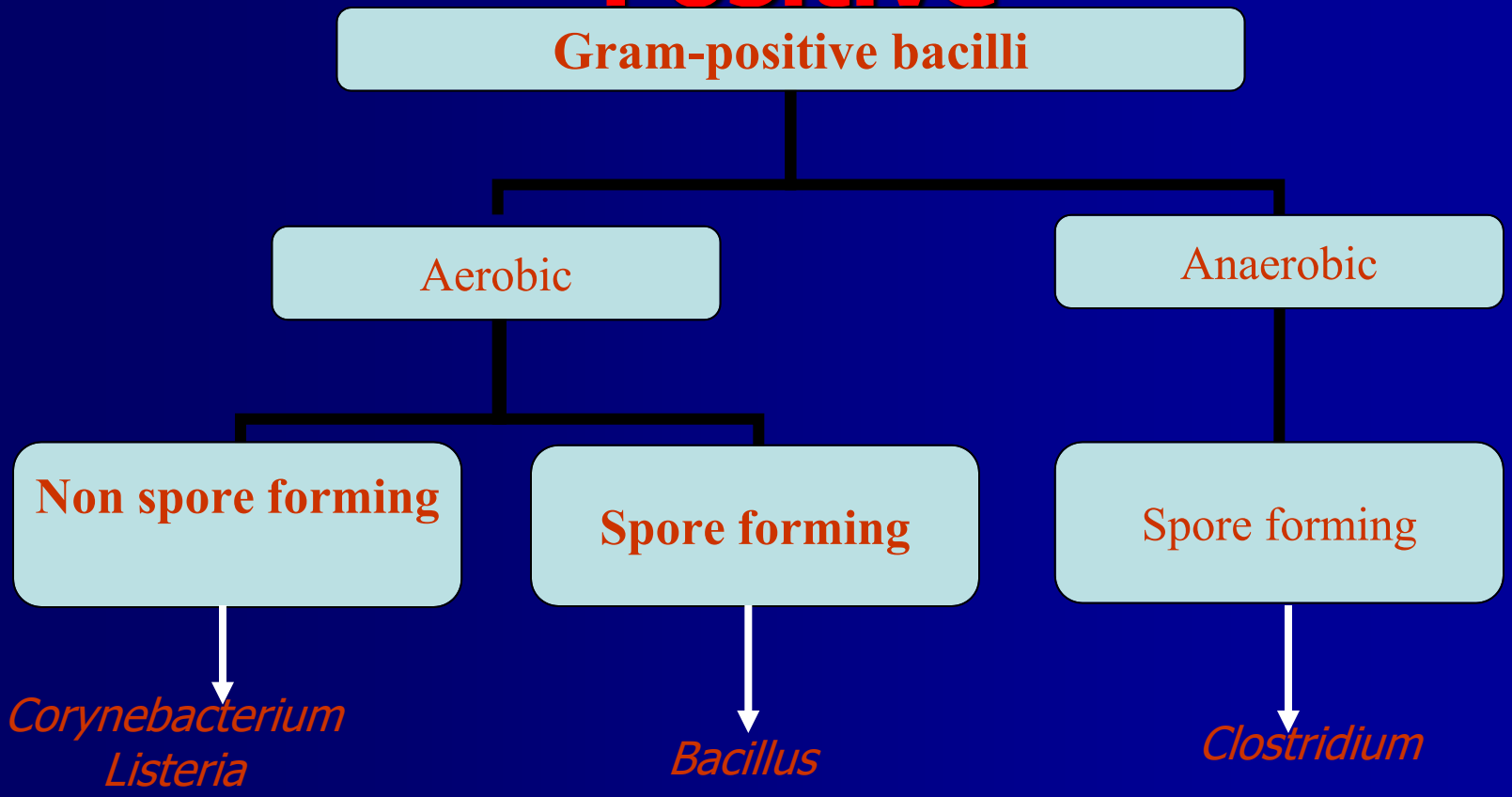
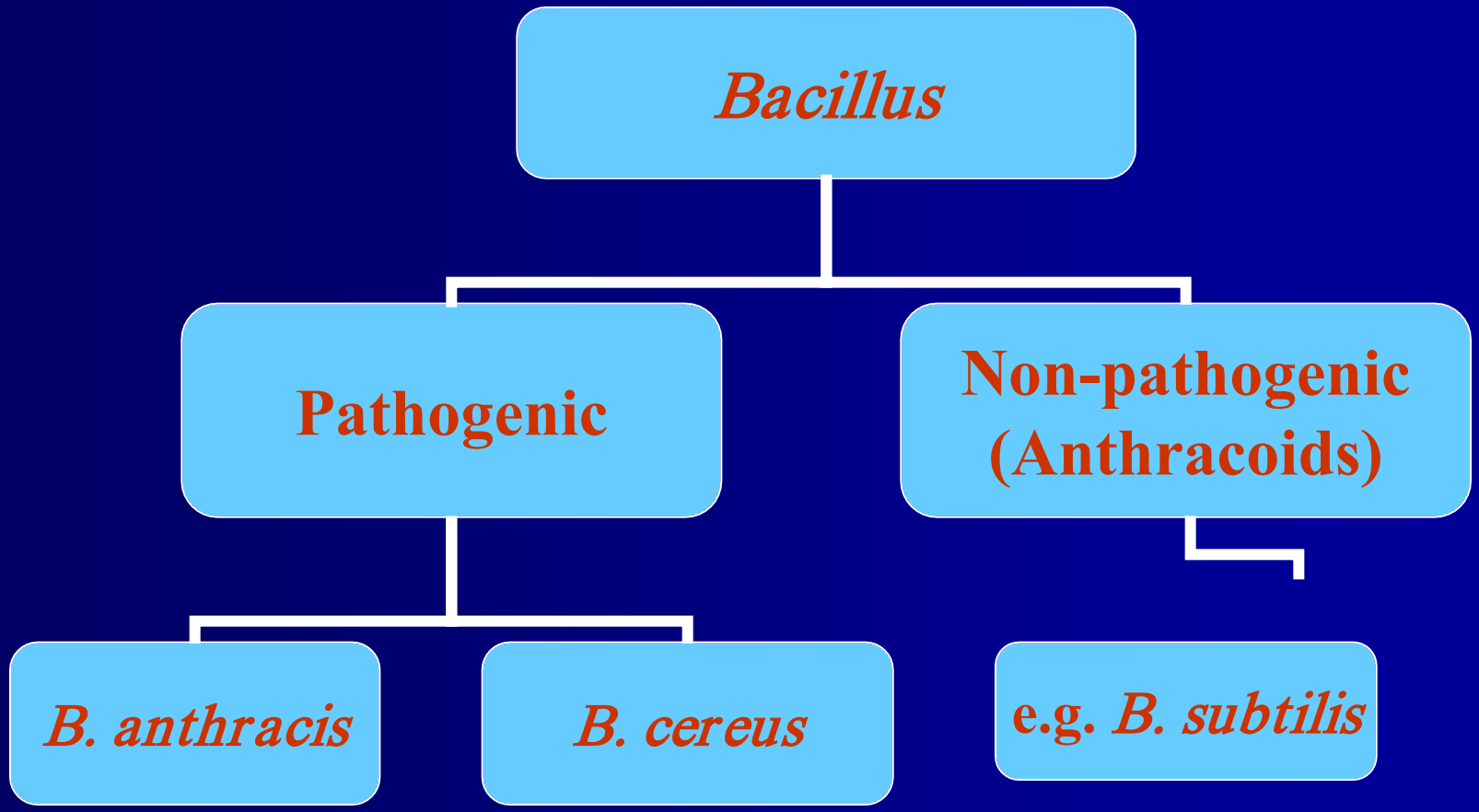
Classification of Gram-Positive

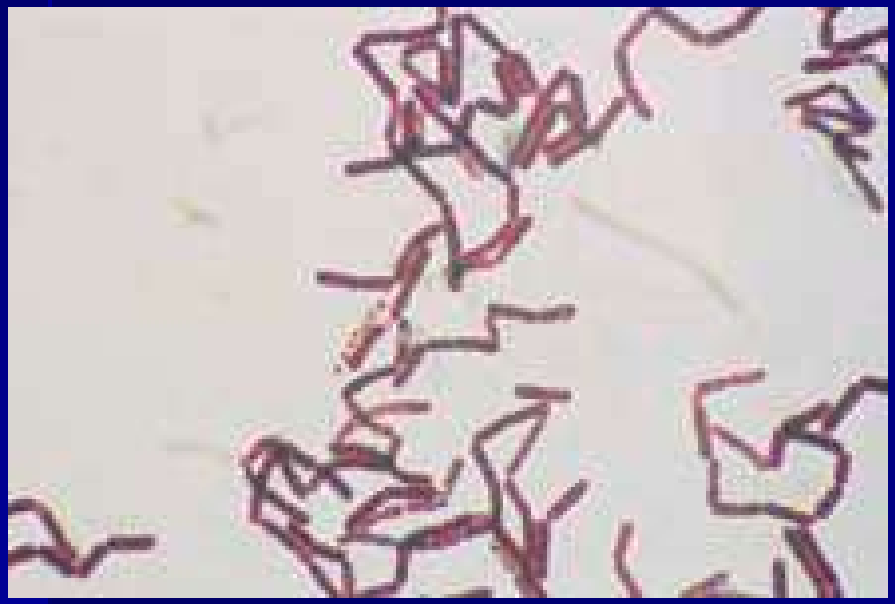


Aerobic Spore Forming
Bacillus spp



Bacillus Species: General Characteristics

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

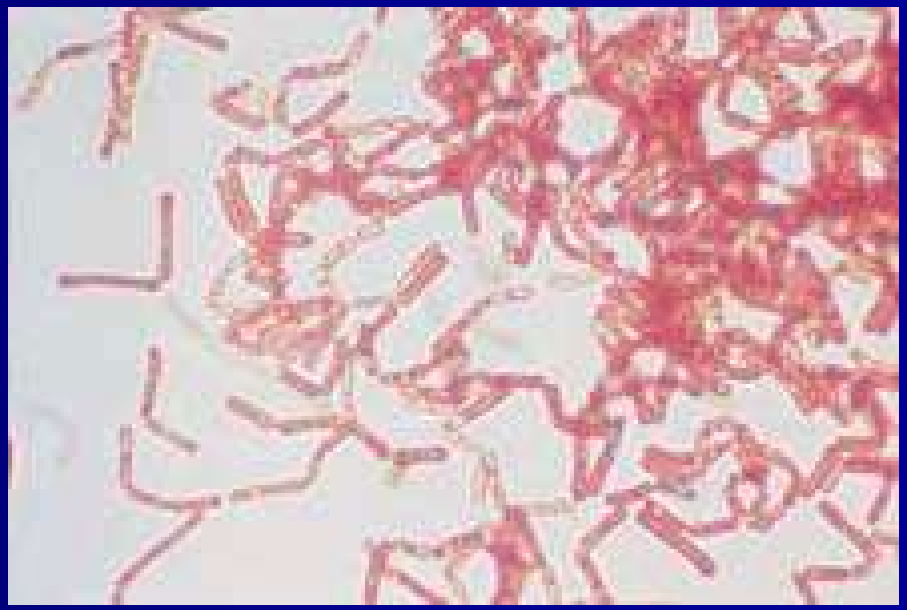


Bacillus sp.

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*

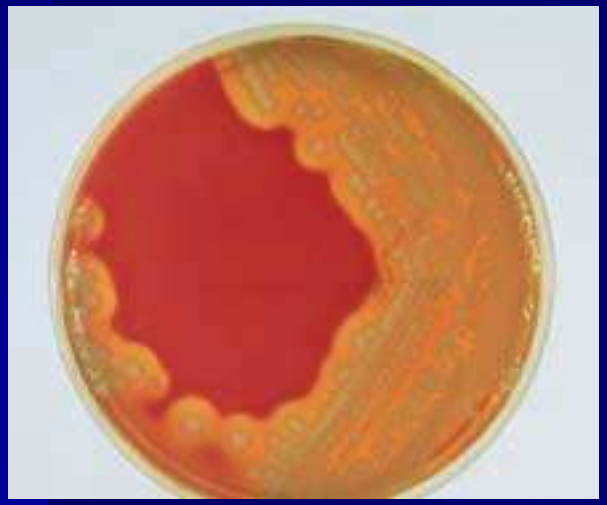
- Very large Gram positive bacilli
- 1-1.2 µm in width x 3-5µm in length
- Arranged in long chains
- Motile **except** *B. anthracis*
- Spore forming (outside the host)
- Capsulated (inside the host)
- Non Fastidious
- Facultative anaerobic
- Breakdown glucose by oxidative and fermentative i.e. O+/F+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

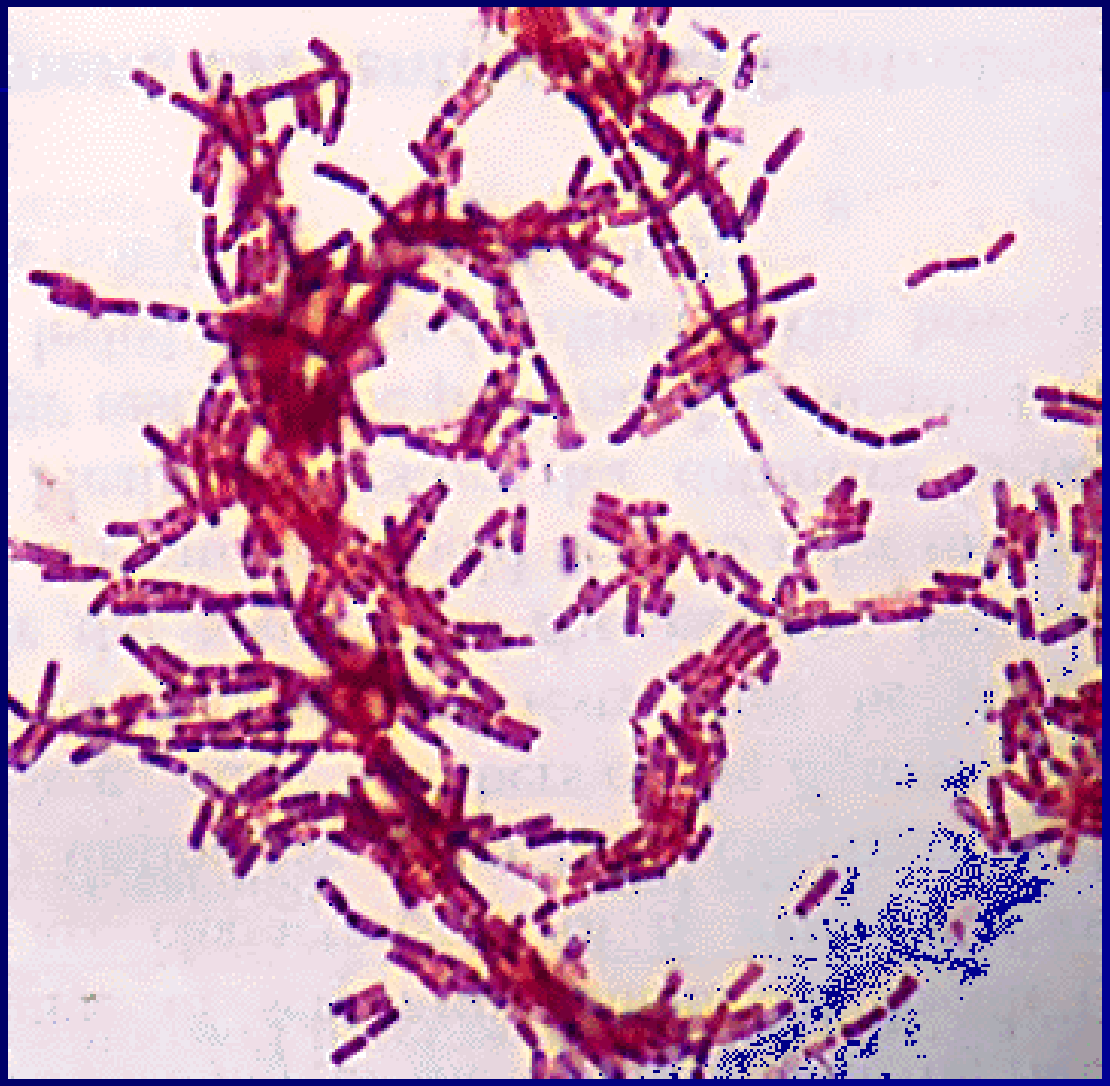
Other *Bacillus* species

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

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

	B. anthracis	*B. cereus*
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

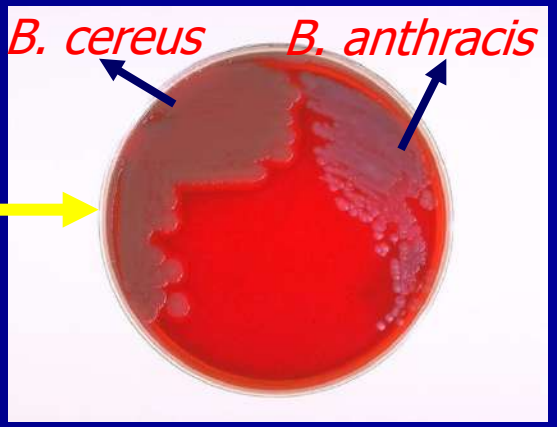
Identification of *Bacillus Spp.*

- **Cultural Characteristics**
  - **Grow on nutrient Agar**
    - On ordinary medium
      - Grow aerobically at 37C with characteristic mucoid or smooth colonies, which indicates the pathogensity 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

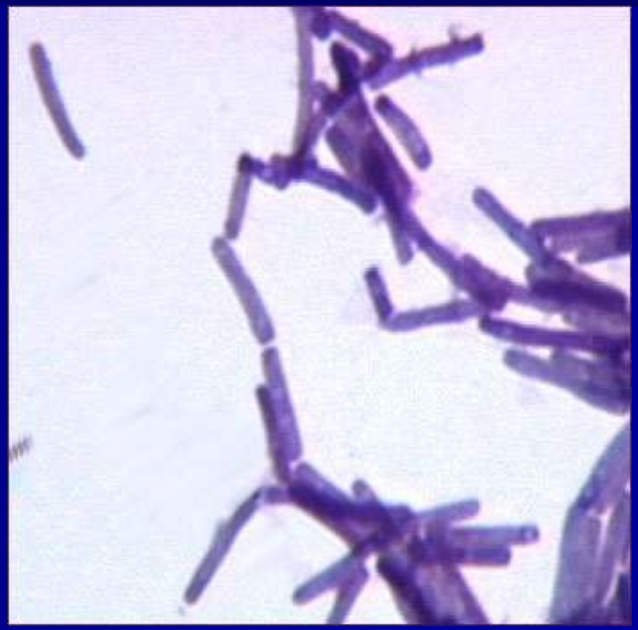
Nutrient Agar

Blood Agar



Identification of *Bacillus Spp.*

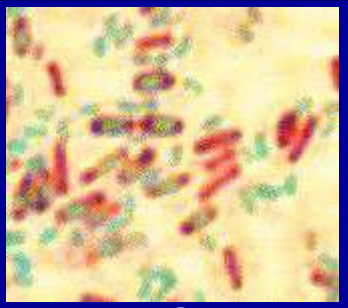
- **Morphology**
  - **Microscopical**
    - **Stain**
      - **Gram Stain**
        - Gram positive bacilli
        - Found in chains
        - Non motile
        - Capsulated inside the host
        - Sporulated outside the host
        - Spore is central, oval and non-bulging



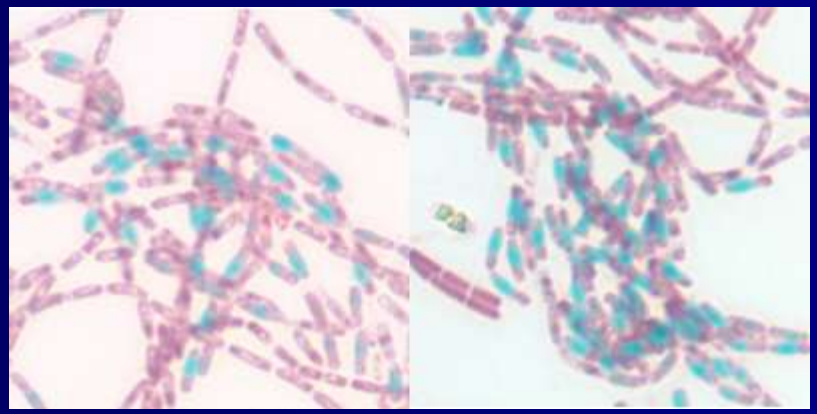
Spore Stain Procedure

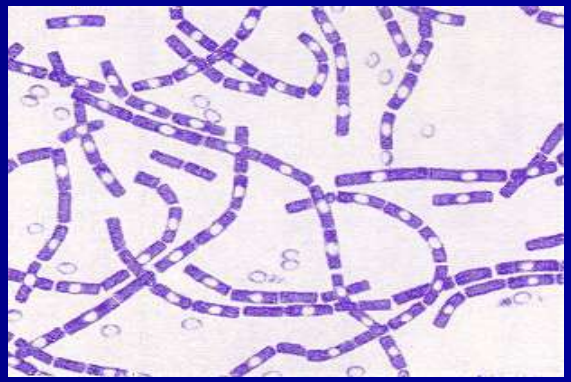
1. Make a heat fixed smear of *Bacillus*
2. Place the slide on the slide rack
3. Cover the smear with malachite green stain
4. Apply heat for 3-5 min without boiling and drying of the slide
5. Wash the slide gently in running water about 20 S
6. Counterstain with safranin for one minute
7. Gently rinse 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.





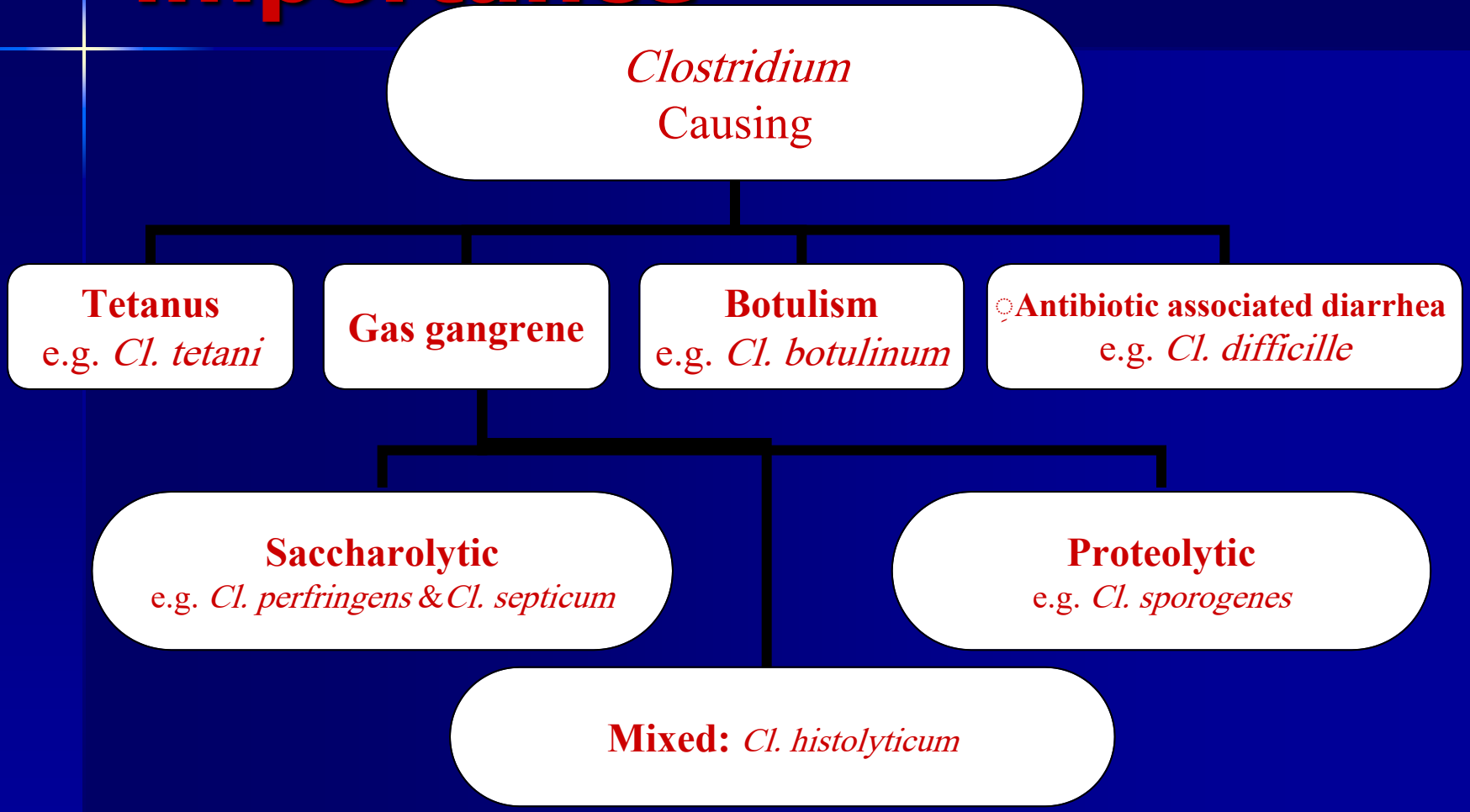
Biochemical Tests: 1- Catalase Test

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

Starch Hydrolysis (Amylase Activity)

- **Principle**
  - Starch + Iodine → blue color
  - Glucose + Iodine → No reaction
- Nutrient Agar containing 1% Starch + M.O → (Amylase) Glucose → (Iodine) Appearance of colorless zone around the growth
- **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

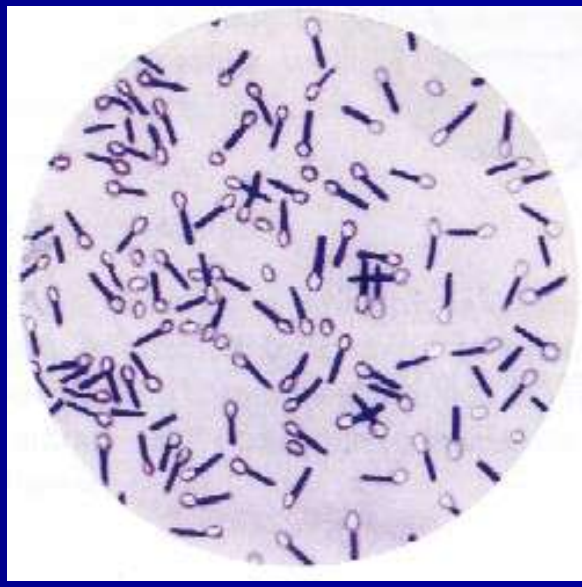


Clostridia

- 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*

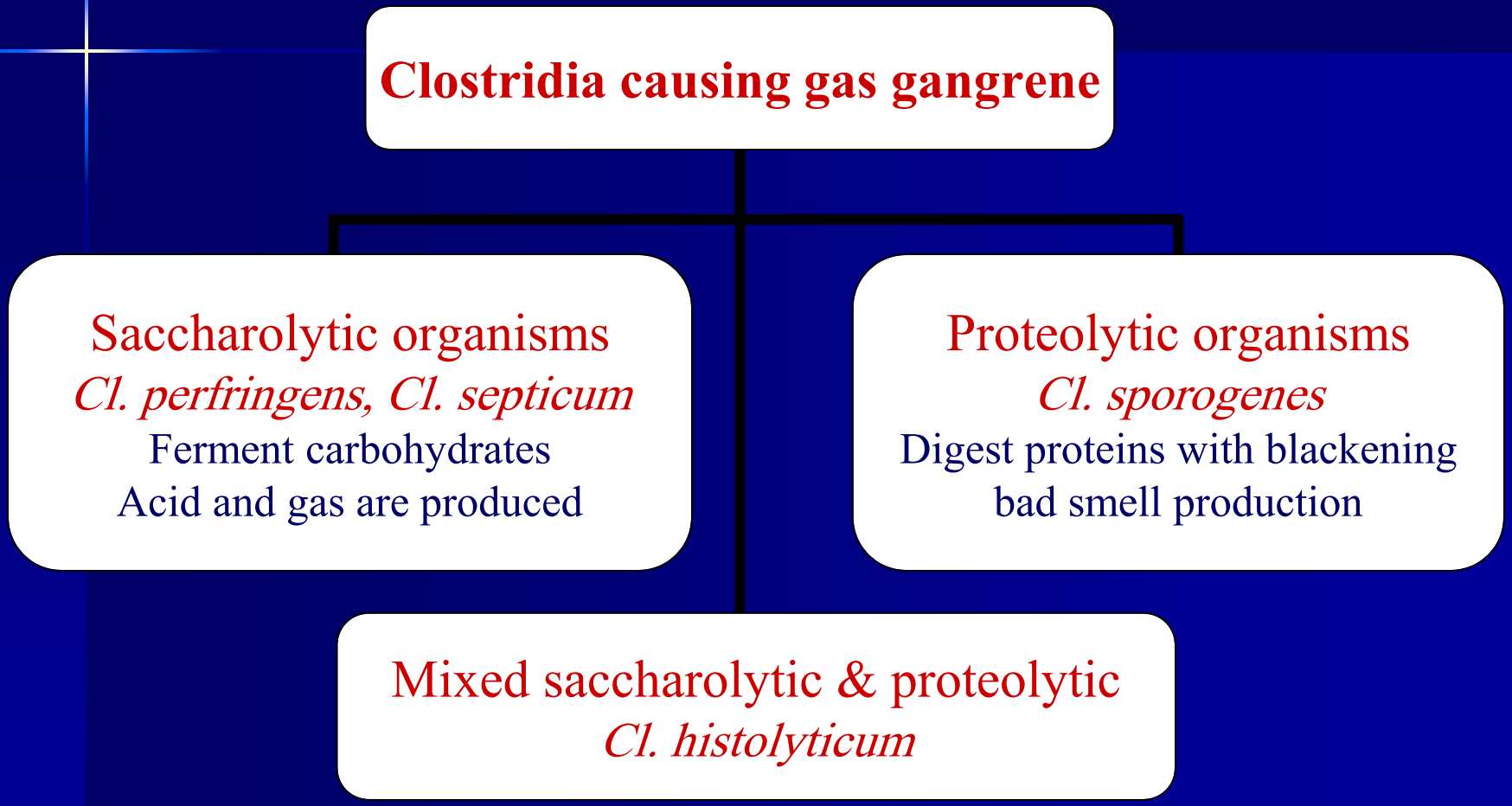
- Gram positive, straight, slender rod with rounded ends
- All species form endospore (drumstick with a large round end)
- Fermentative
- Obligate anaerobe
- Motile by peritrichous flagella
- Grows well in cooked meat broth and produces a thin spreading film when grown on enriched blood agar
- Spores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



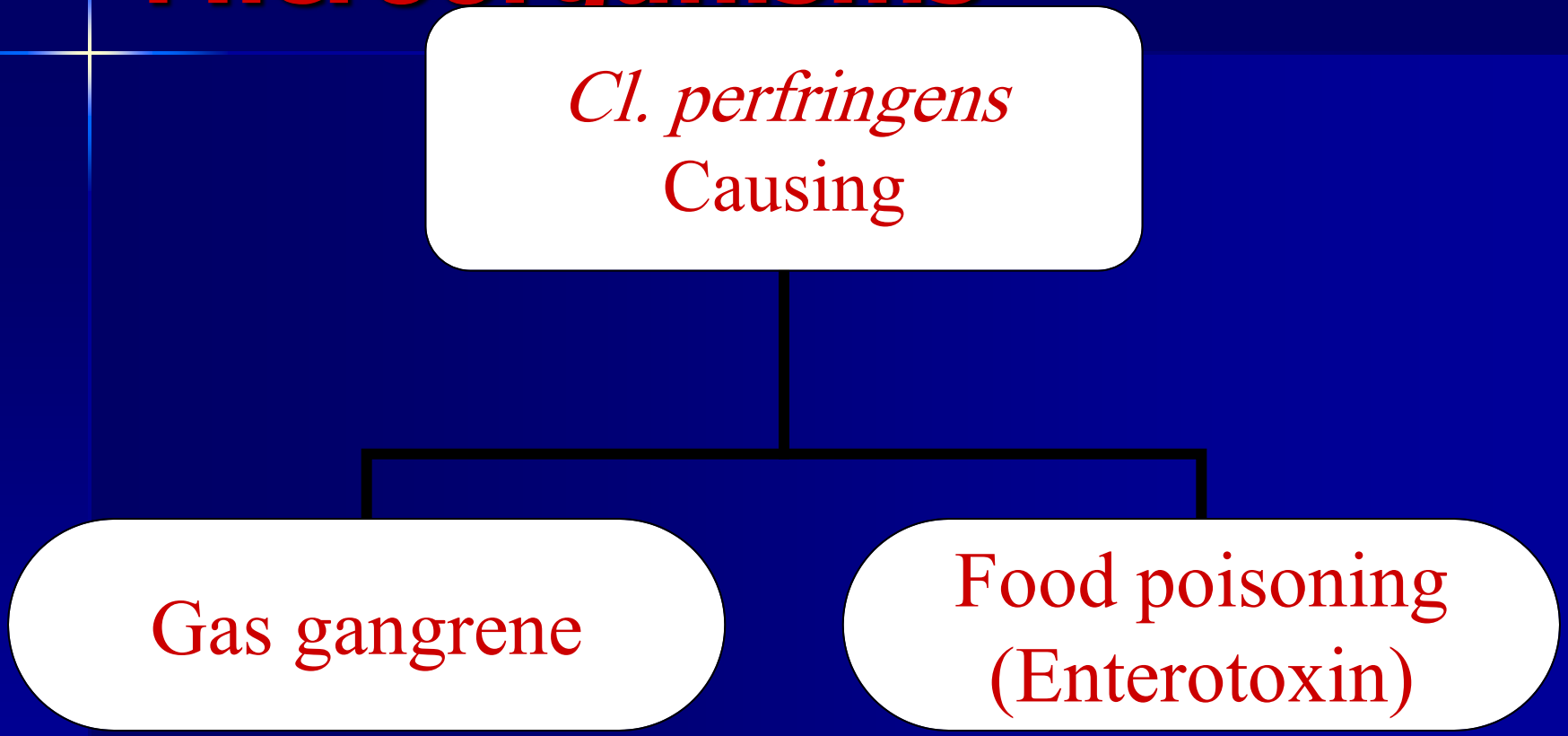
Laboratory Diagnosis of Tetanus

- 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



Saccharolytic Microorganisms



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

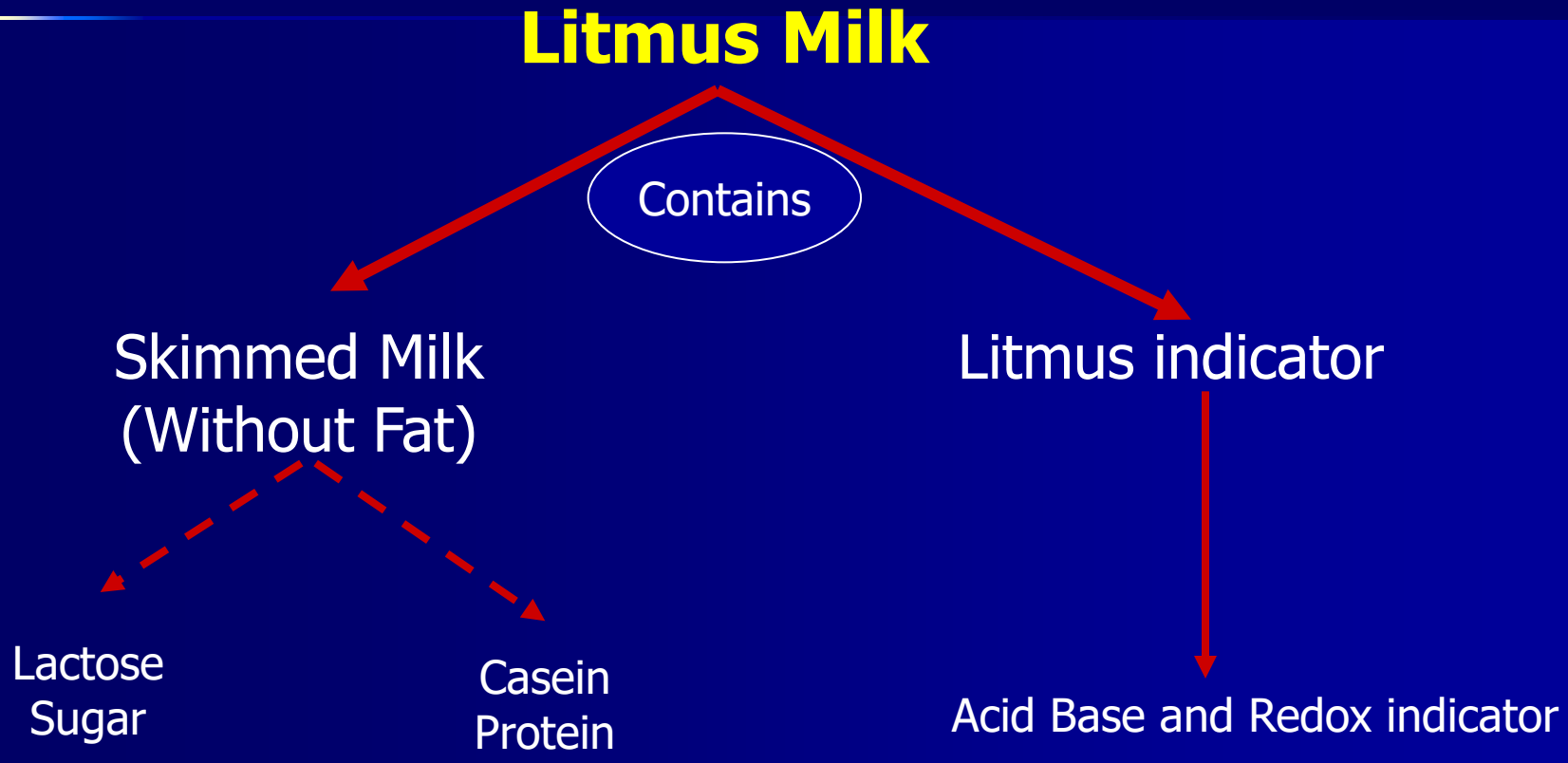
Laboratory Diagnosis

- **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 observed with the production of H_2S and NH_3
  - **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



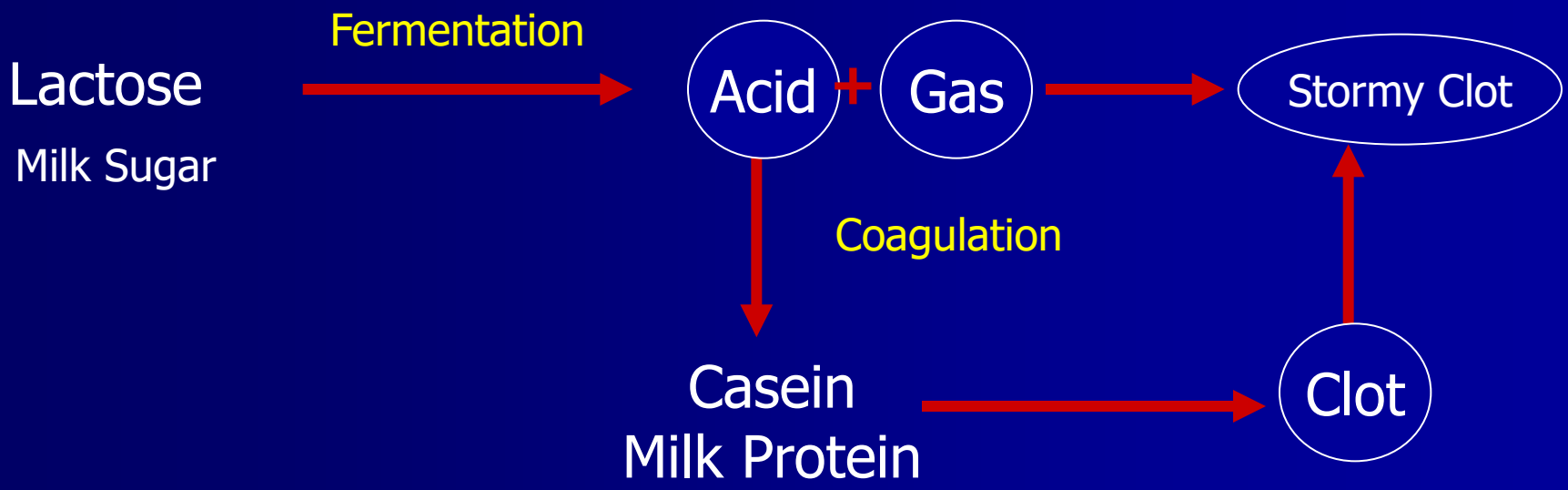
Reaction on Litmus Milk

1- Acidic Reaction

2- Basic Reaction

Reaction on Litmus Milk

Stormy Clot Formation

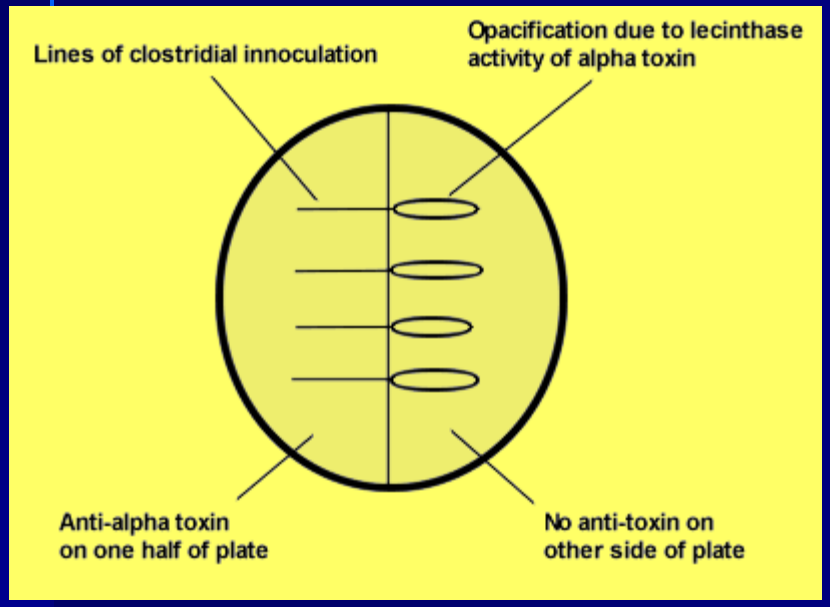


Reaction on Litmus Milk

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



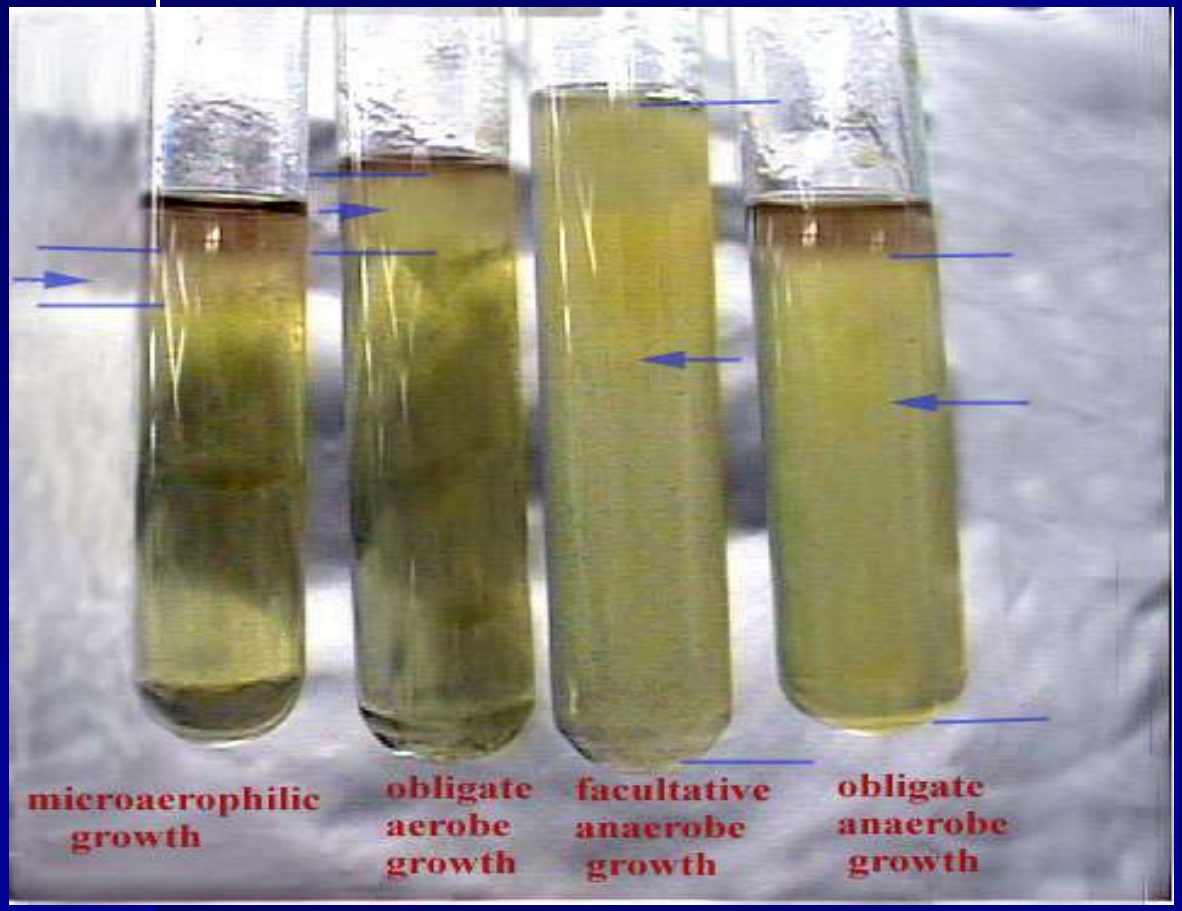
Procedure of Nagler Reaction

Positive Nagler Reaction

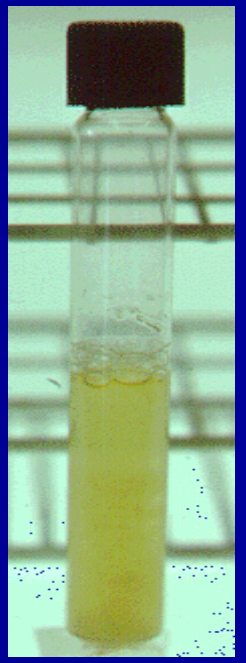
Anaerobic Cultivation

- 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

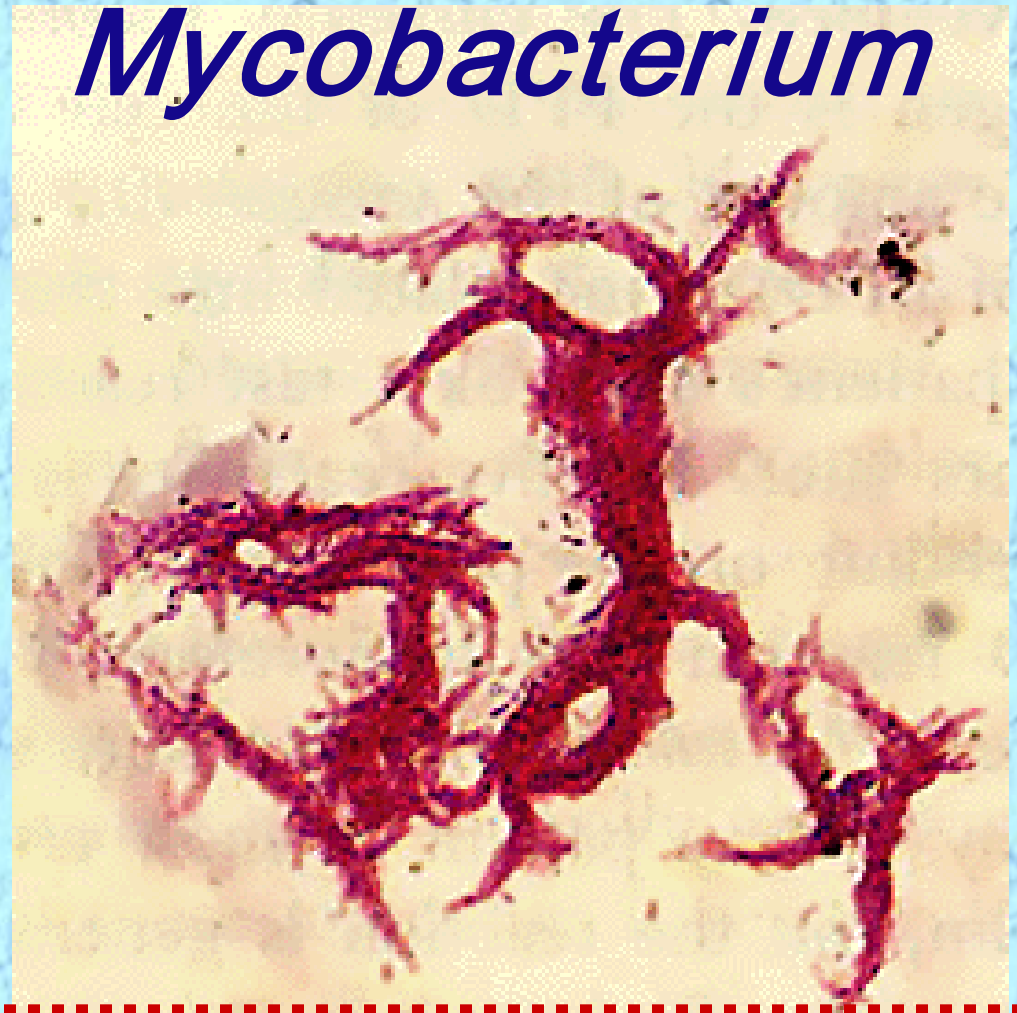
Important Human Pathogens

Mycobacterium tuberculosis

Mycobacterium leprae (uncommon)

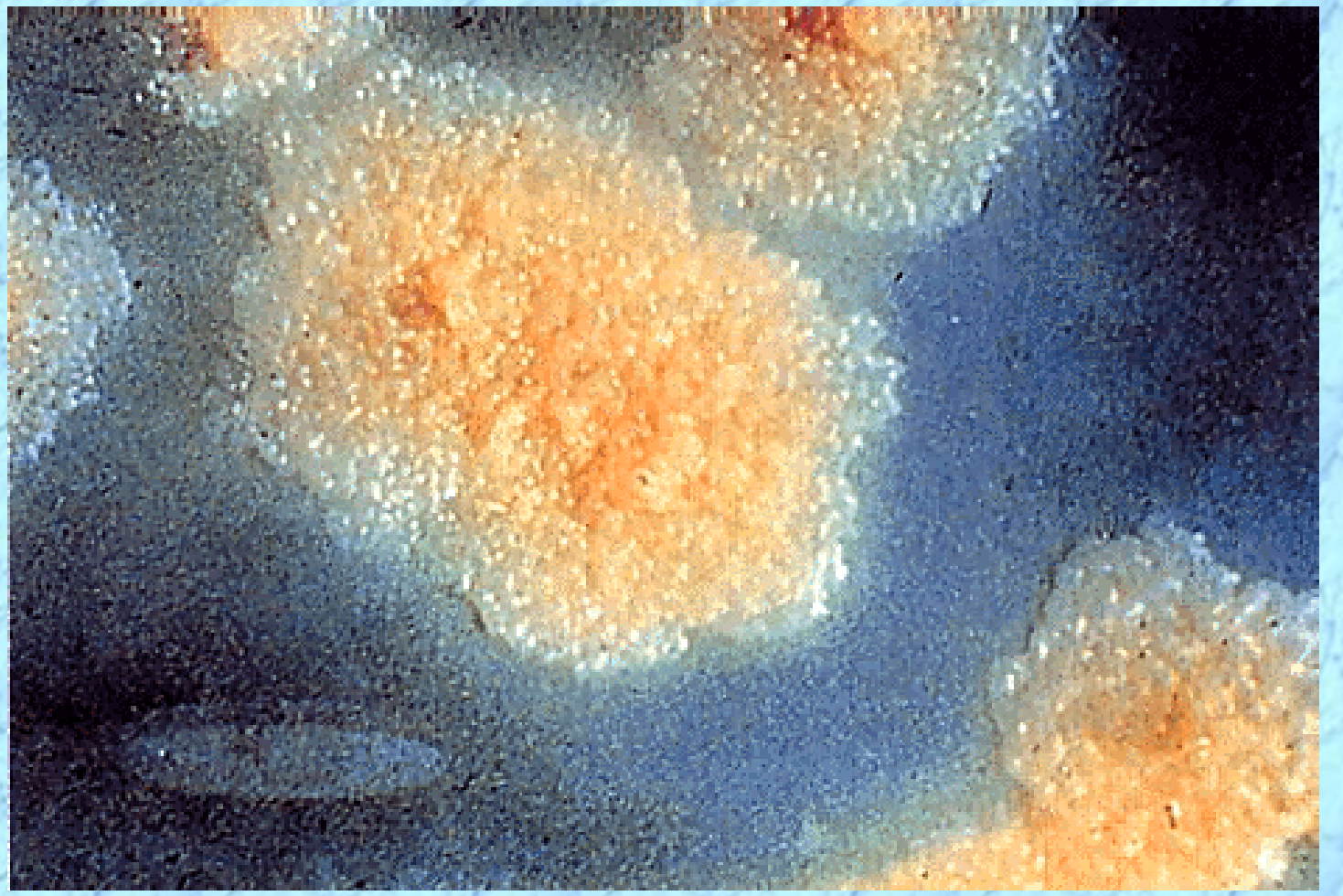
Mycobacterium avium-intracellulaire Complex (MAC) or (M. avium)

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

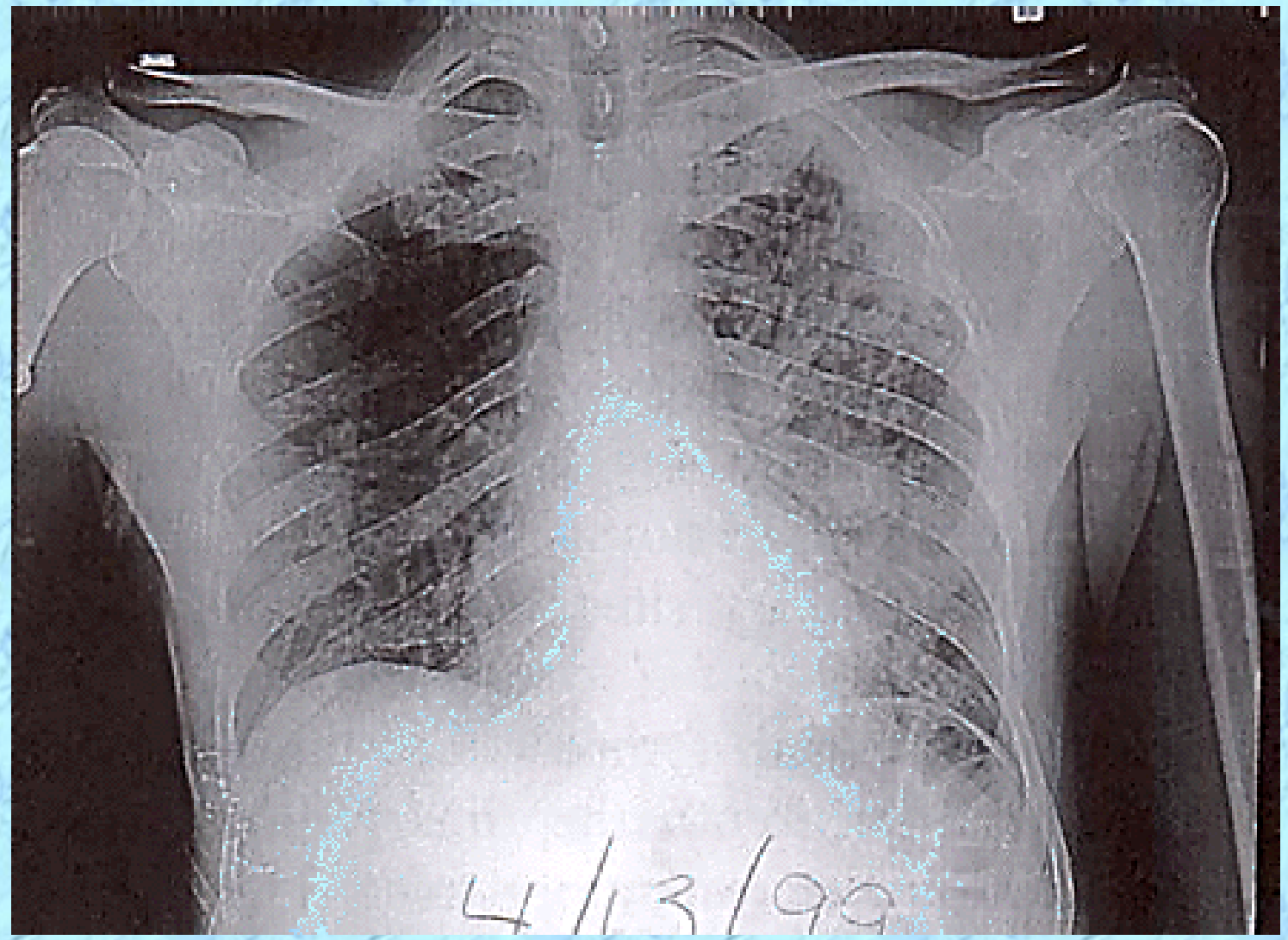
Nucleic acid probes

Nucleic acid sequencing

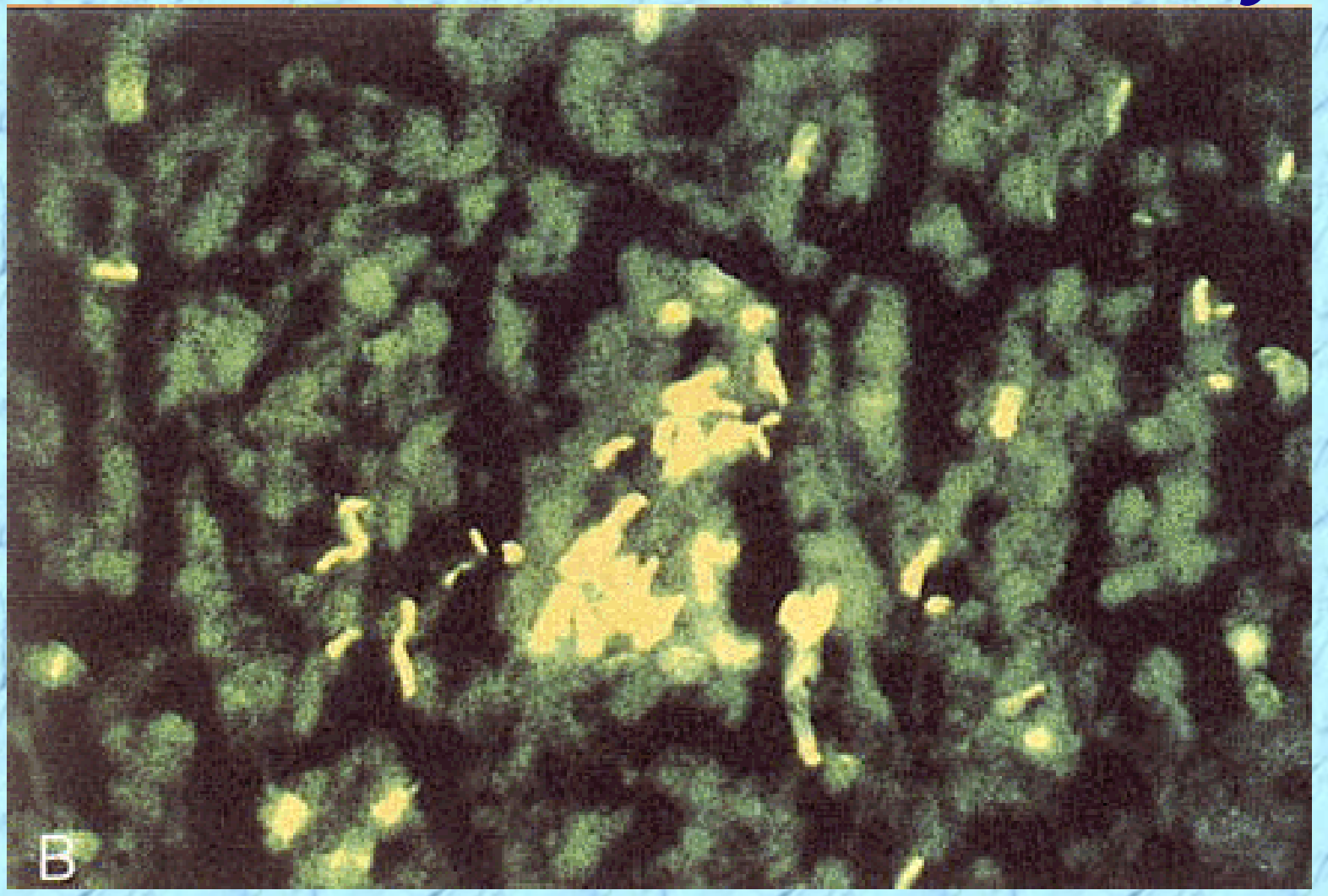
Differential Characteristics of Commonly Isolated Mycobacterium spp.

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

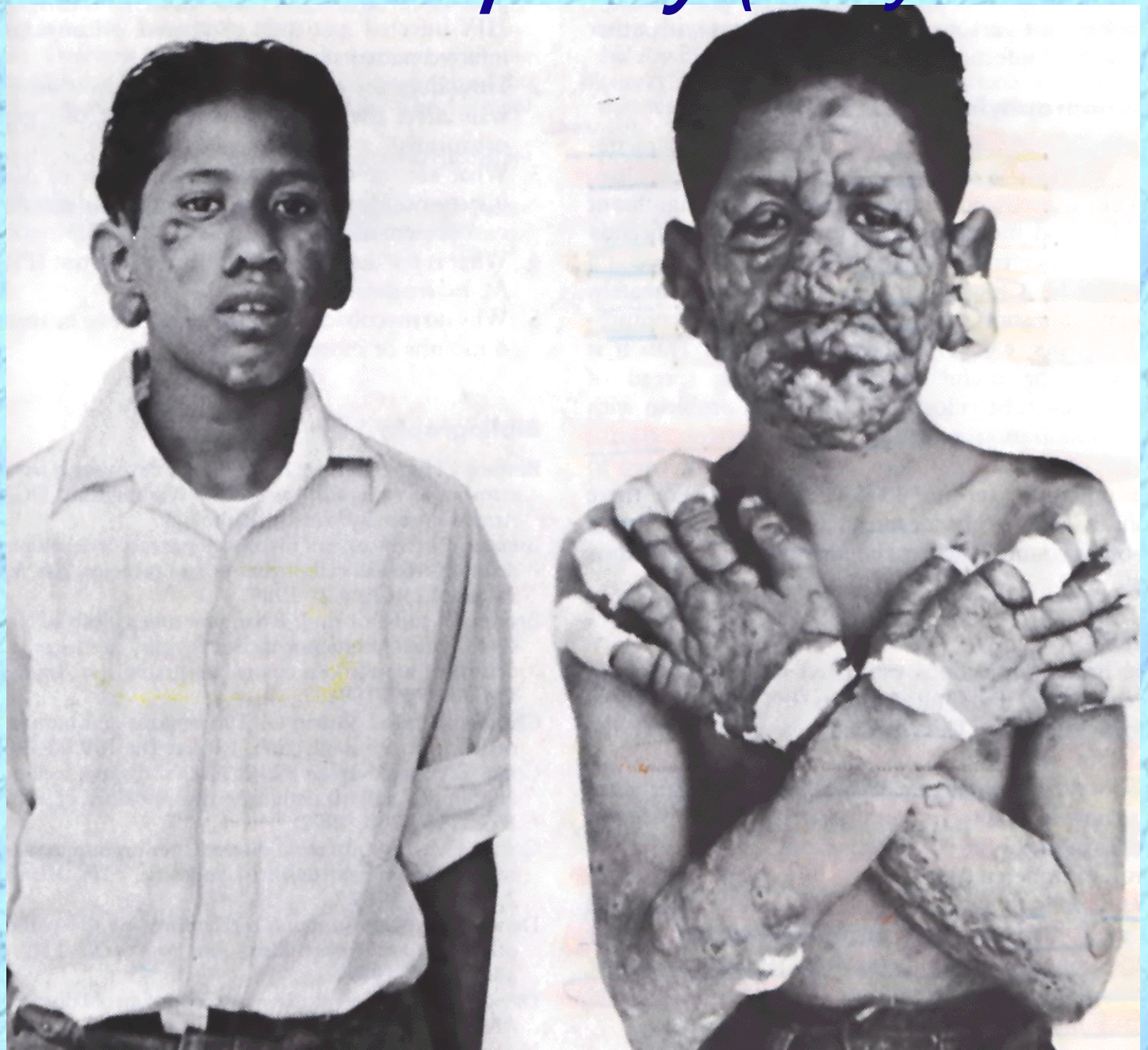
Dapsone with or without rifampin is used to treat the tuberculoid form of disease; clofazimine is added for the treatment of the lepromatous form. Therapy is prolonged.

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

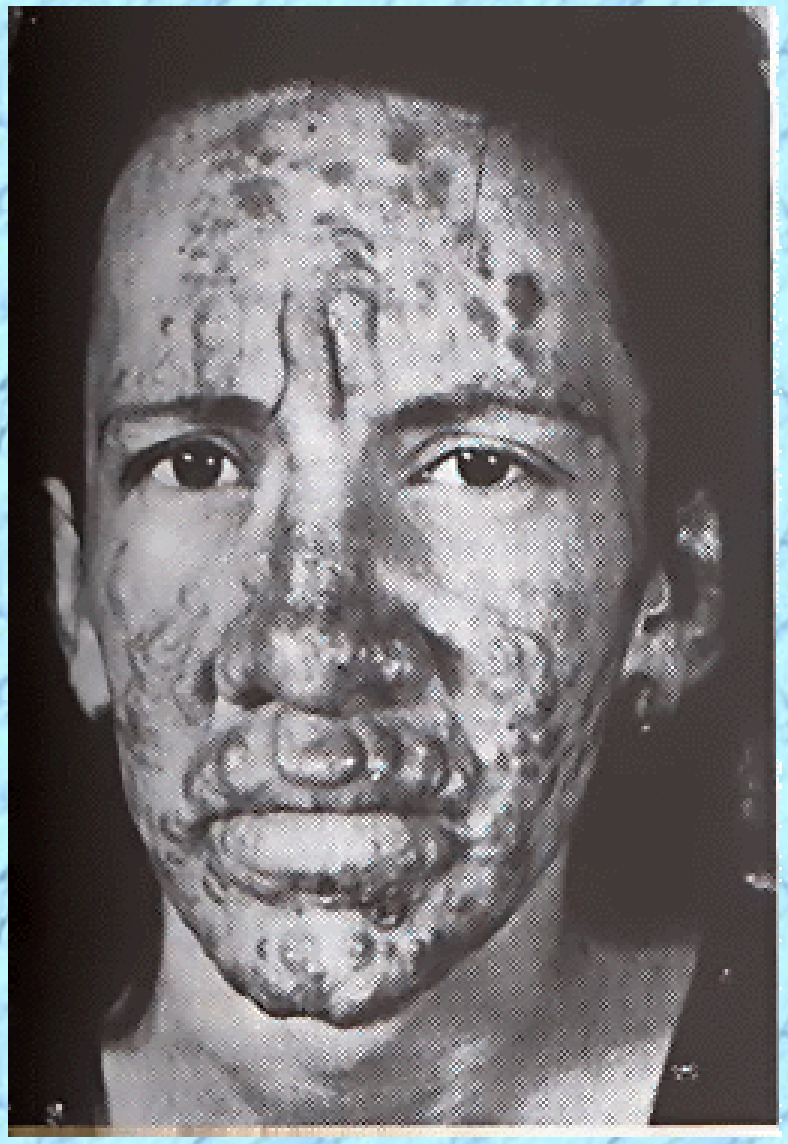
Disease is controlled through the prompt recognition and treatment of infected people.

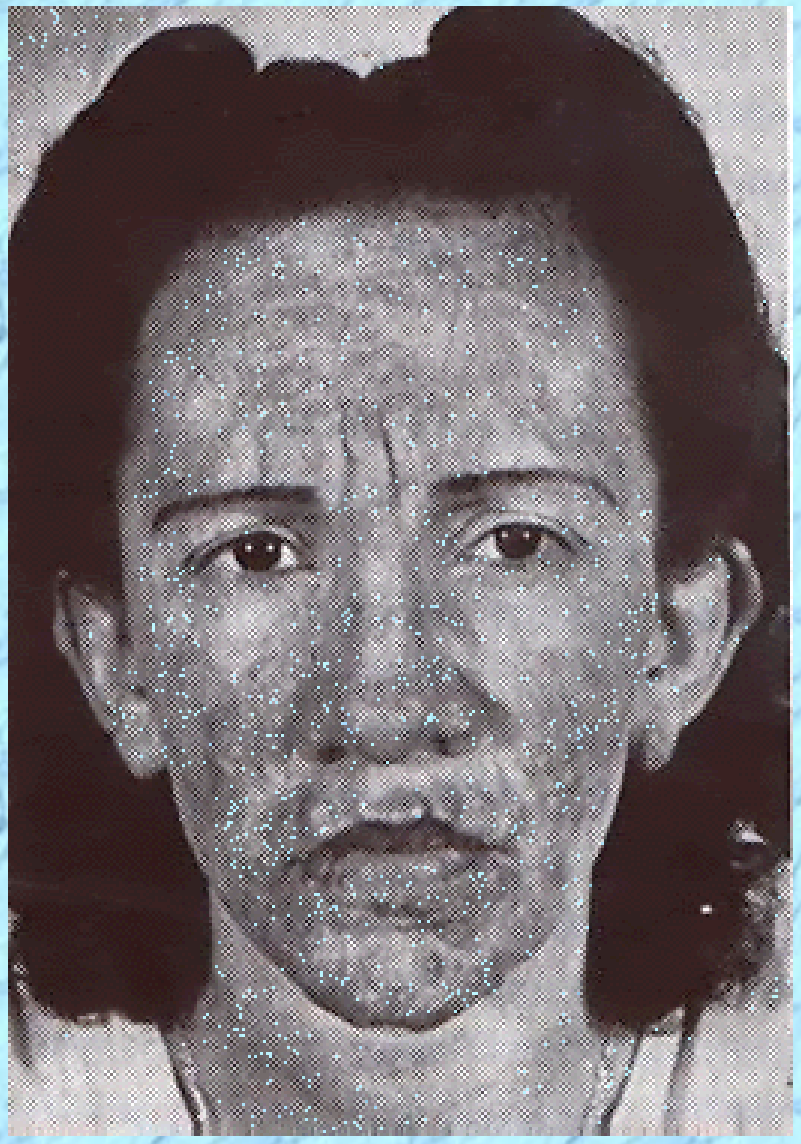
REVIEW

Lepromatous Leprosy (Early/Late Stages)



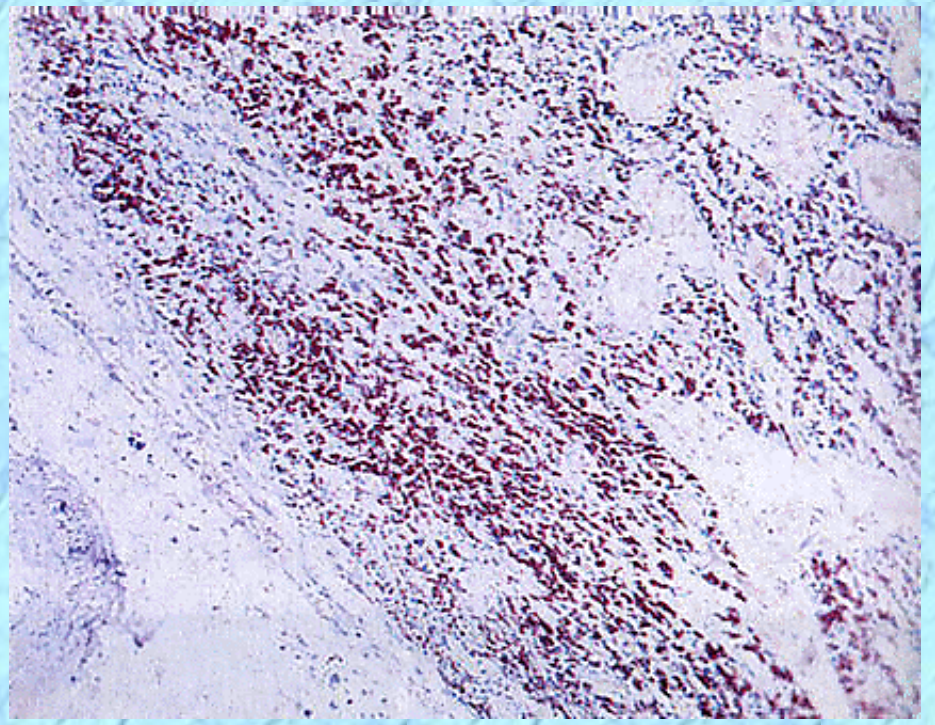
Lepromatous Leprosy Pre- and Post-Treatment



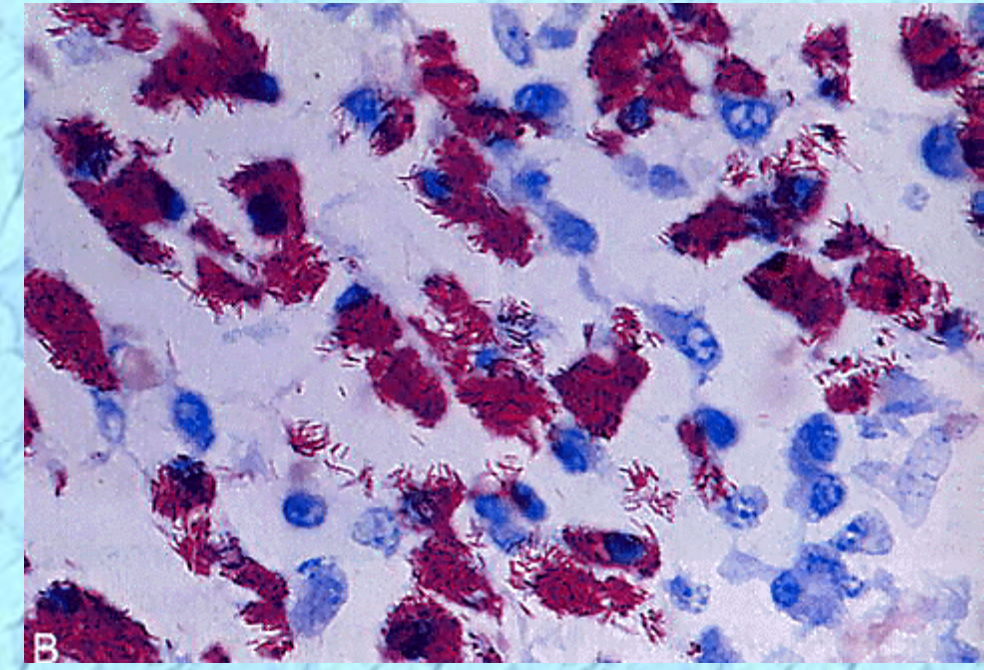


Mycobacterium avium-intracellulaire Complex (MAC)

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.

REVIEW

Aerobic Actinomycetes

Aerobic Actinomycetes:Nocardia species

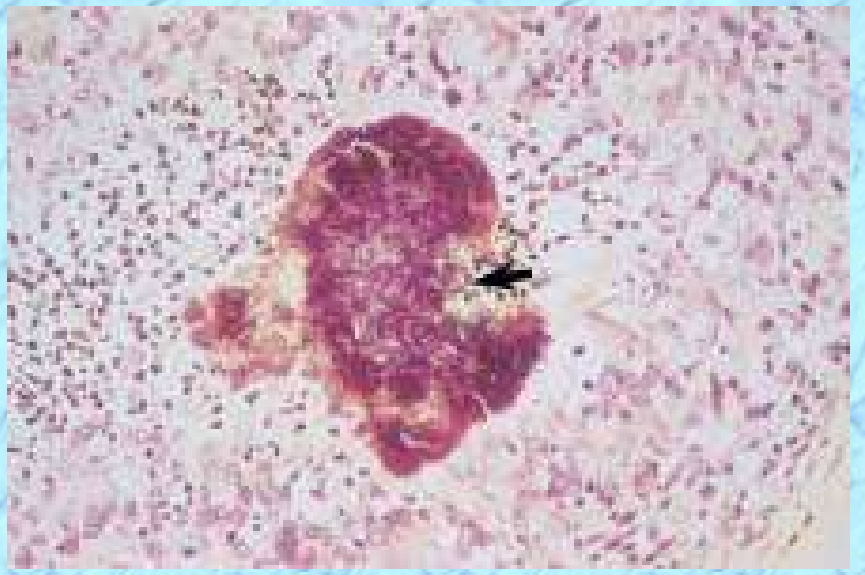
- **General Characteristics**
  - ✓ **Aerobic, gram-positive, filamentous rods, sometimes resembling branched hyphae**
  - ✓ **Weakly acid-fast and may stain gram-variable**
  - ✓ **Morphologically resemble fungi, both in culture and in types of infections produced**
  - ✓ **Generally found in the environment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

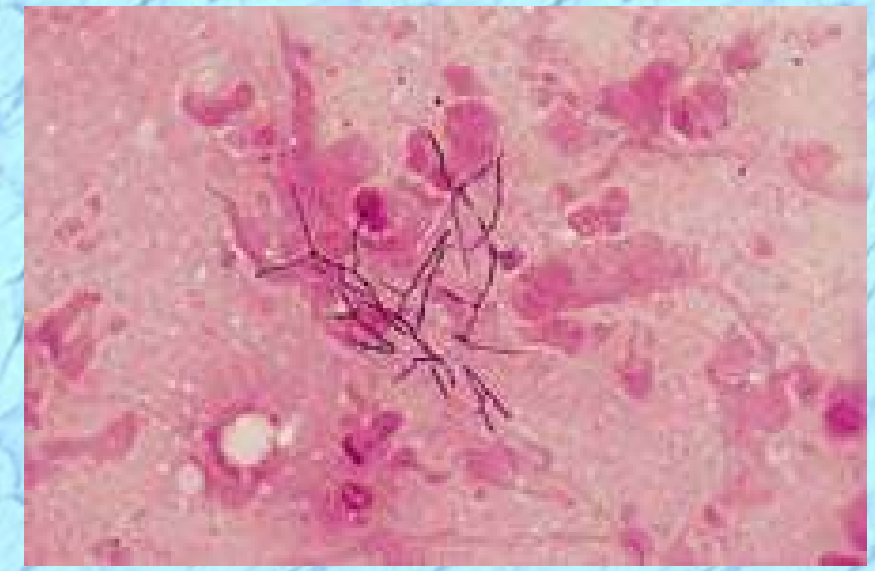
- **Clinical infections**
  - ✓ **Pulmonary form**
  - ✓ **Mycetomas**



Sulfur granules collected from draining sinus

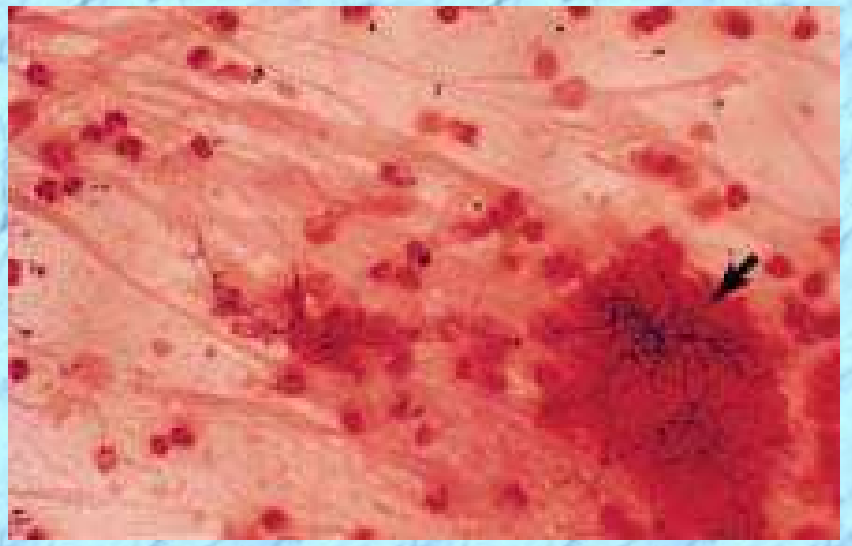
Laboratory Diagnosis: *Nocardia, Actinomadura, and Streptomyces species*

- **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**

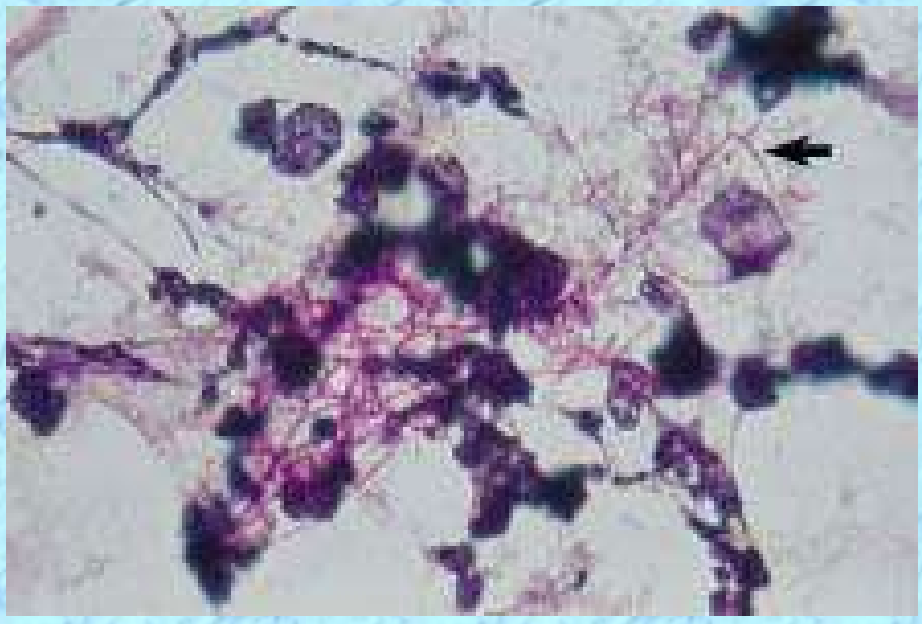
Laboratory Diagnosis:

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

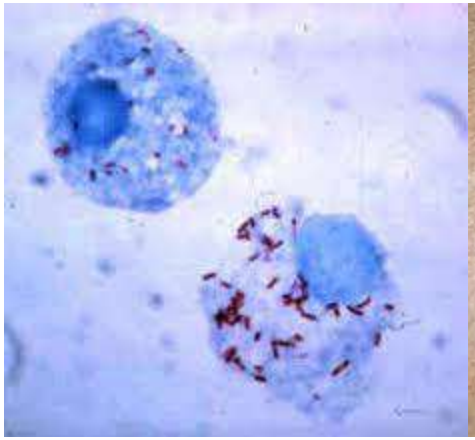
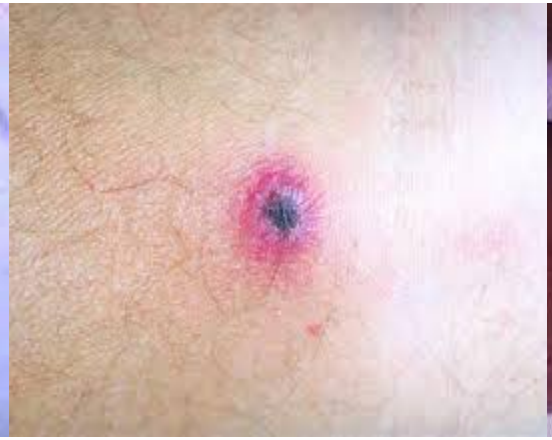
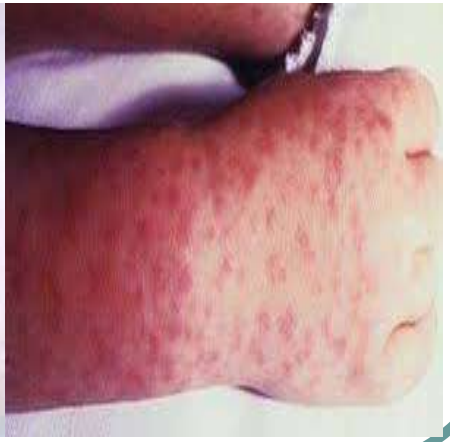
Rickettsia and *Orientia* species are transmitted by the bite of infected ticks or mites or by the feces of infected lice or fleas. From the portal of entry in the skin, rickettsiae spread via the bloodstream to infect the endothelium and sometimes the vascular smooth muscle cells. *Rickettsia* species 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.**

Mycoplasmal pneumonia

- **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.**

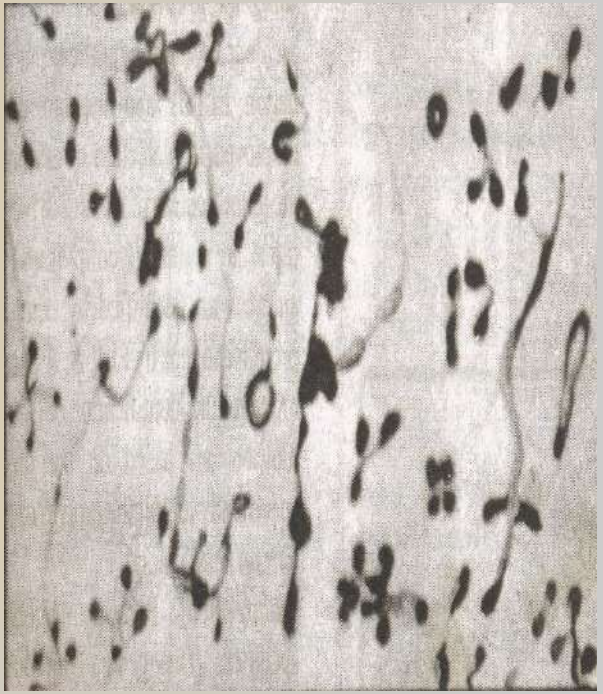
Laboratory Diagnosis

- **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 acredine 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% CO_2 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

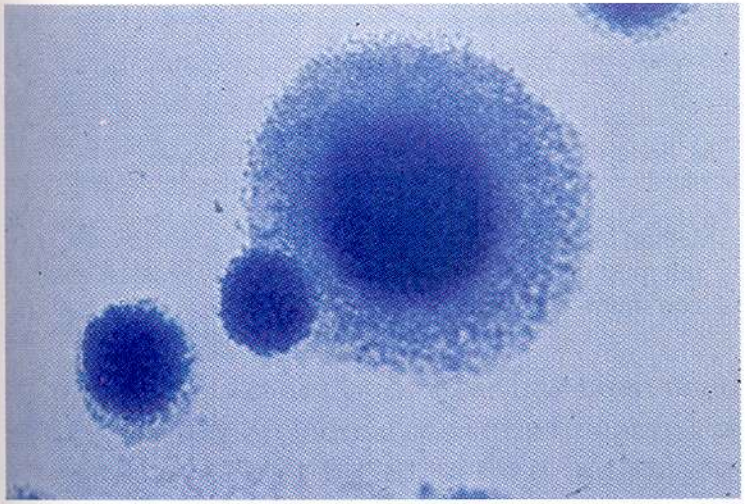


Figure 21-16

Diene's stain of *Mycoplasma* spp. colonies demonstrating typical "fried egg" appearance.

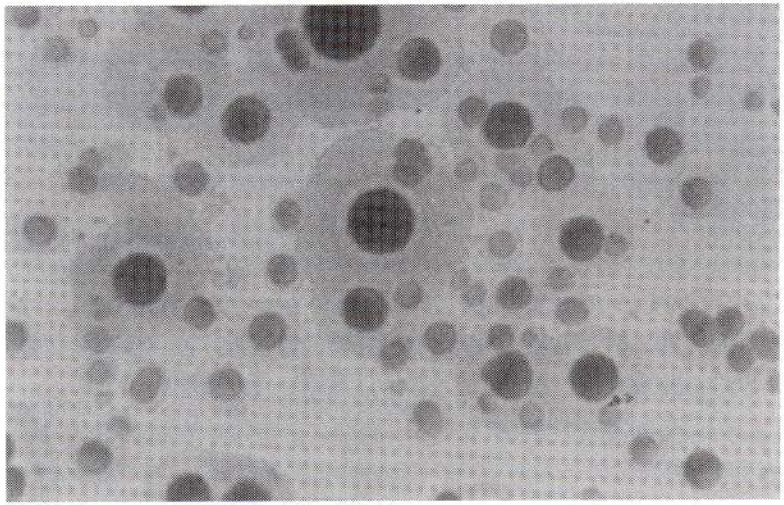
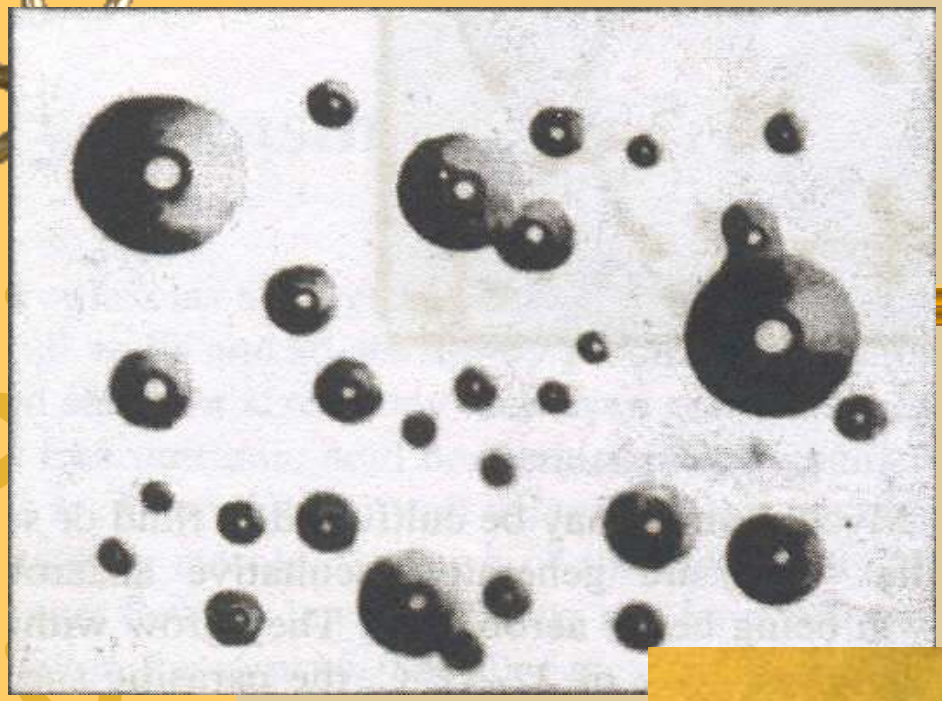
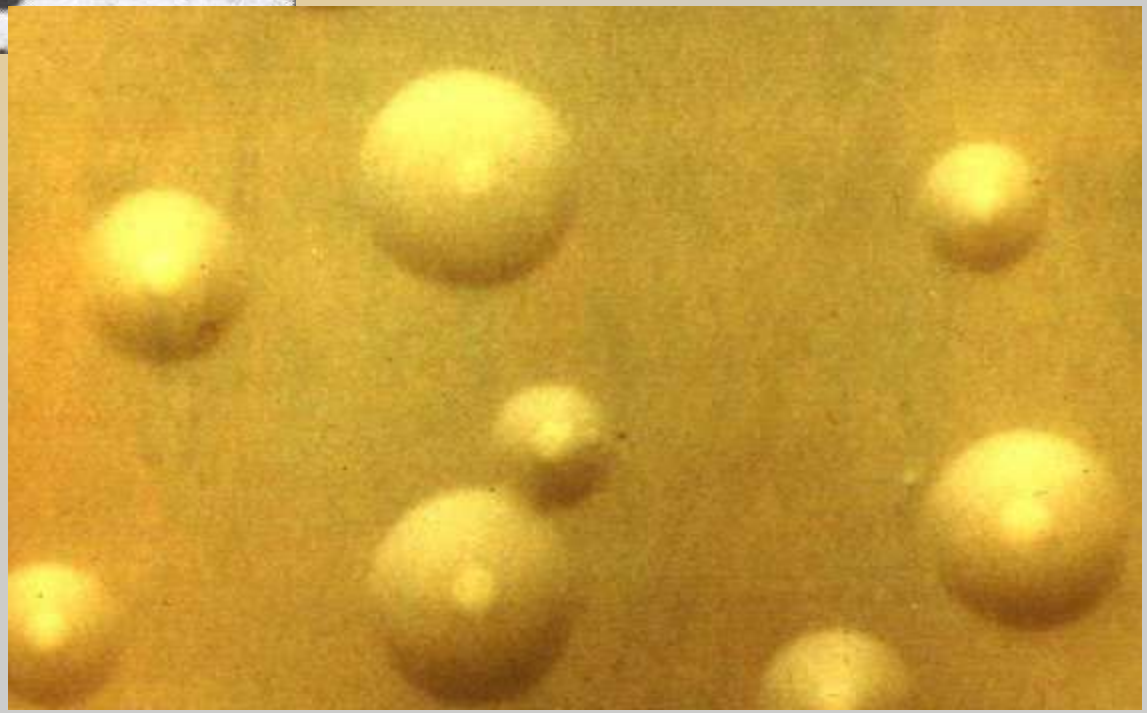


Figure 21-17

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

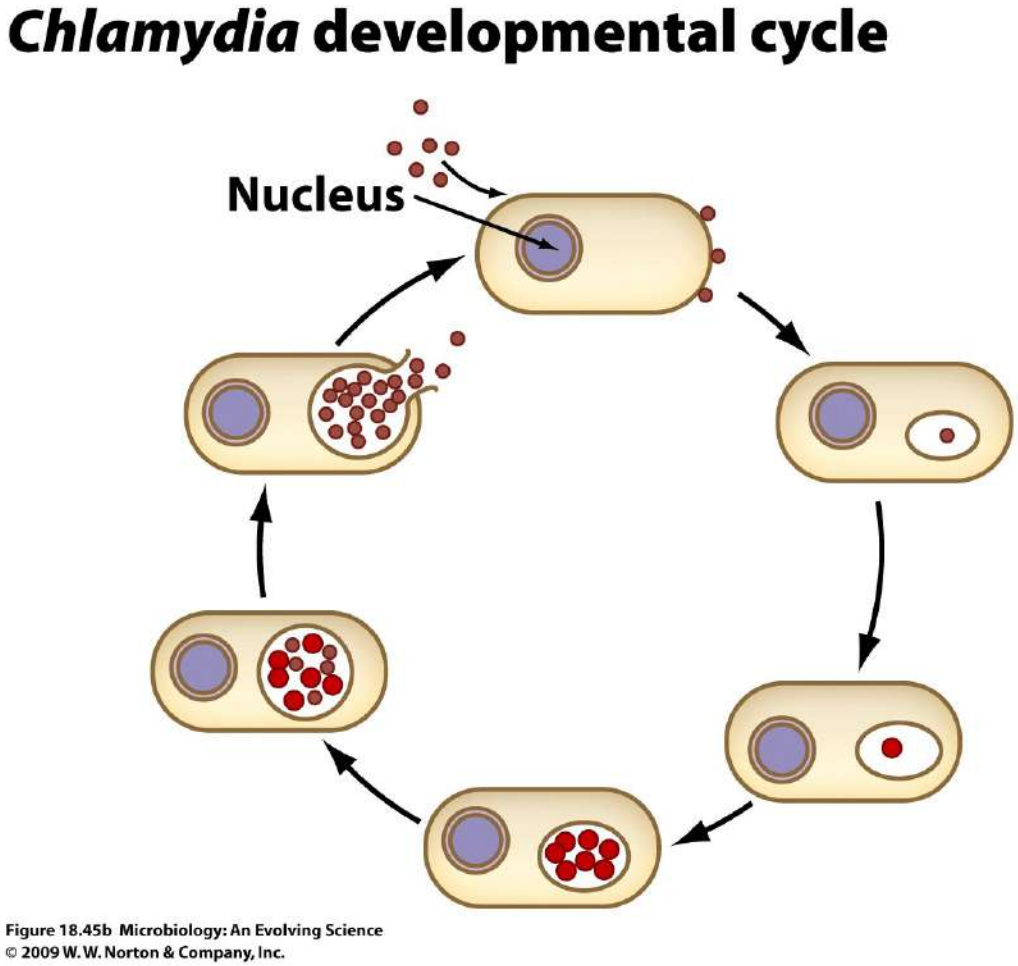


Figure 18.45b Microbiology: An Evolving Science

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

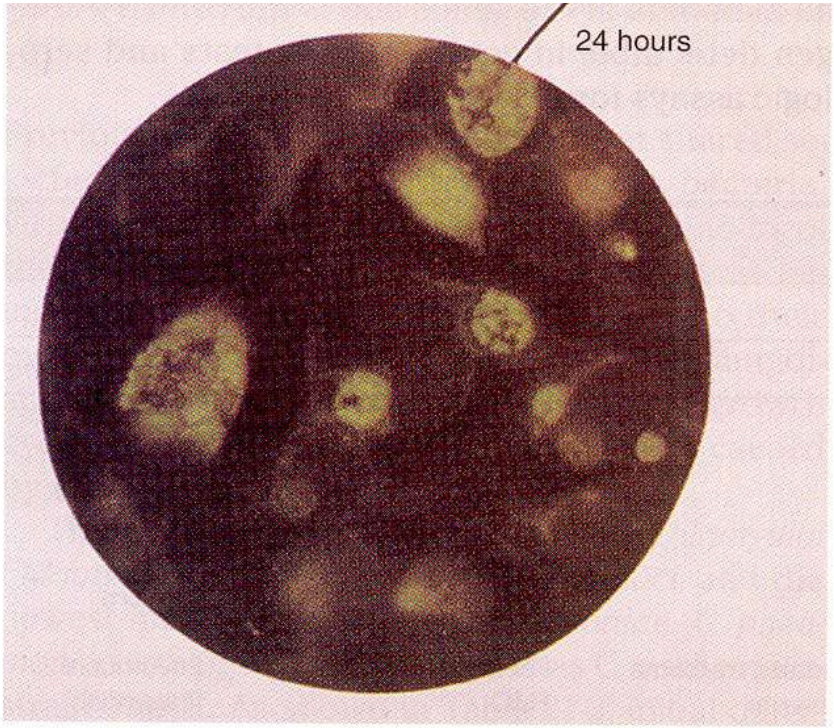


Figure 21-2

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

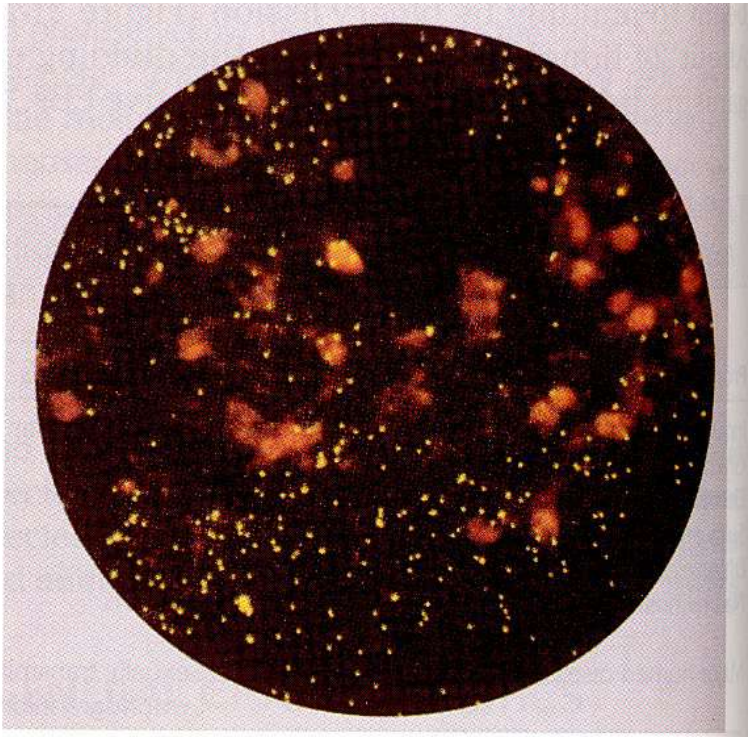
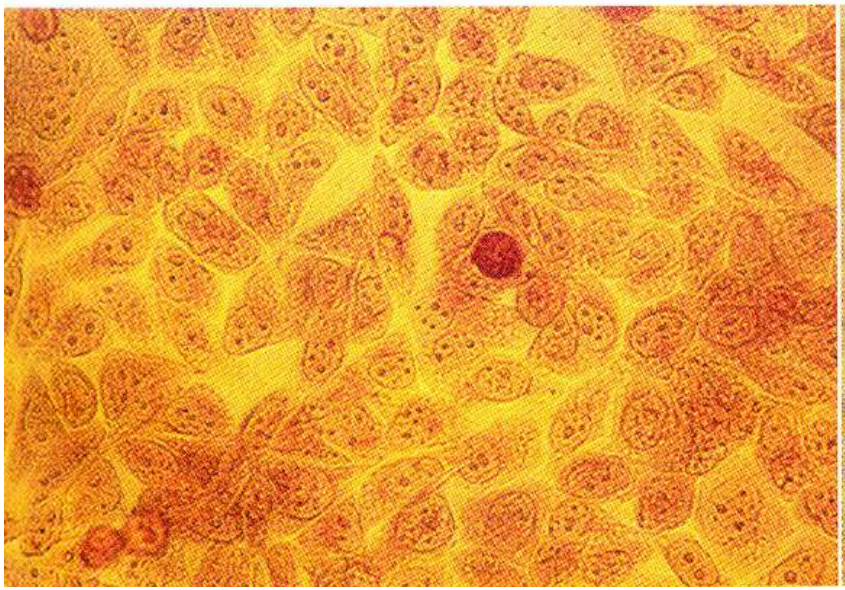
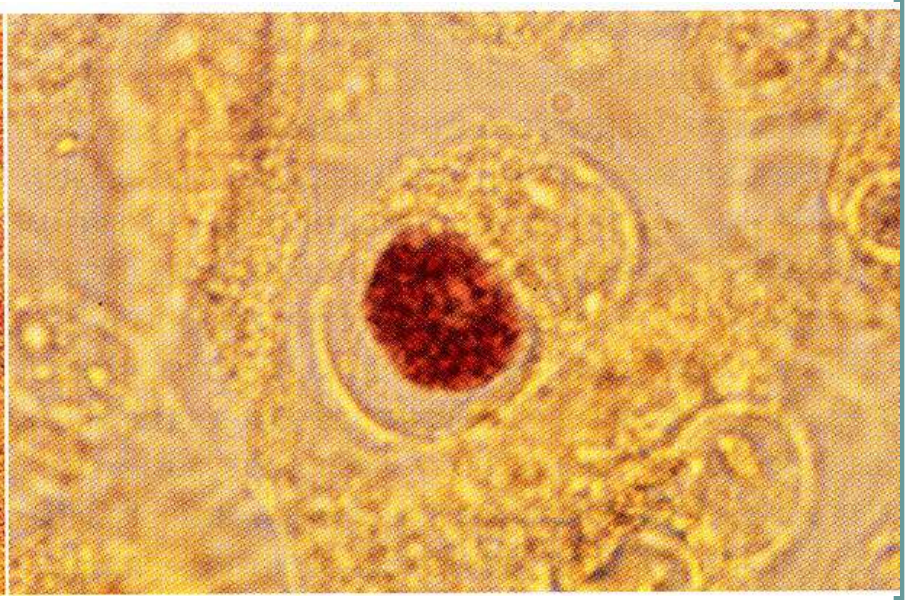


Figure 21-3

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.

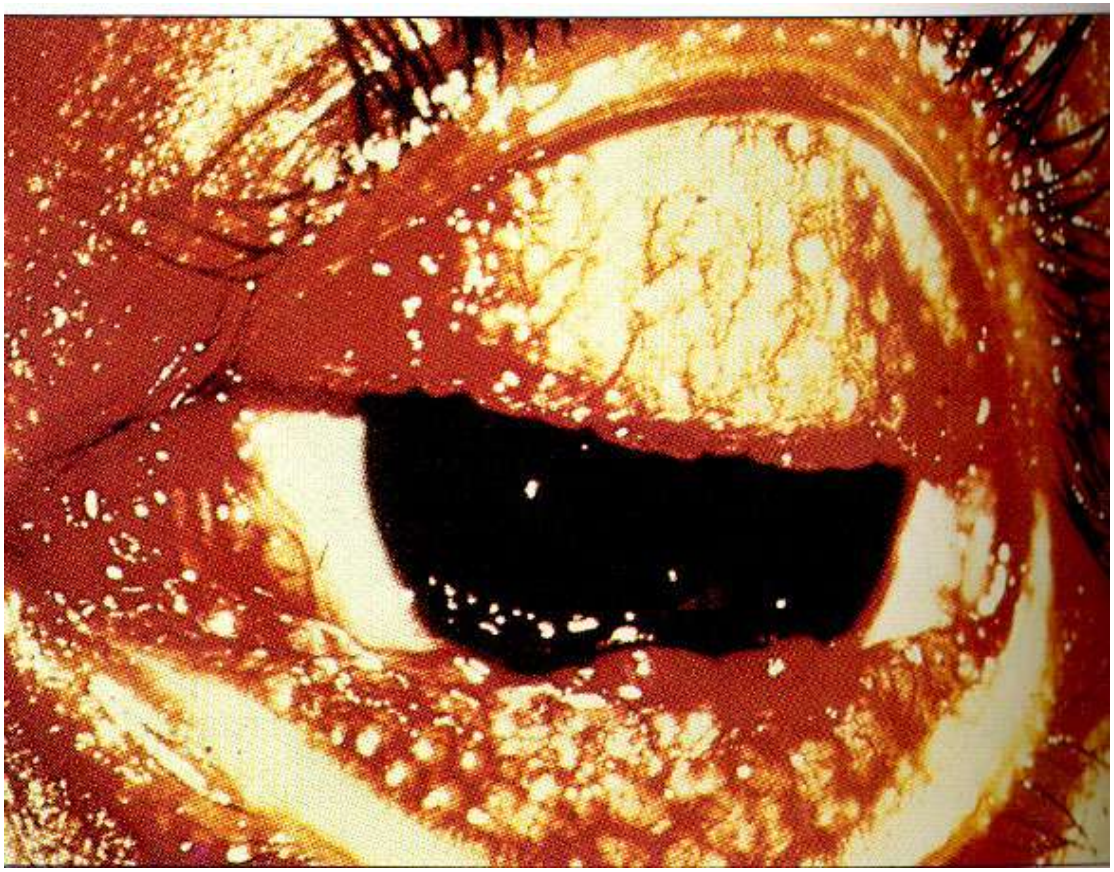
- Inclusion conjunctivitis – this occurs in both newborns and adults and a genital tract infection is the source of the infection (serotypes D-K); is a benign, self-limited conjunctivitis which heals with no scarring
  - Newborns – are infected during the birth process and the infection manifests 1-2 weeks after birth as a mucopurulent discharge that lasts2 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 $_{L1, 2, 3}$) 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/antimicrobic 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 paraluiscuniculi 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 lipoproteinsThe 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.

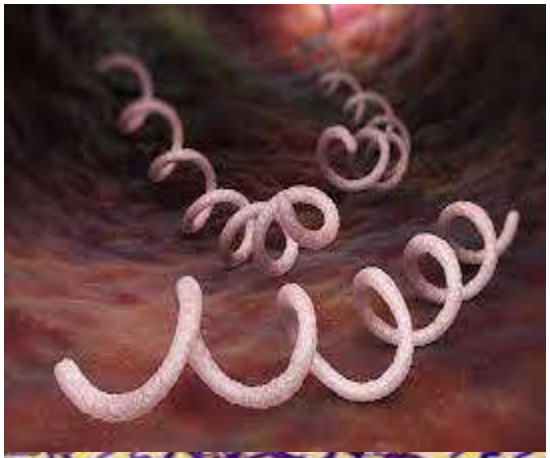
Laboratory identification

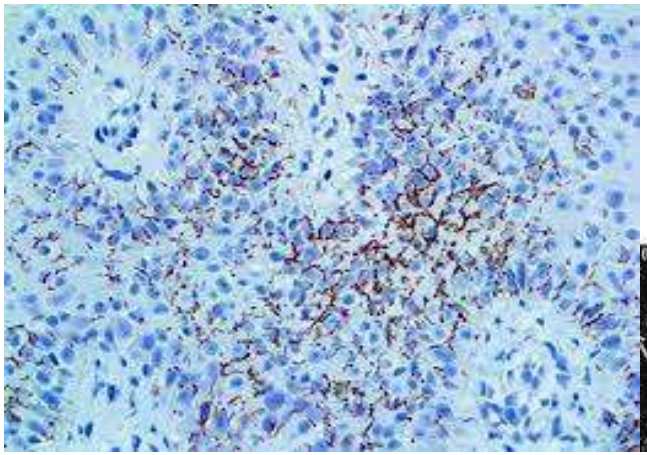
Micrograph showing T. pallidum (black and thin) – Dieterle stain

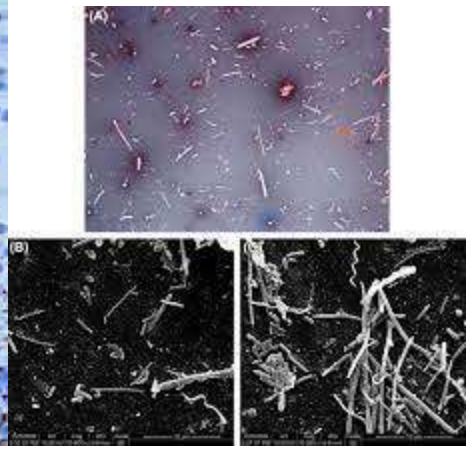
Treponema pallidum was first microscopically identified in syphilitic chancres by Fritz Schaudinn and Erich Hoffmann at the Charité in Berlin in 1905.This bacterium can be detected with special stains, such as the Dieterle stain. T. pallidum is also detected by serology, including nontreponemal VDRL, 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



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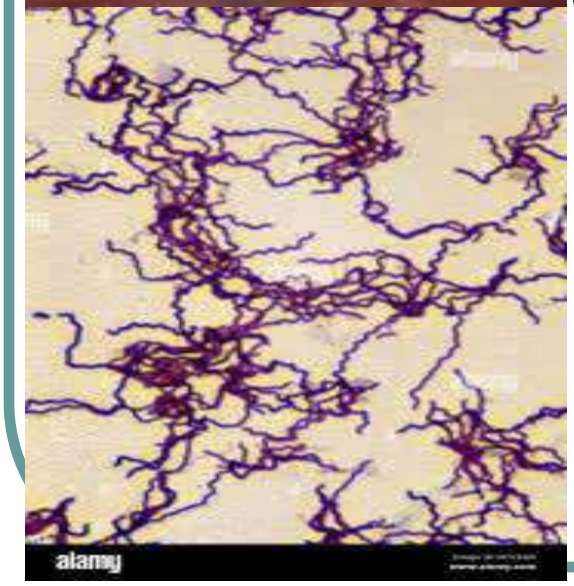
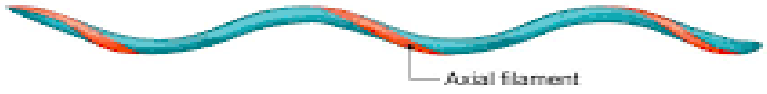
(B)


alamy

Treponema


Endoflagella
Axial filament
Ribosomes
Cytoplasm
Nucleoid
Cell wall
Periplasmic space
Outer membrane


Axial filament

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 periodontitisT. denticola is related to the syphilis-causing obligate human pathogen,

Adherence and cytotoxicity

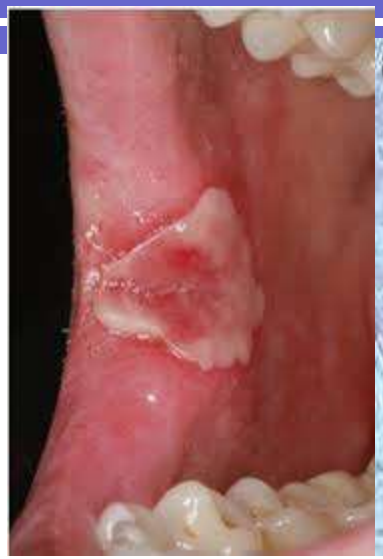
The main site for T. denticola habitation in the oral cavity is the gingival crevice. These spirochetes attach to proteins (including fibronectin and collagen) of local gingival fibroblasts, binding to their plasma membrane. A 53-kDa surface protein on T. denticola is responsible for transporting its 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

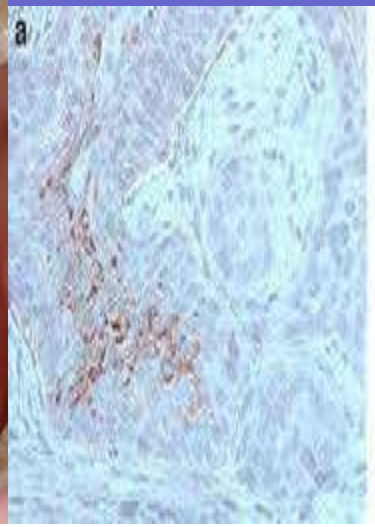
role in Periodontal disease

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 cancerIt 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 producedThe 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)

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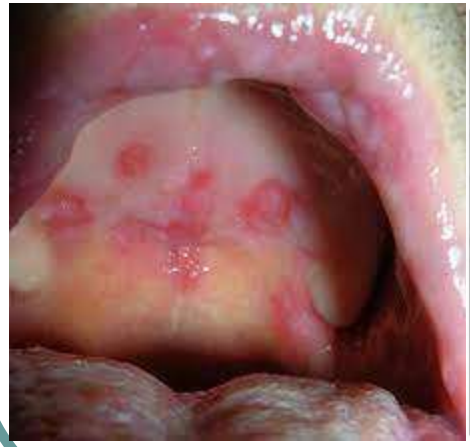

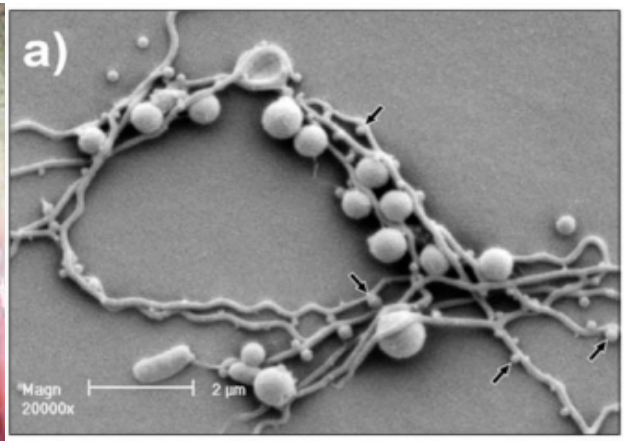
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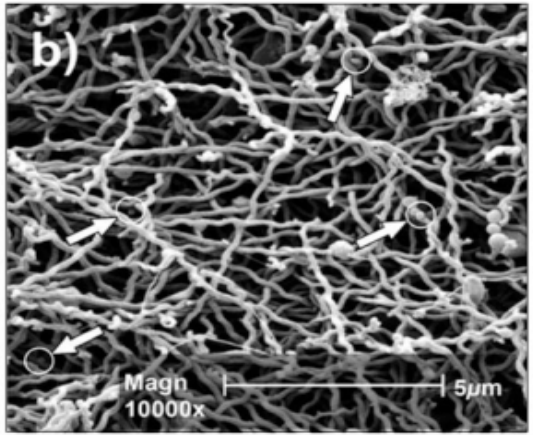
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ا.م.د جتين عزالدين علي ا.د هديل مزهر يونس

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- conversation 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) .

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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 distrupts 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 dur to the opsonization of mutans streptococci with IgA ,IgG antibodies.

- the vaccination trails on humans have been unsuccessful because of fears of possible sie effects ,which would be unacceptable as caries is not a life threating 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 trails very difficult).

-A caries vaccine could,however,be usefulfor 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 chlorehexidine, 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.

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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.

Bacteroidesfragilis

Habitat and transmission

Bacteroides species are the most predominant flora in the intestine (1011 cells per gram of faces), 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-sporing 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* inhibits 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 fuso-spirochaetal infections at other sites in humans ,although the lack of choline binding protein confines the organism to the mouth and not the nasopharynxFusibacteria 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. Fusibacterium varium has phosphatase activity , esculin hydrolysis ,indole, production propionate from lactate and ONPG activity negative but bile resistance ,production propionate from therionine 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/tazobactum,ticarcillin/clavulanate, amoxicillin/sulbactum, ampicillin/sulbactum, ertupenem, imipenem, meropenem, clindamycin, and cefoxitin 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 (H_2S)- 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

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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 singl 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 nuclic 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-nucic acid compostion(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

- Herps 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

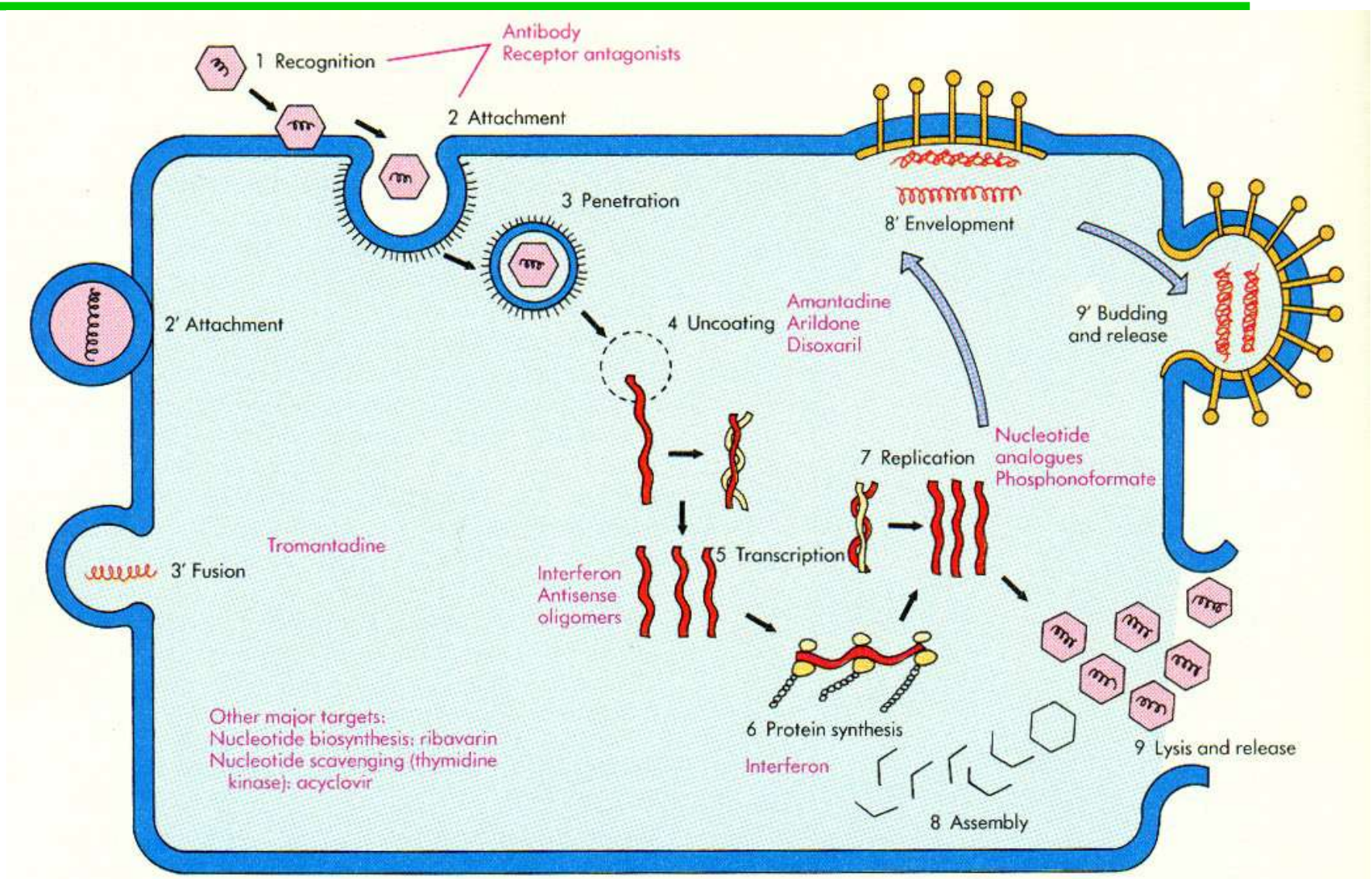
1. overall

	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

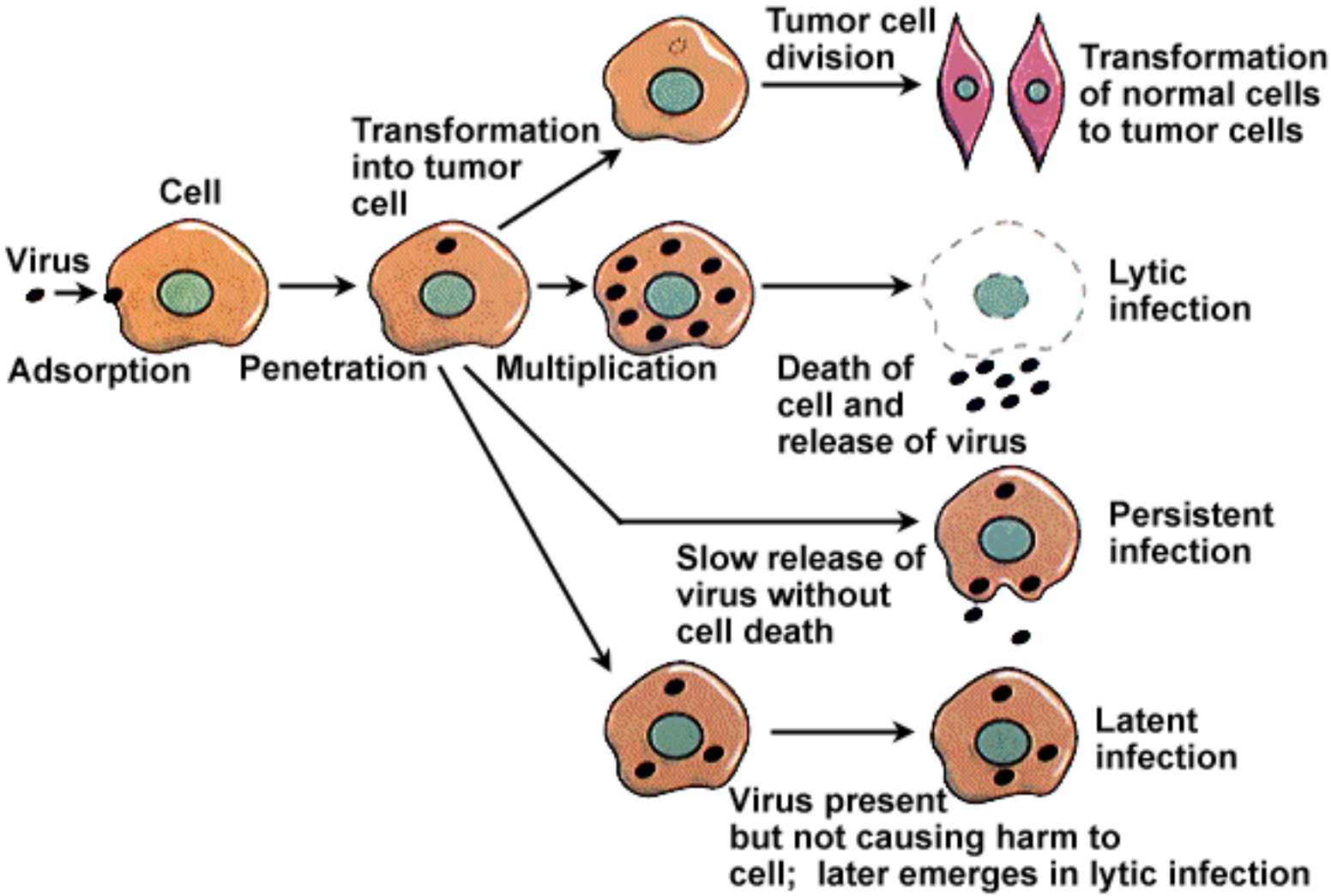
How viruses multiply (replication)

- 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

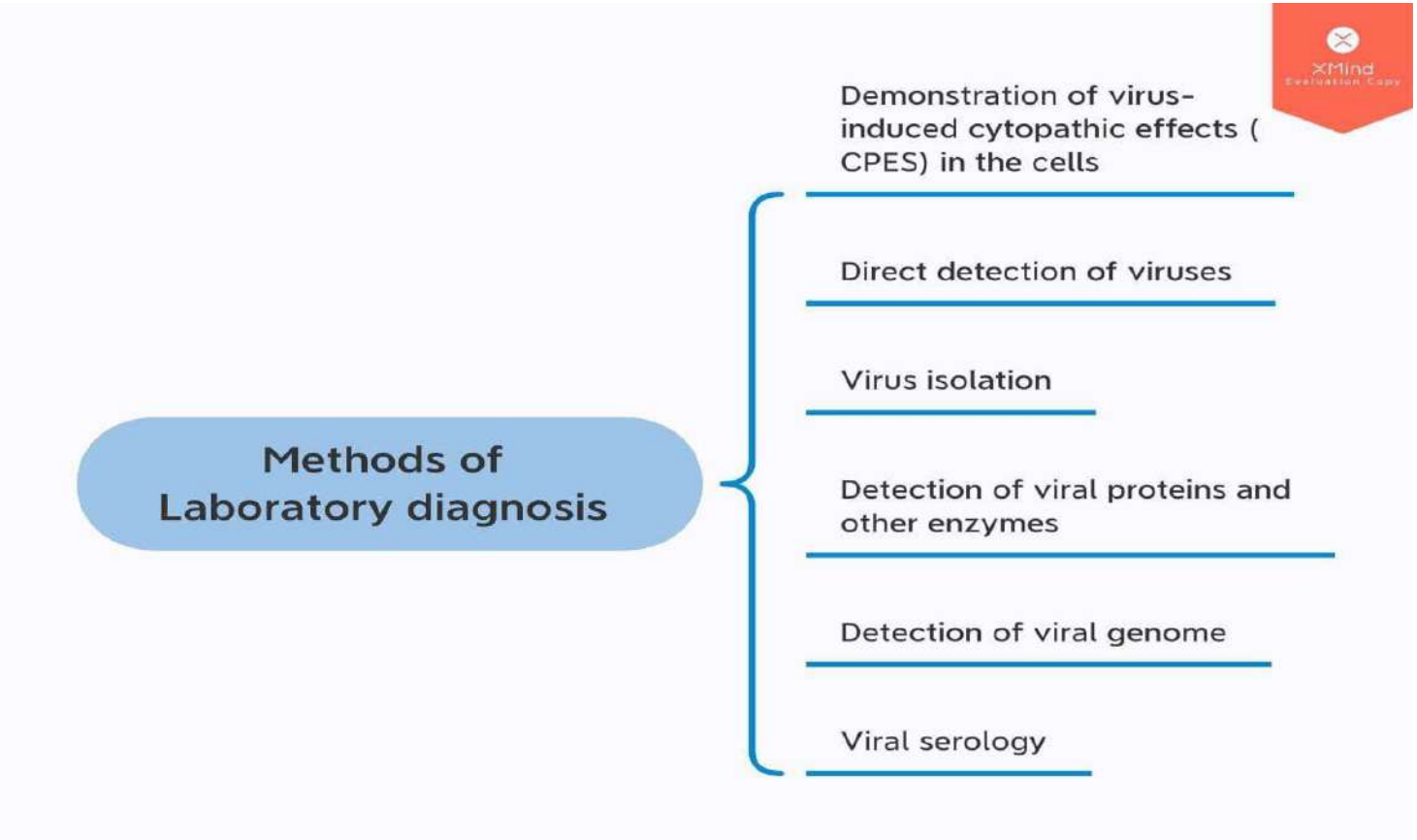
Animal Viruses



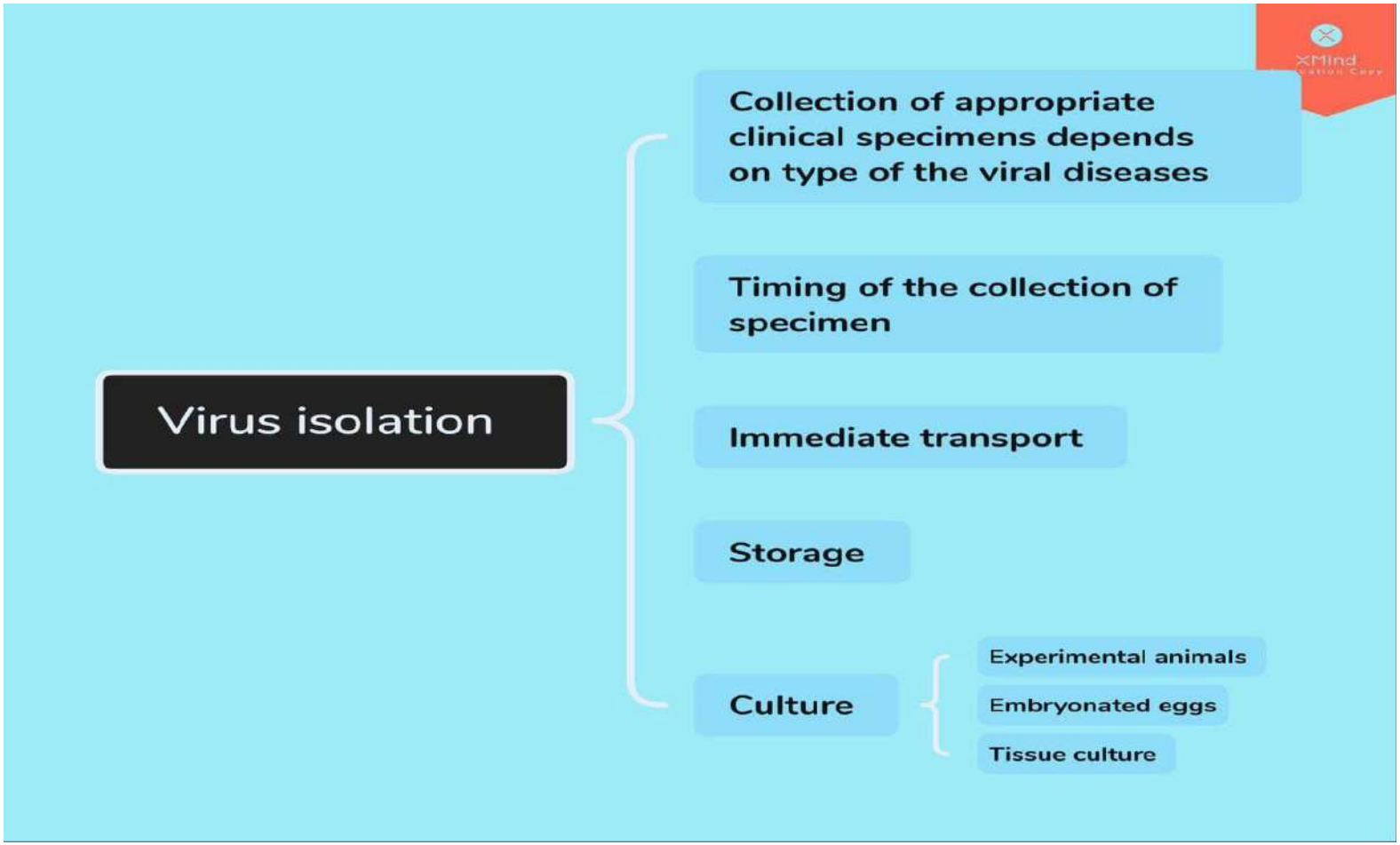
Summary of effects of viral infection on cells



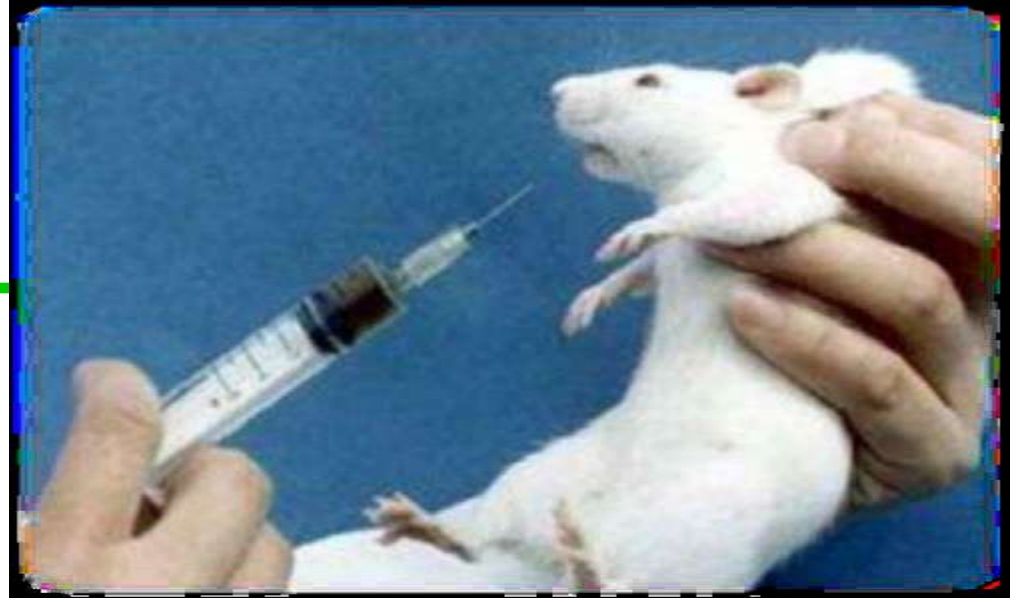
Methods of lab diagnosis



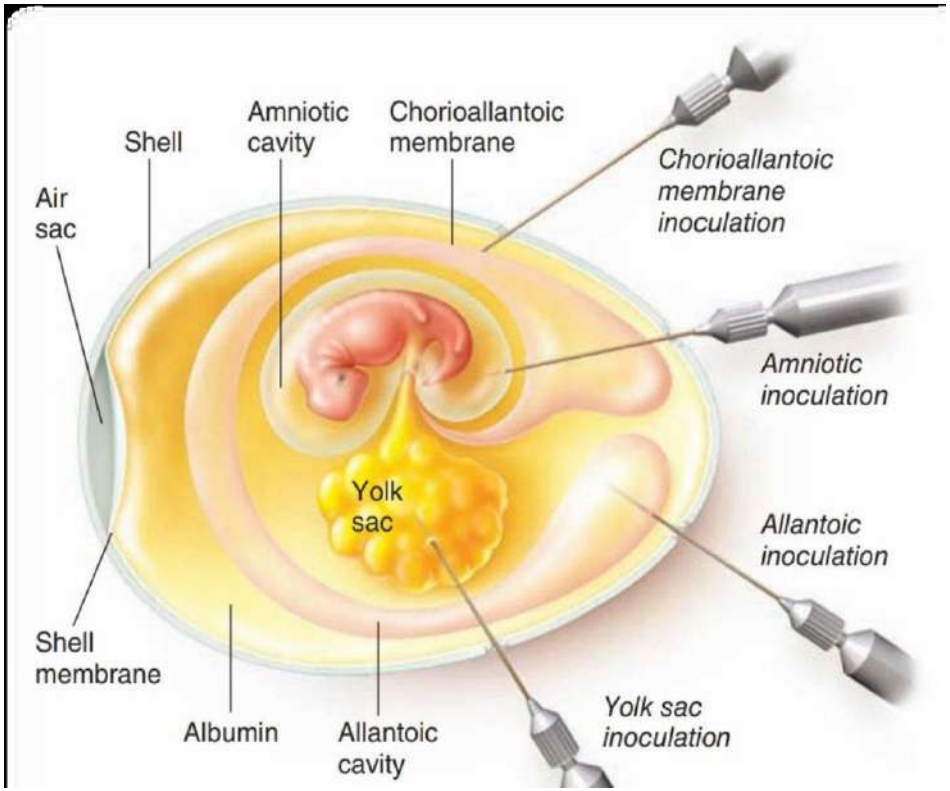
Viruses isolation

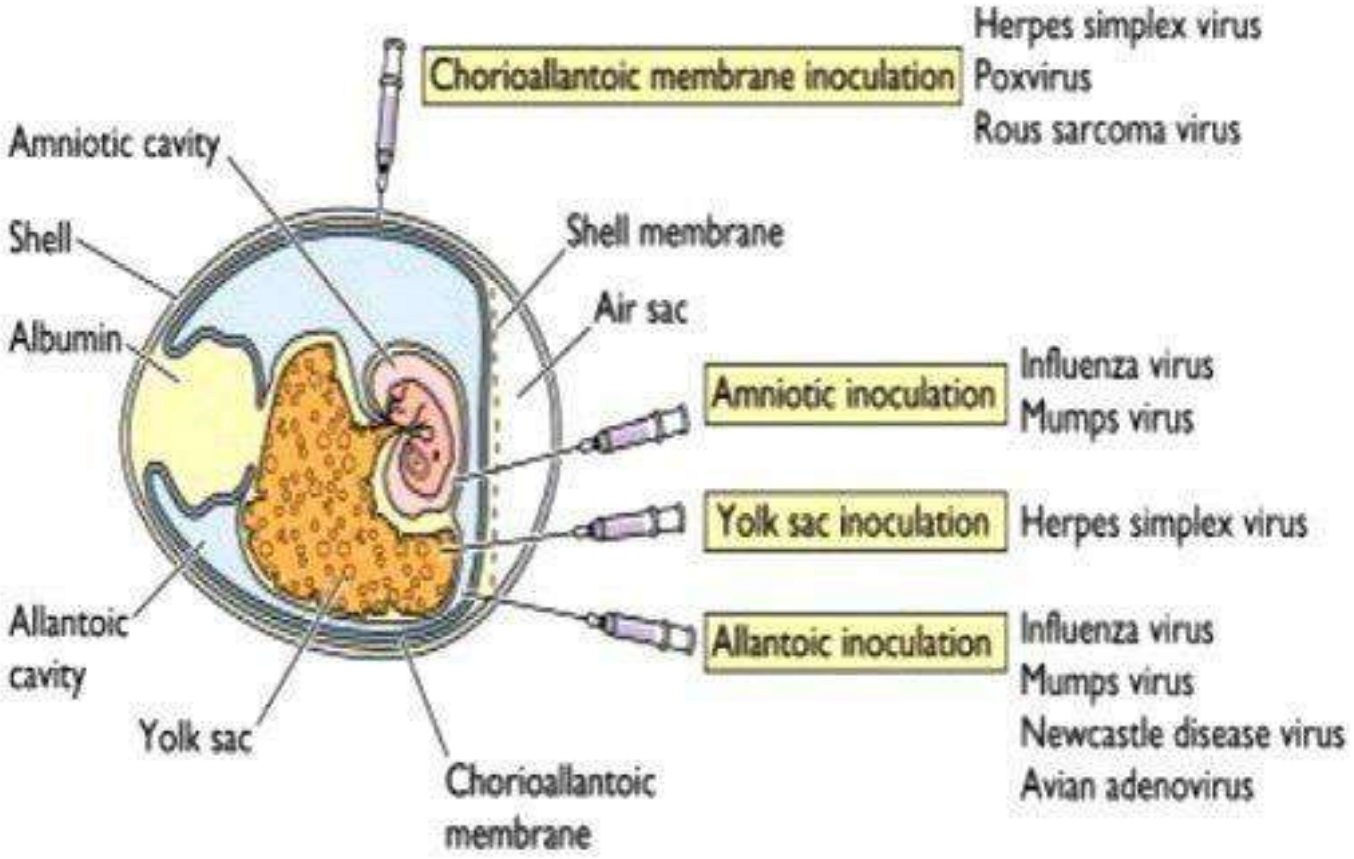


Animal inoculation



- Embryonated egg




Chorioallantoic membrane inoculation
Herpes simplex virus
Poxvirus
Rous sarcoma virus
Amniotic cavity
Shell
Albumin
Shell membrane
Air sac
Amniotic inoculation
Influenza virus
Mumps virus
Yolk sac inoculation
Herpes simplex virus
Allantoic cavity
Allantoic inoculation
Influenza virus
Mumps virus
Newcastle disease virus
Avian adenovirus
Yolk sac
Chorioallantoic membrane

Tissue culture

Cell culture

Explant

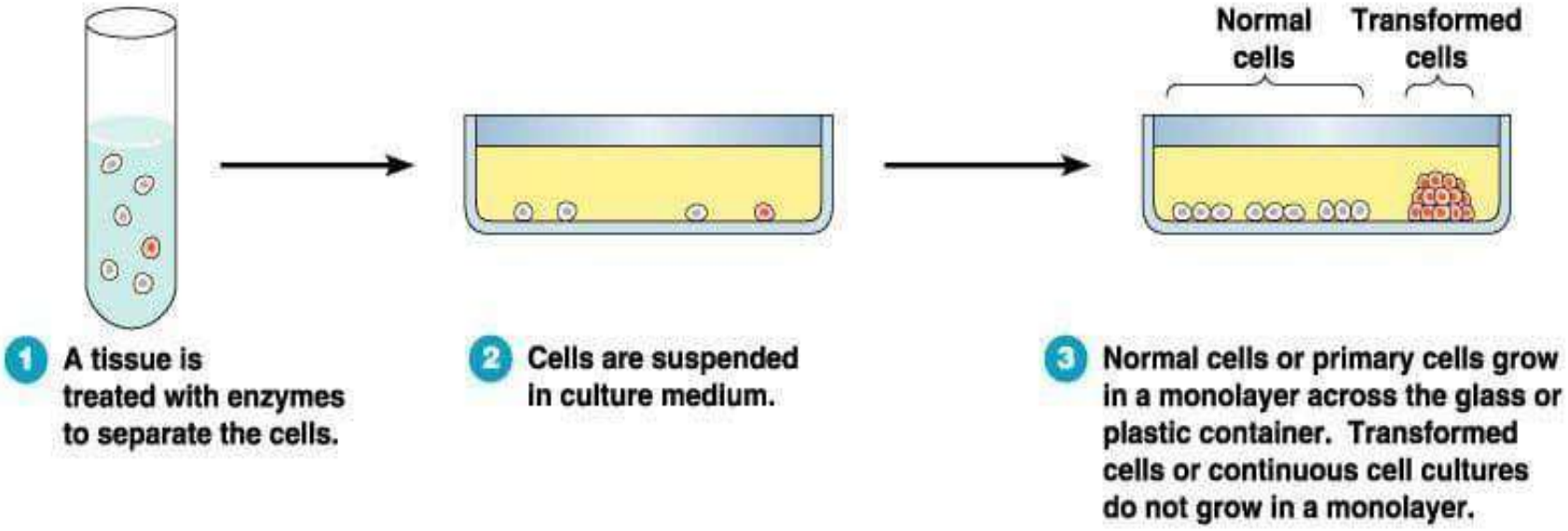
- Fragments of minced tissue can be grown as 'explant' embedded in plasma clots.
- They may be cultivated in suspension.
- Example: Adenoid tissue explants cultures used for isolation of adenovirus.

Organ

- 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

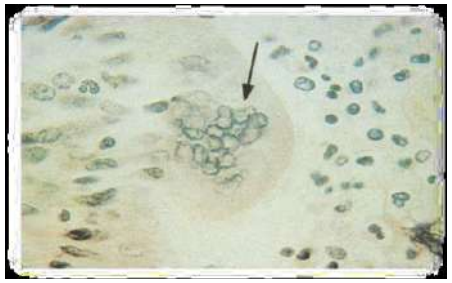
Because no one cell culture type can support the growth of all medically relevant viruses, virology laboratories must maintain several different cell culture types.

- The minimum requirements are:

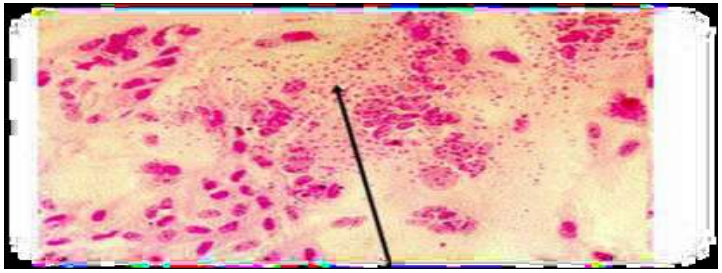
➢ Primary monkey kidney cell line: used for the isolation of respiratory and enteroviruses
➢ Human fibroblast line : Used for the isolation of cytomegalovirus (CMV), varicella-zoster virus (VZV), and rhinoviruses.
➢ A continuous human epithelial cell line such as HEp-2: required for the isolation of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

DETECTION OF VIRUS GROWTH IN CELL CULTURE

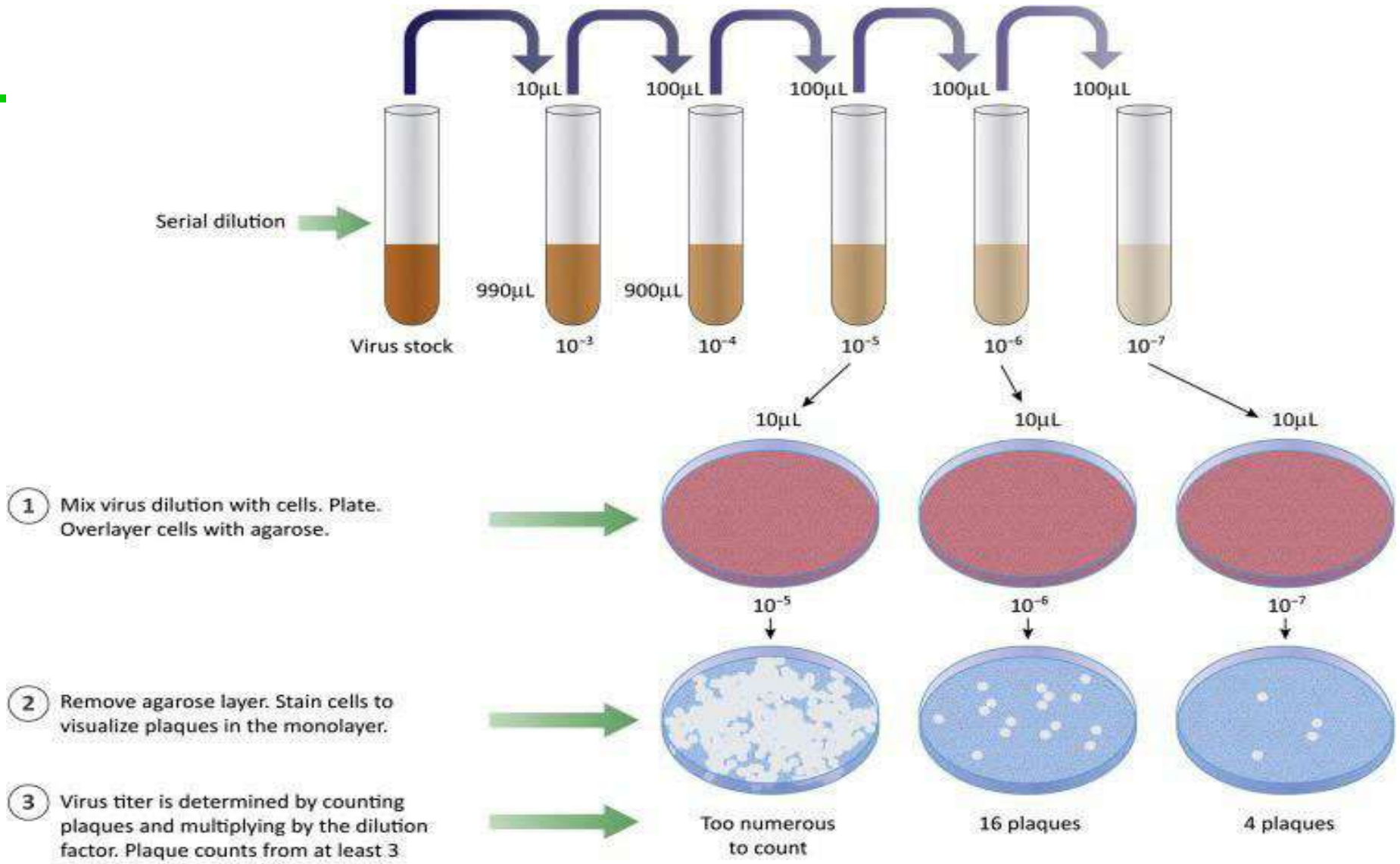
- Cytopathic effect



- Hemadsorption



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


10µL
100µL
100µL
100µL
100µL
Serial dilution
990µL
900µL
Virus stock
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10µL
10µL
10µL
1 Mix virus dilution with cells. Plate. Overlayer cells with agarose.
10^{-5}
10^{-6}
10^{-7}
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 fluores- cent 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.

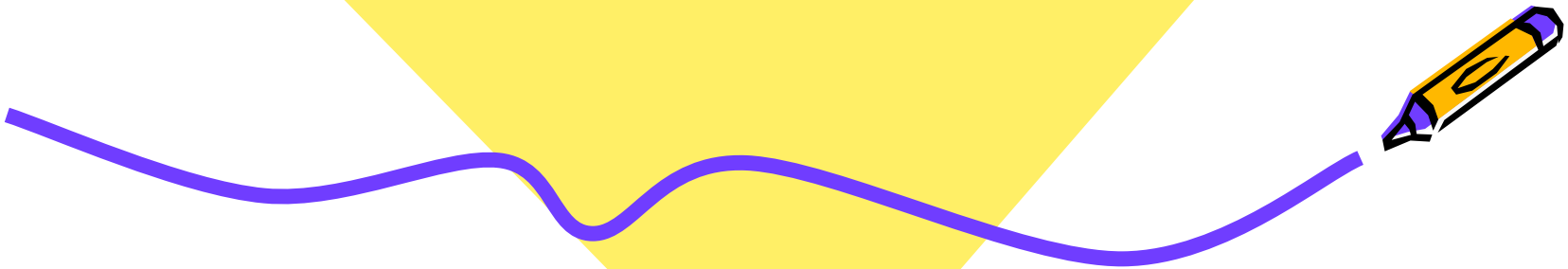
The limitations of serological tests in viral diseases are the following:

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 um--------1um
- Nucleus.
- Cytoplasm
- Cell membrane,

  Sterol---absent in bacteria
- Cell wall,

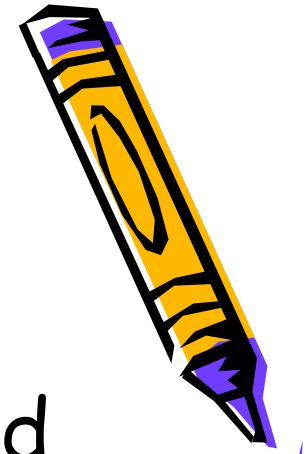
  Chitin ----peptidoglycane

- 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-acetyleglucasamine.

- 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, amphtericin B & azole group

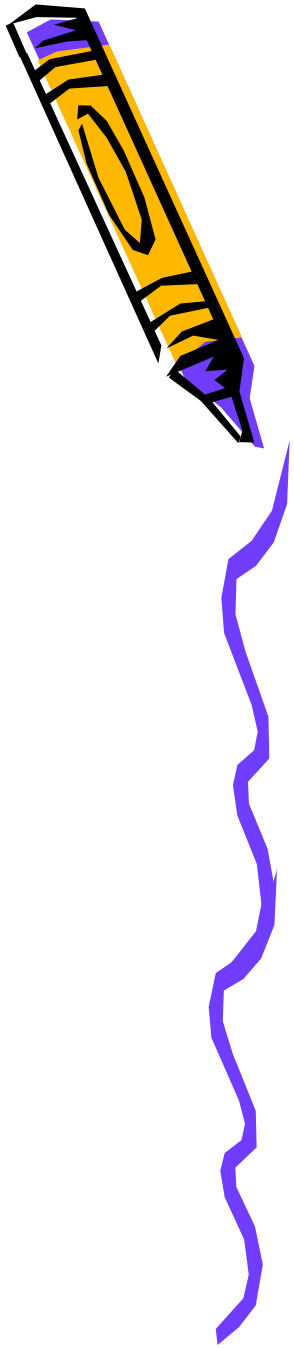
Atmospheric & carbon source requirements

- 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.

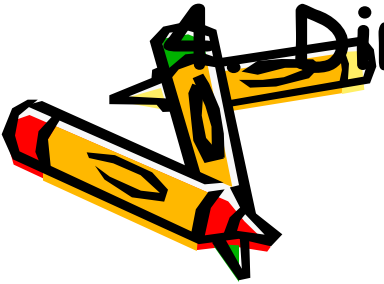
Natural habitat

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

Morphology of Fungi



1. Filamentous fungi (molds)
2. Yeasts
3. Yeast-like fungi
4. 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.

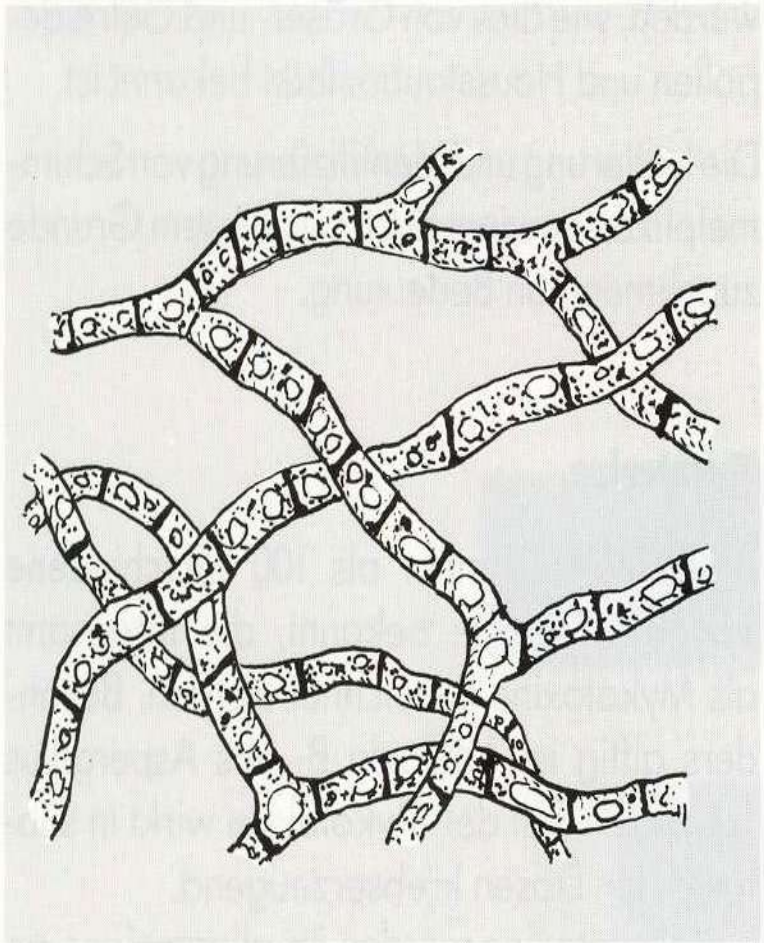


Abb. 47: Septiertes Myzel

mycelium: septate

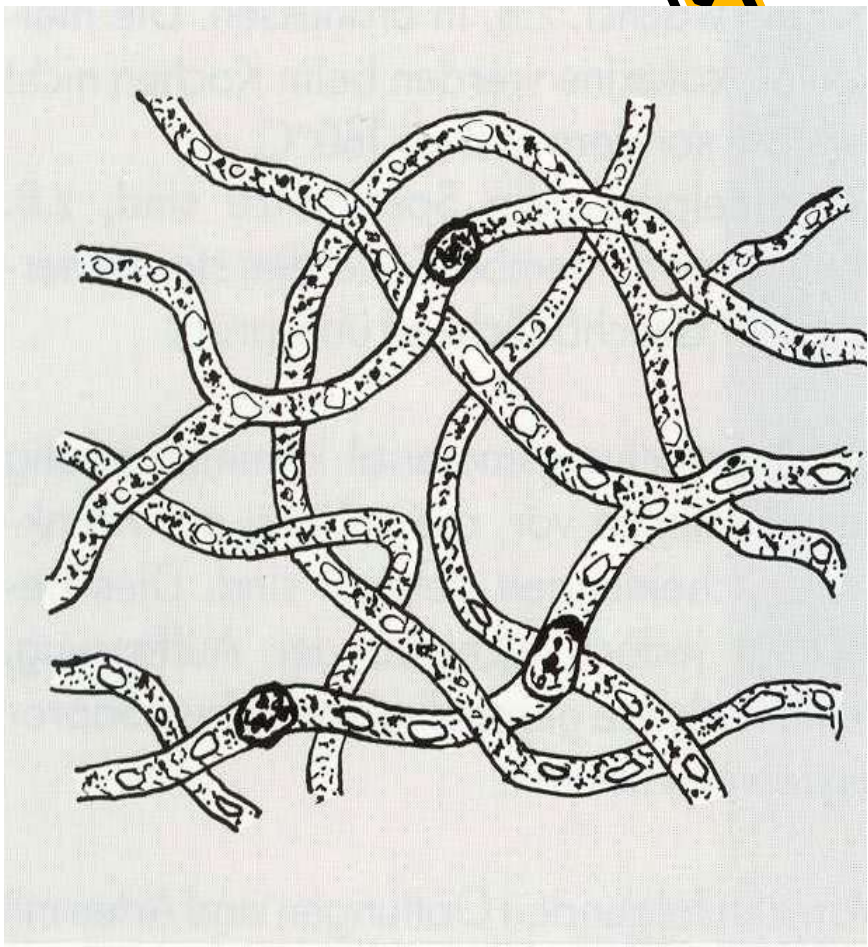
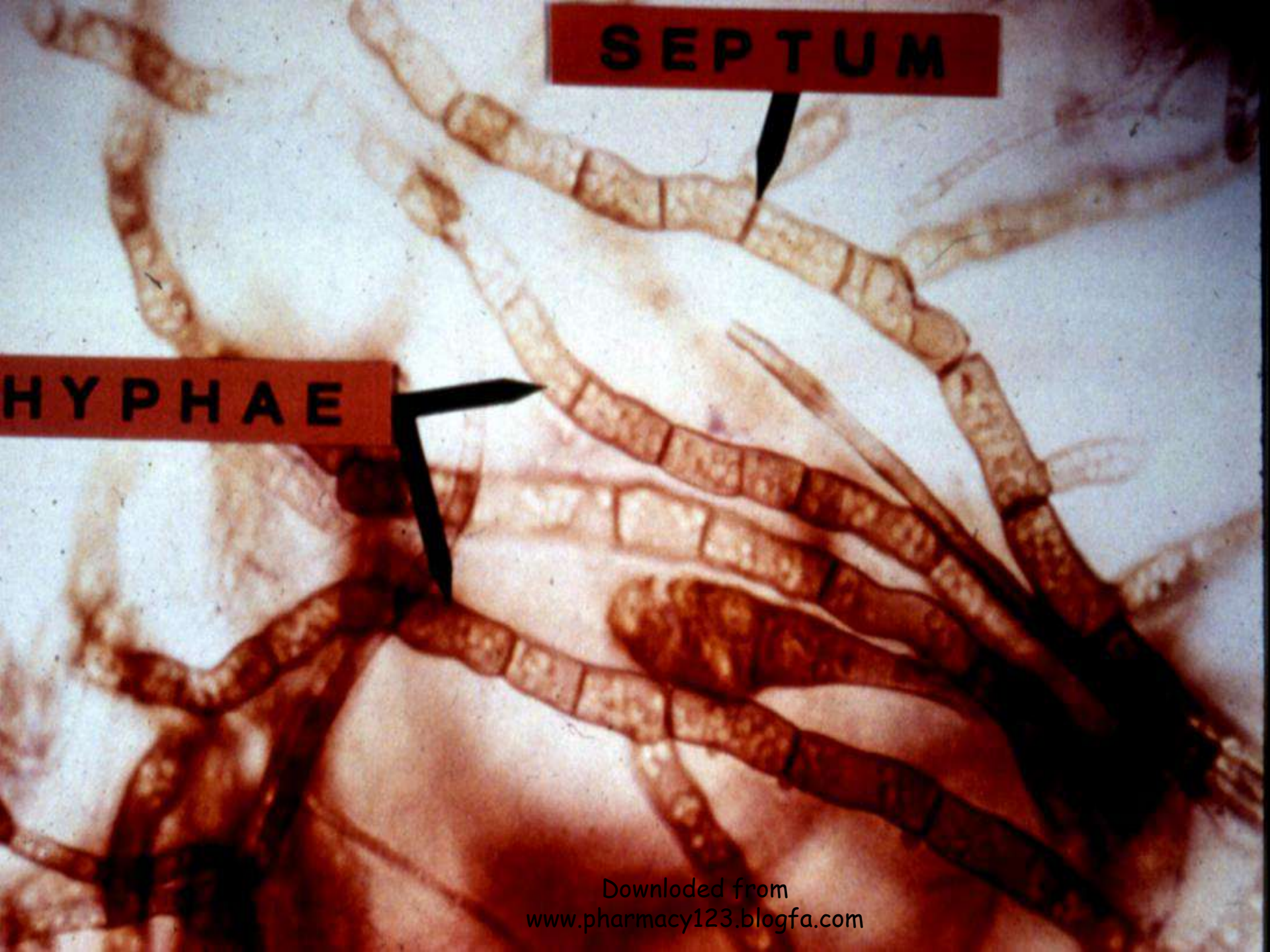
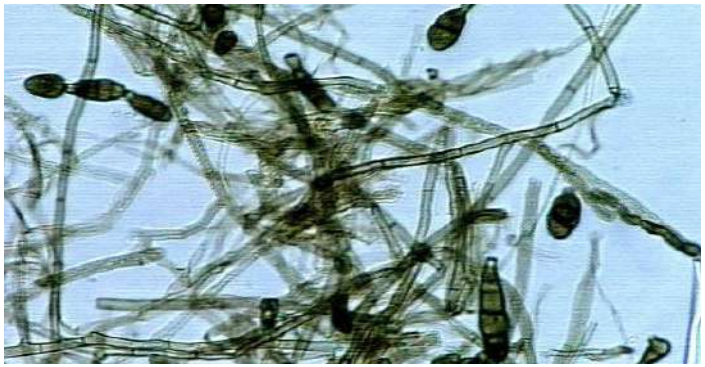


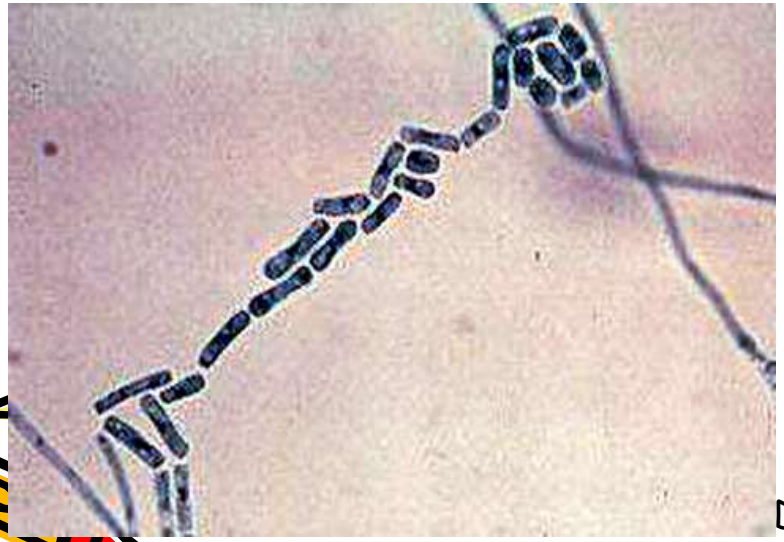
Abb. 48: Unseptiertes Myzel

mycelium: non septate



Mycelia & Conidia

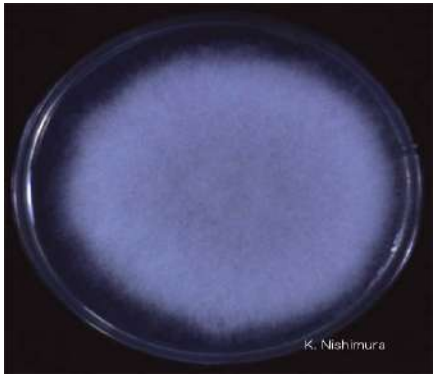


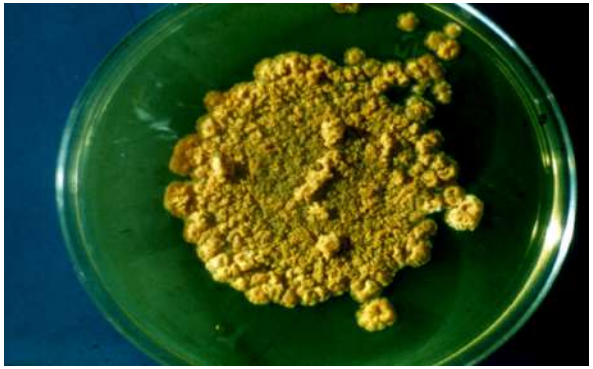


3. The surface of these colonies may be powdery, or may show a cottony aerial mycelium.

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

Colony Morphology





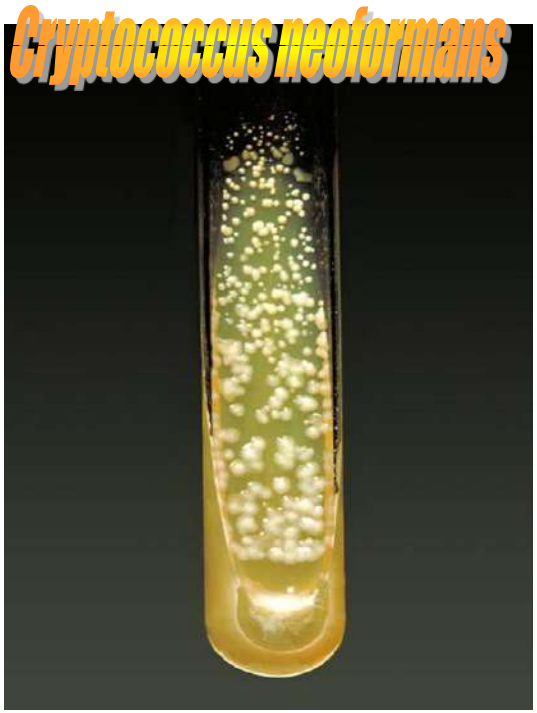
Yeasts

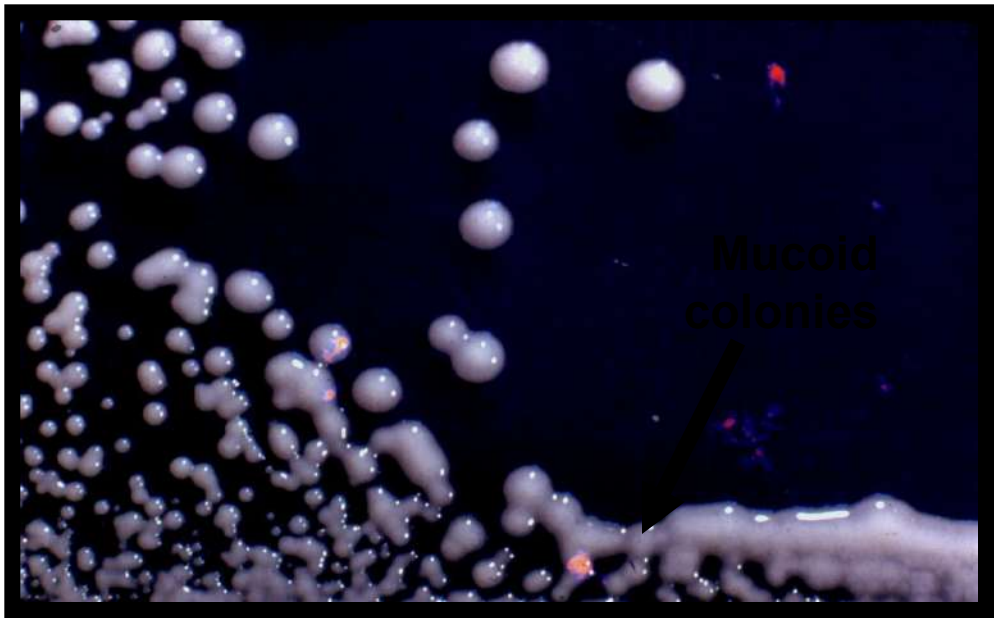
1. These occur in the form of round or oval bodies which reproduce by the formation of buds known as blastospores.

2. Yeasts colonies resemble bacterial colonies in appearance and in consistency.

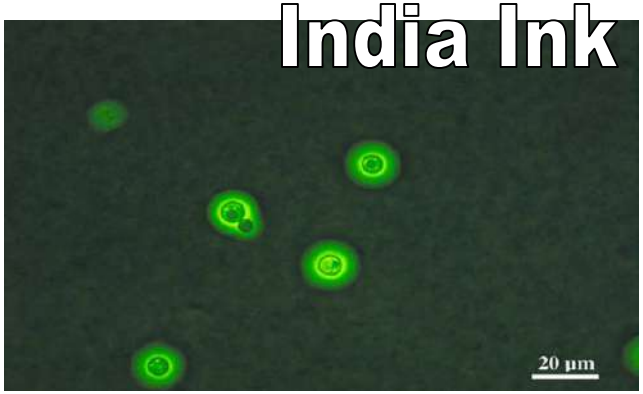
3. The only pathogenic yeast in medical mycology is *Cryptococcus neoformans*.

Yeast colonies





Cryptococcus neoformans

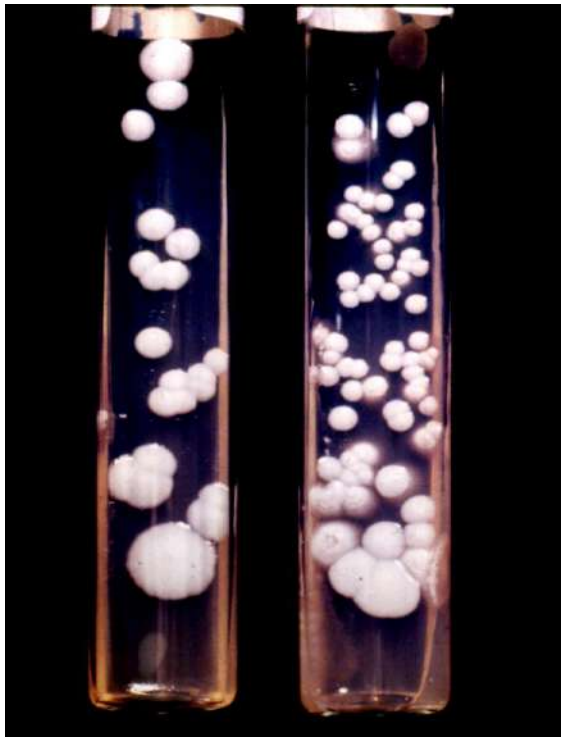


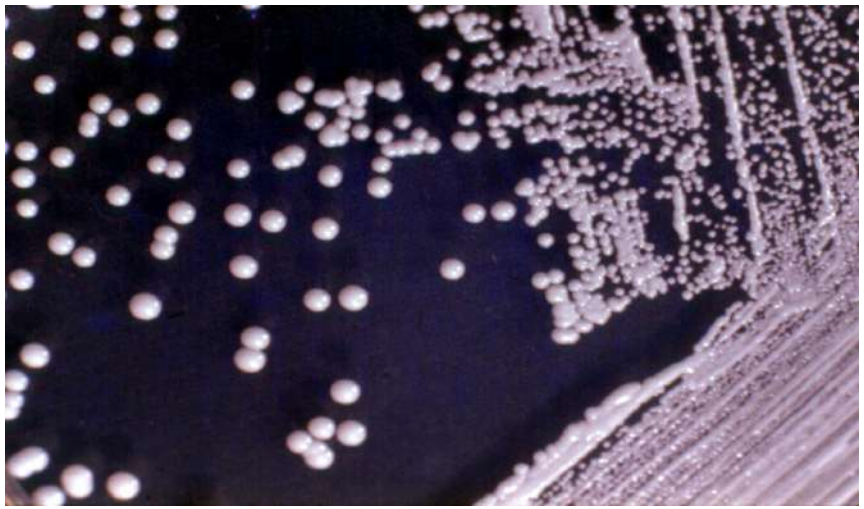
Yeast-Like

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***.

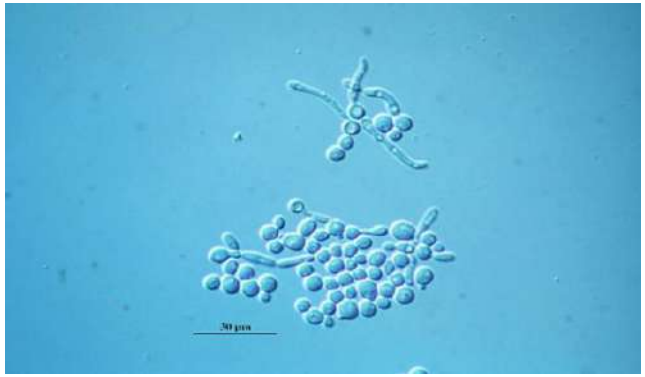
Candida Colonies

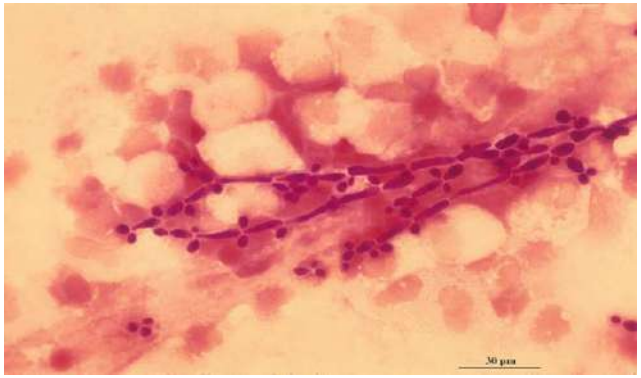


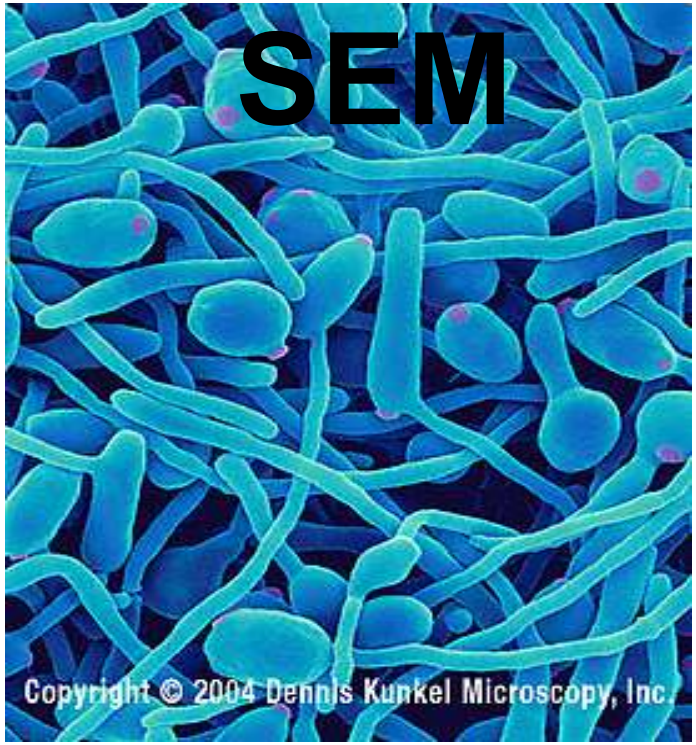


Candida albicans

Candida albicans





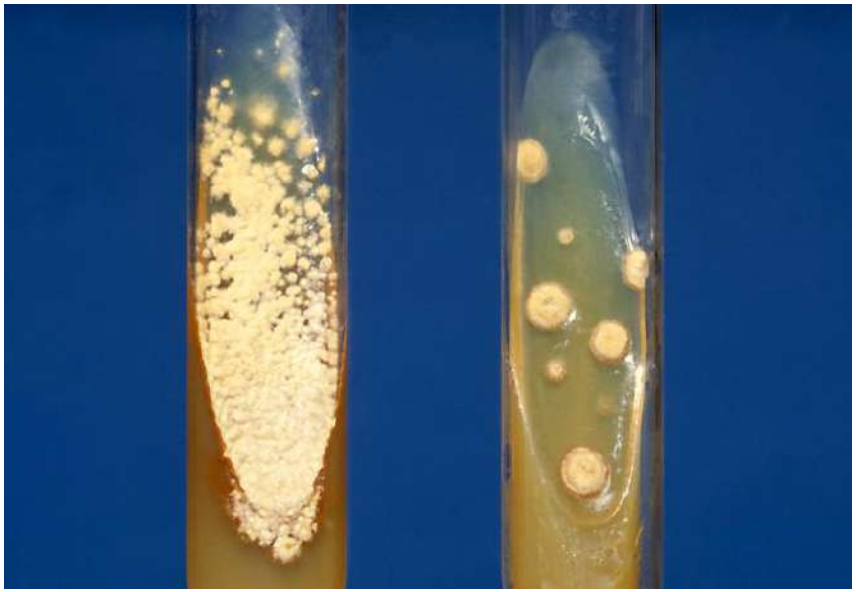


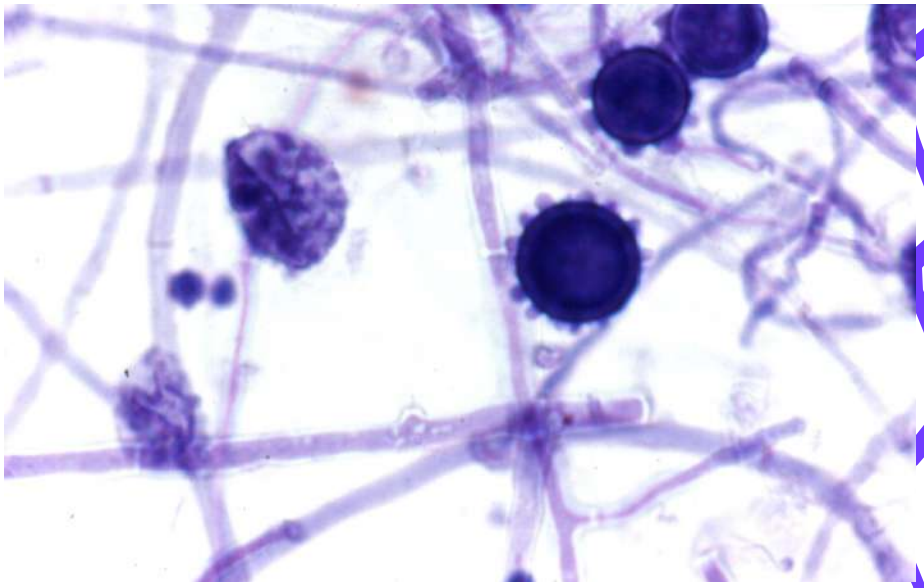
Thermally Dimorphic Fungi

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

Histoplasma capsulatum

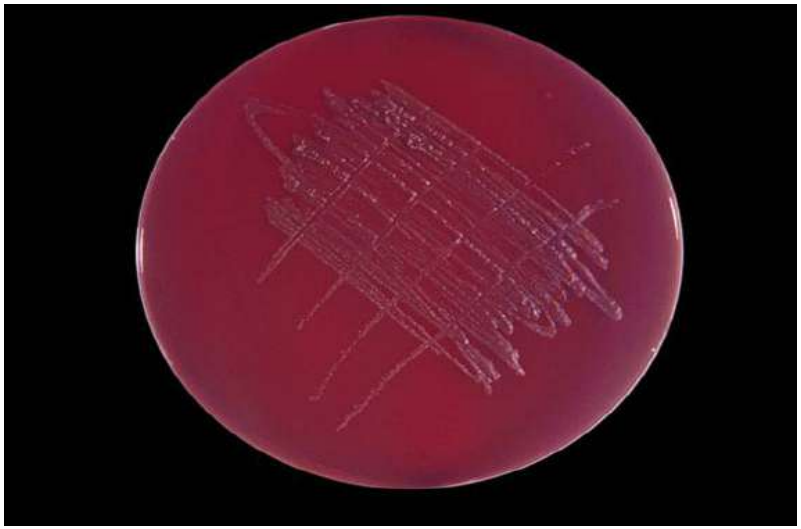
27oC

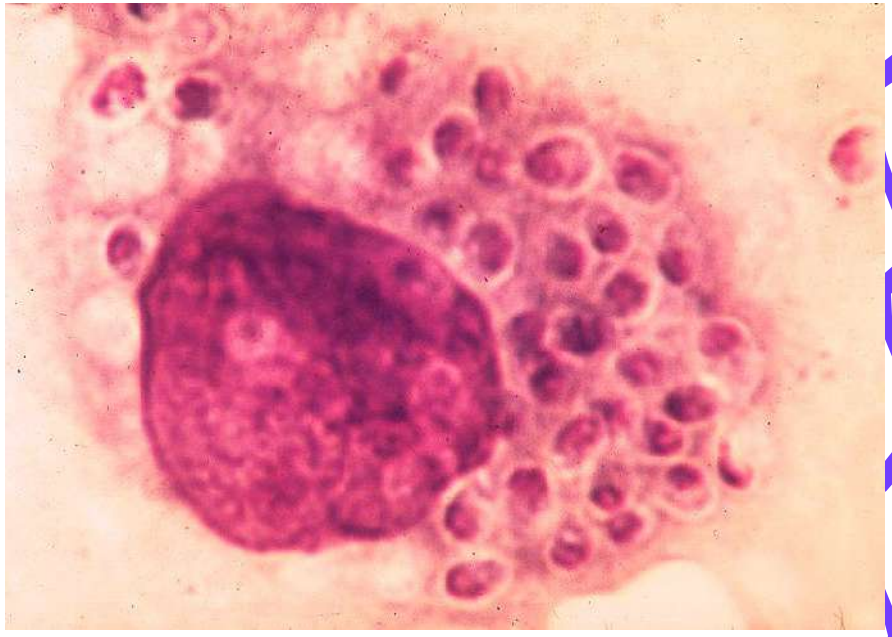




Histoplasma capsulatum

37oc

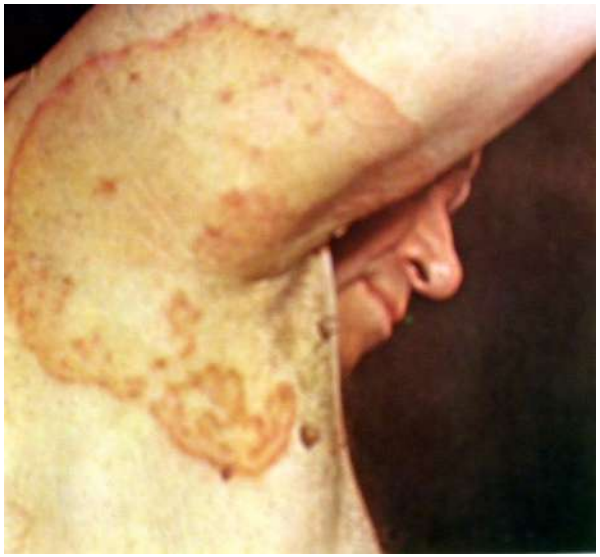


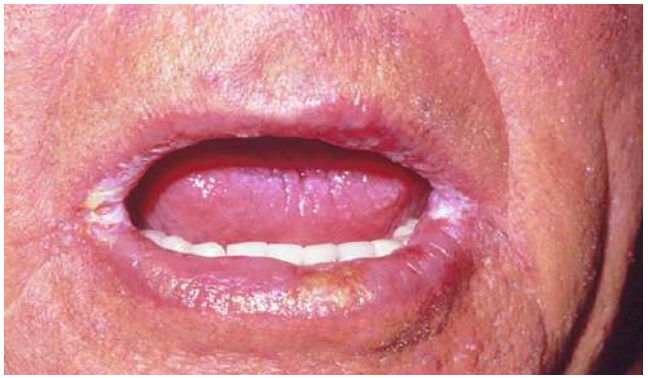


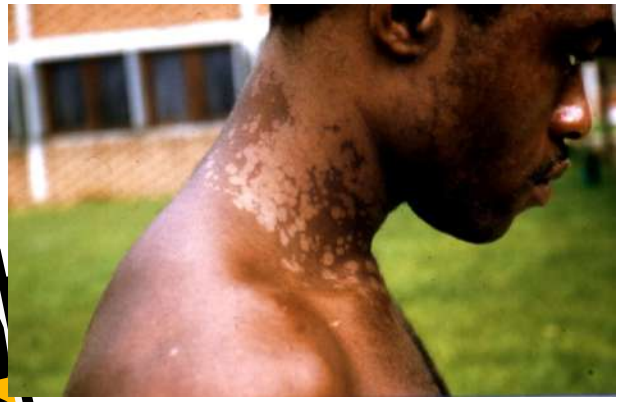
Human fungal infection;

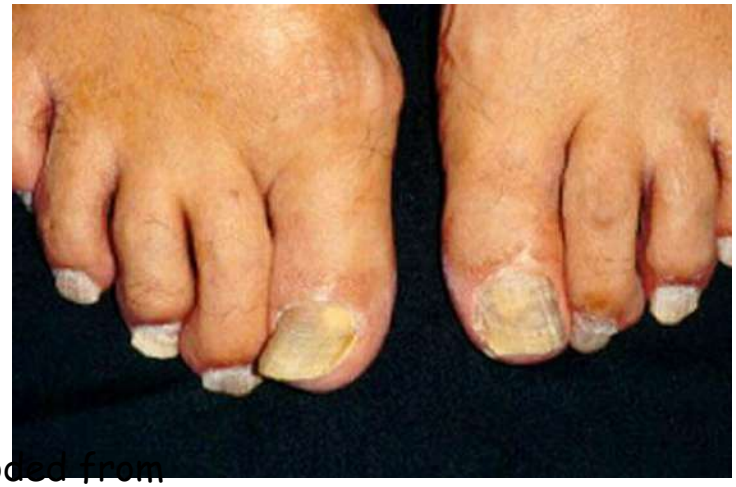
- Superficial
- Subcutaneous
- Systemic

Superficial mycoses

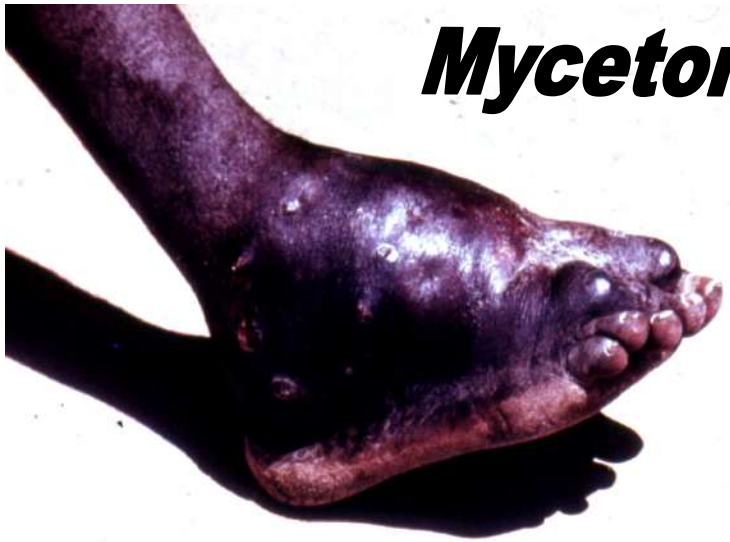








Subcutaneous mycoses



Fusiforms and Spirochaetes -Fusobacterium, leptotichia Treponema and oral Treponema

Dr. chateen I Ali,

prof dr hadeel M.younis

Fusobacteria

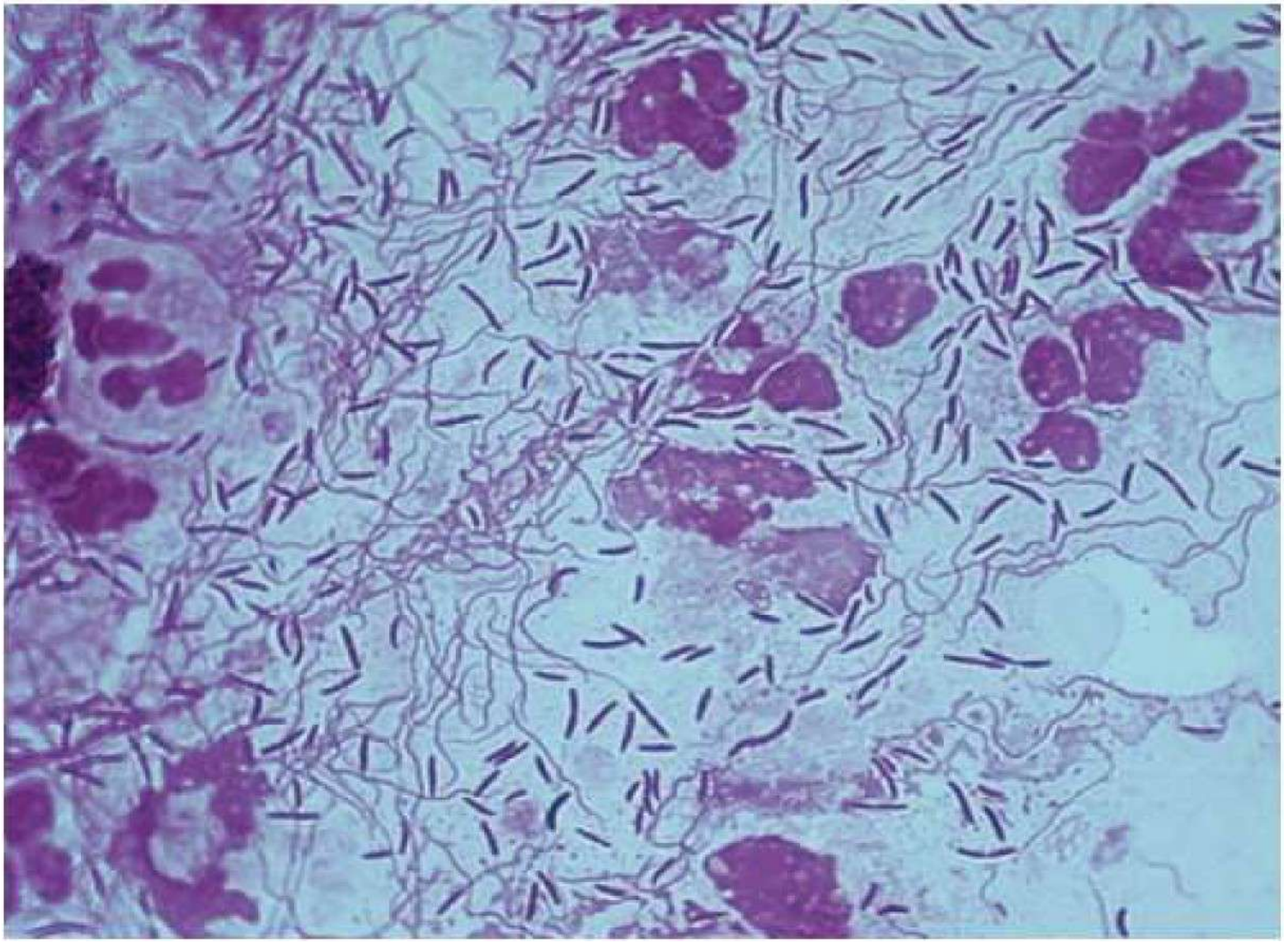
- are non-sporing, anaerobic, non-motile, non or weakly fermentative,
- **spindle-shaped bacilli** (with fusedends: hence the name).
- They are **normal inhabitants of the oral cavity**, colon and female genital tract
- **Types :**
- ***Fusobacterium nucleatum*** (the type species) : Causing **Fusospirochaetal** infections, which they cause in combination with spirochaetes,
- ***Fusobacterium periodontium*** and ***Fusobacterium simiae*** are isolated mainly from periodontal disease sites, and
- others such as ***Fusobacterium sulci*** are sometimes found in the healthy gingival sulcus.

- **Fusobacterium nucleatum**
- **Habitat and transmission**
- Several **subspecies** of F. nucleatum have been identified in **different habitats**.
- These include
- 1- ***F. nucleatum*** *subsp.* ***polymorphum***, found in the **healthy gingival crevice**
- 2- ***F. nucleatum*** *subsp.* ***nucleatum***, recovered mainly from **periodontal pockets**.
- 3- A third subspecies is ***F. nucleatum*** *subsp.* ***vincentii***.
- Infections are almost invariably endogenous.

Characteristics

- Gram-negative, strictly anaerobic, **cigar-shaped bacilli** with **pointed ends** . Cells often have a central swelling.
- A Gram-stained smear from **deep gingival** obtained from a lesion of **acute ulcerative gingivitis** is a simple method of
- demonstrating the characteristic **fusobacteria, together with**
- **spirochaetes** and **polymorphonuclear leukocytes**

Fig. 18.1 A photomicrograph of fusobacteria showing characteristic Gram-negative, cigar-shaped cells with pointed ends.



. **ig..** 8 .. 2 A Gram-stain- d smear obtaine. rom deep gingival pl·aque o a patfen with acute ulc rat[v · gingivitis (s aJso Fig 33.6) showrng 1,he usos **pi**rocha etaJ com **pl**ex. *Note:* the Iarg *e* ce·lJs a re po Iymo rp hs.

Culture and identification

- Grows on **blood agar as granular colonies with an irregular edge**. In *Fusobacterium nucleatum*, "fried egg" colonies after 3 to 5 days of incubation. Depending on the strain, they can be **hemolytic.**
- As fusobacteria can remove **sulphur** from **cysteine and methionine** to produce **odoriferous hydrogen sulphide** and **methylmercaptan**, they are
- thought to be associated with
- **halitosis.**

Pathogenicity

- 1- The **endotoxin** of the organism involved in the **pathogenesis of periodontal disease**.
- It possesses remarkable adherence properties
- 2- Also **fusobacterium adhesin A (FadA)**, which confers this property

- F. nucleatum is usually isolated from **polymicrobial infections**; it is rarely the sole pathogen. Thus, **in combination with oral spirochaetes** (*Treponema vincentii* and others), it causes the classic fusospirochaetal infections.
- These are:
- 1- **acute (necrotizing) ulcerative gingivitis or trench mouth**
- 2- **Vincent's angina**, an ulcerative tonsillitis causing tissue necrosis, often due to extension of acute ulcerative gingivitis •
- 3- **cancrum oris or noma**: a sequela of acute ulcerative gingivitis with resultant gross tissue loss of the facial region.

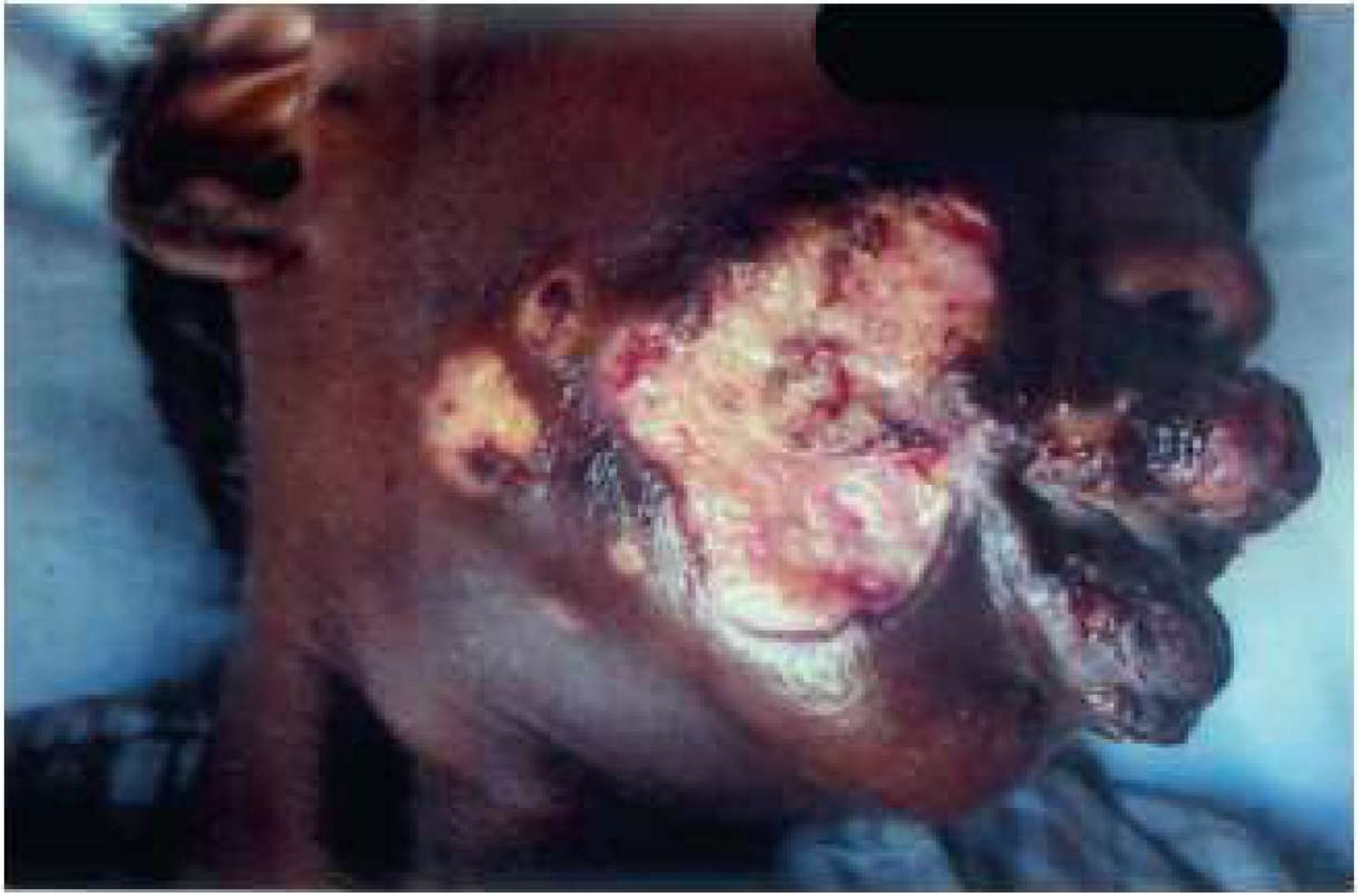


Fig. 33.7 Severe tissue destruction of the orofac1 l region in an Indian child with cancrum oris or noma.

As **fusobacteria coaggregate** with most other oral bacteria, they are believed to be important **bridging organisms** between **early and late colonizers during plaque formation**

Antibiotic sensitivity and prevention

Fusobacteria are uniformly sensitive to **penicillin** and, being strict anaerobes, are sensitive to **metronidazole**. Regular oral hygiene and antiseptic mouthwashes are the key to prevention of oral fusobacterial infections in susceptible individuals.

Leptotrichia

- ***Leptotrichia spp***. are **oral commensals** previously thought to belong to the genus **Fusobacterium**. They are :
- Gram-negative, strictly anaerobic, slender, filamentous bacilli, usually with one pointed end.
- ***Leptotrichia buccalis***, present in low proportions in **dental plaque**, is the sole representative of this genus.

Spirochaetes

- Spirochaetes are a diverse group of **spiral**, **motile organisms** three genera are human pathogens:
- 1-*Treponema* causes syphilis, and, in the oral cavity, acute necrotizing ulcerative gingivitis (together with fusobacteria)
- 2- *Borrelia* causes relapsing fever and Lyme disease •
- 3-Leptospira causes leptospirosis.

- Spirochaetes are helical organisms with a central protoplasmic cylinder
- The cell wall is **similar to Gram-negative bacteria** but stains poorly with the Gram stain.
- Underneath the cell wall run **three to five axial filaments** that are fixed to the extremities of the organism. **Contractions of these filaments** the bacterial cell body to give it its **helical shape**.
- The organism moves either by rotation along the long axis or by flexion of cells.
- immunofluorescence is more useful for identification purposes.
- All spirochaetes are strictly **anaerobic or microaerophilic.**

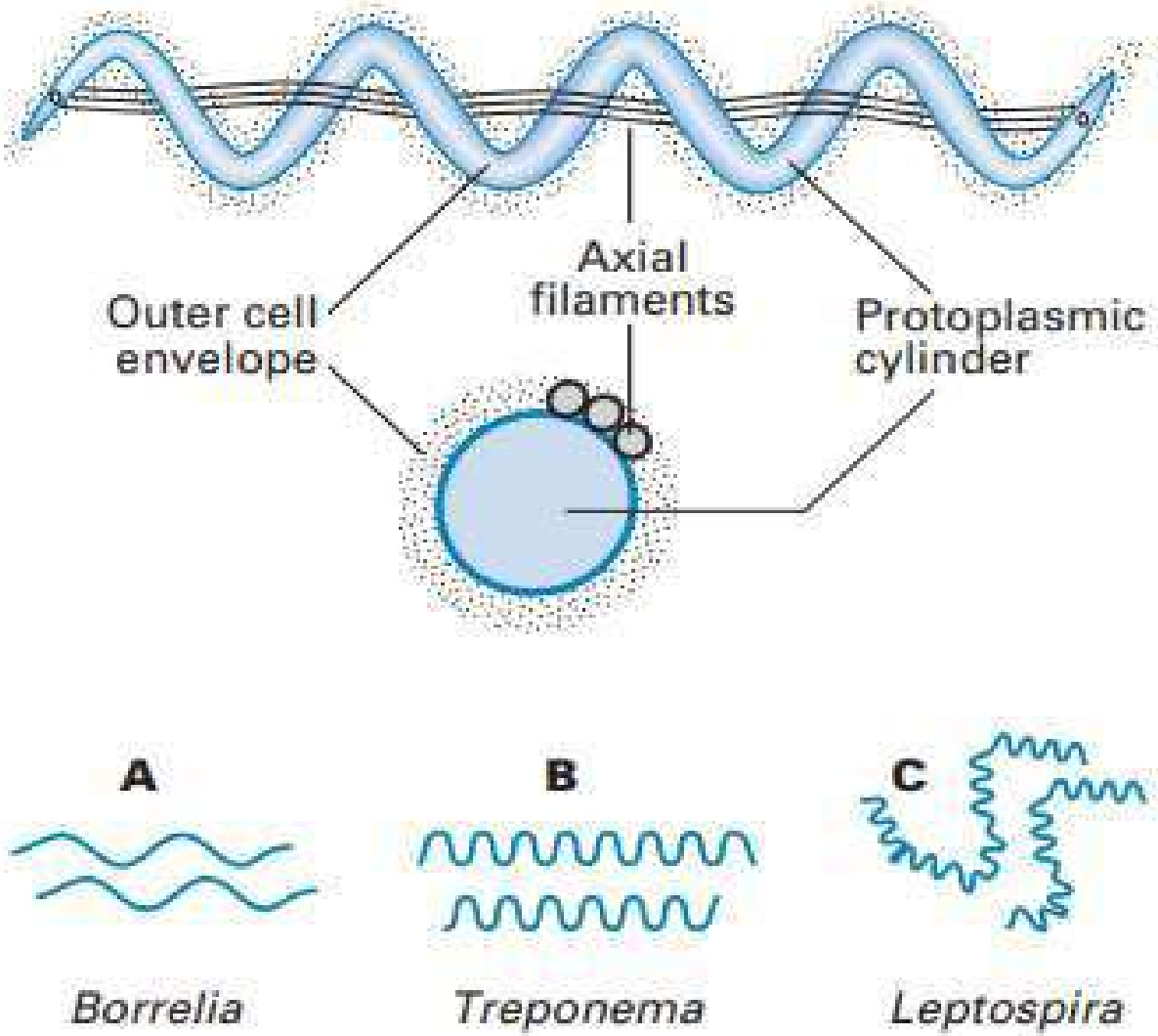


Fig. 18.3 Structure of a spirochaete (top) and the morphology of the three major genera of spirochaetes.

Treponema

- The coils of Treponema are regular . A number of species and subspecies are recognized,
- some of which are important **systemic pathogens**,
- while others are **oral inhabitants** implicated in periodontal disease.
- ***Treponema pallidum***
- **Habitat and transmission**
- **Lesions of primary and secondary syphilis**
  Transmission is by 1-**direct contact** with lesions usually during **sexual contact**, 2-**body secretions**, 3-**blood, 4-emen and 5-saliva**,, and 6- from mother to fetus by **placental transfer**.

Characteristics

- Slender, corkscrew-shaped cells with 6–12 evenly spaced coils, 6–14 × 0.2 μm;
- **strictly anaerobic** and extremely sensitive to **drying and heat**, hence dies rapidly outside the body

Culture and identification

- ***Treponema pallidum*** Cannot be cultured in **vitro**, but can be propagated in the testes of rabbits;
- tissue fluid from primary and secondary clinical **lesions** helps identification, but serological tests are the mainstay of diagnosis.

Pathogenicity

- Causes syphilis, a sexually transmitted disease
- The **virulence factors** of *T. pallidum* are not well characterized..
- Antibiotic sensitivity and control **Penicillin is the drug of choice**; for allergic patients, **tetracycline is an alternative**. Prevention of syphilis is based on early detection.

Oral treponemes

- All oral spirochaetes are classified in the genus *Treponema*.
- Although **many species** have been described, only **four have been cultivated** :
- 1- *Treponema denticola,*
- *2- Treponema vincentii,*
- *3- Treponema pectinovarum and*
- *4- Treponema socranskii.* Habitat and transmission Predominantly, the oral cavity of humans and primates, at the gingival margin and crevice in particular. Transmission routes are

Habitat and transmission

- Predominantly, the **oral cavity of humans and primates**, at the **gingival margin and crevice** in particular.
- Transmission routes are **unknown**. Infections are **endogenous**.
- Characteristics Motile, helical rods, 5–15 × 0.5 μm, with irregular (three to eight) spirals, which are less tightly coiled than, for instance, ***T. pallidum*** Cell walls are **Gram-negative but stain poorly**. The size is variable and can be used as a basis for classification (large, medium or small).

Culture and identification

- **In contrast** to *T. pallidum*, oral spirochaetes can be grown **in vitro**.
- They are **strict anaerobes**, slow-growing in **oral treponema isolation (OTI) medium**.
- Suspect lesions of **acute necrotizing ulcerative gingivitis** or **advanced periodontitis** can be examined by obtaining a Gram-stained smear of deep gingival plaque and visualizing the characteristic fusospirochaetal complex under light microscopy alternatively, dark-ground microscopy may be used.

Pathogenicity

- These organisms are
- 1- a component of the **fusospirochaetal complex** of a**cute necrotizing ulcerative gingivitis and Vincent's angina**,
- 2- and are a coagent of **advanced periodontal disease**.
- 3- The ability to **travel** through **viscous environments** enables oral spirochaetes to :
- a- migrate within the **gingival crevicular fluid**

- b - and to **penetrate sulcular epithelial linings** as well as
- C- Penetration of **gingival connective tissue**.
- Virulence factors are little known; **endotoxin** is possibly contributory to disease. **T. denticola** is more 1- proteolytic than other species and 2- degrades collagen and dentine.
- Antibiotic sensitivity and control Sensitive to penicillin and metronidazole. Prevention of infection is achieved by good oral hygiene practices.

Borrelia burgdorferi

- Pathogenicity
- The agent of Lyme disease,
- a generalized infection with **neurological and cardiac manifestations and arthritis**. One of the earliest and most common neurological manifestations is **unilateral facial palsy**.
- Antibiotic sensitivity Sensitive to tetracycline and amoxicillin.

Normal oral microflora

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Oral Microflora

- Oral flora comprises a diverse array of organisms and includes :
- **eubacteria,**
- **archaea,**
- **fungi,**
- **mycoplasmas,**
- **protozoa**
- and possibly a **viral flora** that may persist from time to time.
-

- These organisms usually live in **harmony** in a range of habitats including :
- the **teeth,**
- **gingival sulcus,**
- **tongue,**
- **cheek,**
- **hard and soft palate**
- **and tonsils.**
- Collectively the oral flora have been termed **oral microbiota**, and more recently, the **oral microbiome**

- **Bacteria** are by far the **predominant group** of organisms, and there are probably some **500 to 700** common oral species of which only **50 to 60%** are **cultivable**.
- The remaining **unculturable flora** are currently being identified using **molecular techniques.**

- In fact that the oral cavity has a **wide range** of **sites (habitats)** with **different environmental conditions**, makes the study of oral microbiology complex and difficult.
- despite the enormous **diversity** and **complexity** of the oral flora, many organisms commonly isolated from **neighbouring ecosystems** such as the **gut** and **skin** are not found in the mouth,
- That indicating the **unique and selective ecology** of the oral cavity with regard to microbial colonization.

- The main bacterial genera found in the oral cavity are well characterized using mostly traditional culture-based techniques. Oral bacteria can be **classified** primarily as **Gram positive** and **Gram-negative** organisms, and secondarily as either **anaerobic** or **facultatively anaerobic** according to their oxygen requirements. Some oral microbes are more closely associated with disease than others

Flora of the oral cavity

- **Gram-positive cocci Genus Streptococcus**
- **mutans group**
- • Main species: Streptococcus **mutans** serotypes c, e, f, k; Streptococcus **sobrinus** serotypes d, g; Streptococcus **ratti** (previous Streptococcus rattus) serotype b.
- • Main intraoral sites and infections: tooth surface, dental caries.

- **salivarius group** •
- Main species: Streptococcus **salivarius**; Streptococcus **vestibularis**.
- Main intraoral sites and infections: **dorsum of the tongue and saliva**; Streptococcus vestibularis mainly reside in the vestibular mucosa (hence the name); not a major oral pathogen.

- **anginosus group**
- • Main species: Streptococcus **intermedius**; Streptococcus **anginosus**.
- Main intraoral sites and infections: **gingival crevice**; dentoalveolar and endodontic infections.
- **mitis group**
- • Main species: Streptococcus **mitis**, Streptococcus **sanguinis** , Streptococcus **gordonii**, Streptococcus **oralis**, Streptococcus **parasanguinis**, Streptococcus **australis**, Streptococcus **infantis**.
- • Main intraoral sites and infections: mainly **dental plaque biofilms**, **tongue** and **cheek**, **dental caries** (?), **infective endocarditis** (except Streptococcus mitis).

Anaerobic streptococci

- • Main species: **Peptostreptococcus anaerobius**, **Micromonas micros** (previously Peptostreptococcus micros), **Finegoldia magnus** (previously Peptostreptococcus magnus)
- group acronym **GPAC** – Gram-positive anaerobic cocci.
- • Main intraoral sites and infections: **teeth,** especially **carious dentine**, **periodontal** and **dentoalveolar abscesses** in mixed culture.

unculturable bacteria

- it is now estimated that only about **50%** of the oral bacteria that can be visualized by microscopy can be cultivated through **traditional laboratory culture** techniques.
- The identity and the role of these so-called **unculturable bacteria** is mostly an enigma.
- There are **two major reasons** that these bacteria cannot be cultured.
- **First**, their nutritional requirements are unknown, and
- second, they **coexist in a supportive ecosystem** with **neighbouring organisms** that sustain them nutritionally as well as physically

Oral protozoa

- **Genus Entamoeba**
- Large, motile amoebae about 12 μm in diameter: • Main species: ***Entamoeba gingivalis***.
- • Main intraoral sites and infections: **periodontal tissues**, especially in patients who have received **radiotherapy** and are **on metronidazole**. Its role, if any, in periodontal disease is unclear.
- **Genus Trichomonas** Flagellated protozoa, about 7.5 μm in diameter:
- • Main species: **Trichomonas tenax**.
- Main intraoral sites and infections: **gingival crevice**; its role in disease is unclear.

The oral ecosystem

- **Ecology** is the study of the **relationships** between **living organisms** and their **environment**.
- An understanding of oral ecology is **essential** in **pathogenesis of diseases**, such as **caries and periodontal disease**, caused by oral bacteria.

- The **oral environment** The human mouth is **lined by stratified squamous epithelium**. This is **modified** in areas according to **function** (e.g. the **tongue**)also containing **teeth and salivary ducts**.
- there is a continuous exudate of **crevicular fluid** from the **gingival crevice**. A **thin layer of saliva** bathes the surface of the oral mucosa.
- The **community of microbial residents** in our body is called the microbiome. So The **community of microbial residents** in oral cavity is called the oral microbiome. **Oral microbiome**, **oral microbiota** or **oral microflora** refers to the microorganisms found in the human oral cavity

- 1-This **commensal (or indigenous, or resident)** flora exists always in its ecosystem and habitat in harmony with the host, but when this relationship is broken disease condition can develop .
- The predominant dental diseases in humans (caries and periodontal disease) are caused in this manner.
- 2-In addition to the **commensal flora**, there are others (such as **coliforms**) **that survive in the mouth only for short periods** (**transient flora**). These transient flora cannot get a **foothold** in the oral environment due to the **ecological pressure**, i.e. the **colonization resistance** exerted by the **resident flora**.
- Indeed, the latter are considered critical in defending the key portal of entry into the digestive system, by offending pathogens.

- **3-Supplemental flora**: It comprises species that are **nearly always present**, but in low numbers **(<1%).**
- These organisms may become indigenous if the **environment changes**. A single bacterial species can be supplemental species in one mouth and indigenous species in another.

oral ecosystem

- The oral ecosystem comprises the
- 1- oral flora,
- 2-the different sites of the oral cavity where they grow (i.e. habitats) and
- 3-the associated surroundings.
- Oral habitats The **major oral habitats** are:
- **1-buccal mucosa**
- **2-• dorsum of the tongue**
- 3-tooth surfaces (both supragingival and subgingival)
- 4-• crevicular epithelium
- 5-• prosthodontic and orthodontic appliances, if present.

Buccal mucosa and dorsum of the tongue

- the **tongue** is **highly colonized** because of the safe refuge provided by the papillae. The **papillary surface** of the tongue has a **low redox potential** (Eh), promoting the growth of **anaerobic flora**, and thus may serve as a **reservoir** for some of the **Gram-negative anaerobes** implicated in **periodontal disease.**
- Further, the **keratinized and non-keratinized** mucosae may offer refuge to variants of oral flora.

Teeth

- The surfaces of the teeth are the only **non-shedding area** of the body that harbours a microbial population. Large masses of bacteria and their products accumulate on tooth surfaces to produce **dental plaque**, present in both health and disease. Plaque is a classic example of a **natural biofilm** and is the **major agent initiating caries and periodontal disease**.
- A range of habitats are associated with the tooth surface . : **smooth surfaces** are colonized by a **smaller number of species** than **pits and fissures**; **subgingival surfaces** are more anaerobic than **supragingival surfaces.**

Factors modulating microbial growth

- Different microenvironments in the mouth support their own microflora, which differ from each other
- The reasons for such variations are complex and include :
- 1- **Anatomical factors**
- A-• the shape of the teeth
- B- • the topography of the teeth (e.g. occlusal fissures)
- C-• poor quality of restorations (e.g. fillings and bridges)
- D-• non-keratinized sulcular epithelium.
- These areas are **difficult to clean**, either by the natural flushing action of saliva or by tooth-brushing

2- Saliva

- The major organic constituents of **saliva** are **proteins and glycoproteins** (**such as mucin**), which **modulate bacterial growth** in the following ways:
- A- • **adsorption on the tooth surfaces** forms a **salivary pellicle**, a conditioning film that facilitates bacterial adhesion
- B- • acting as a primary source of food (**carbohydrates and proteins**)
- C- • **aggregation of bacteria**, thereby facilitating their clearance from the mouth, or deposition on surfaces, contributing to plaque formation
- D- • **growth inhibition** of exogenous organisms by nonspecific defence factors, e.g. **lysozyme**, **lactoferrin** and **histatins**, **which are bactericidal**
- **E-•** maintenance of pH with its excellent buffering capacity (acidic saliva promotes growth of cariogenic bacteria)

3- Gingival crevicular fluid

- There is a continuous but slow flow of gingival crevicular fluid in health, and this increases during inflammation (e.g. gingivitis). The composition of crevicular fluid is similar to that of **serum**
- Crevicular fluid can influence the ecology of the crevice by:
- A- • flushing microbes out of the crevice
- B- • acting as a primary source of nutrients:
- C-• maintaining pH conditions
- D- providing specific and non-specific defence factors: IgG predominates (IgM and IgA)
- E- • phagocytosis: 95% of leukocytes in the crevicular fluid are neutrophils.

4- Microbial factors

- Microbes in the oral environment can **interact with each other** both in **promoting and suppressing** the neighbouring bacteria.
- Mechanisms that accomplish this include:
- A-• **competition for receptors** for adhesion and occupation of colonizing sites and prevention of attachment of '**late-comers**'
- B-• **production of toxins**, such as **bacteriocins**, that kill cells of the same or other bacterial species; e.g. **Streptococcus salivarius** produces an inhibitor (**enocin**) that inhibits Streptococcus **pyogenes**
- C- • production of **metabolic end products** such as short chain carboxylic acids, which lower the
- D-• use of **metabolic end products** of other bacteria for nutritional purposes (e.g. Veillonella spp. use acids produced by Streptococcus mutans)

- E- **coaggregation** with the same species (**homotypic**) or different species (**heterotypic**) of bacteria, e.g. corn-cob formation (Fig.).
- These **mechanisms**, which **enable** the commensal oral flora to **suppress or inhibit the growth of exogenous**, **non-oral organisms** and thereby exclude them from their habitat, are called **colonization resistance.**

Fig. 31.1 Scanning electron micrograph of supragingival plaque showing corn-cob formation: cocci aggregated around an axial filamentc (x5000).

Microbiology of periodontal disease

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- Periodontal diseases can be defined as disorders of **supporting structures** of the teeth, including the **gingivae**, **periodontal ligament** and **supporting alveolar bone.**
- Everyone suffers from various degrees of periodontal disease at some point, and it is one of the major diseases afflicting humankind.

Classification of periodontal disease

- Periodontal disease can be broadly categorized into **gingivitis** and **periodontitis**.
- These are yet again **subdivided** into **numerous categories**; a recent classification of periodontal diseases is given in Table 33.1.
- classification of periodontal disease and the **clinical descriptors** used relate to:
- 1- • the rate of disease progress (e.g. chronic, aggressive)
- 2- • lesion distribution (e.g. localized, generalized)
- 3-• age group of the person (e.g. prepubertal, juvenile, adult)
- 4- • association with systemic or developmental disorders. Periodontitis usually develops from a pre-existing gingivitis; however, not every case of gingivitis develops

Table 33.1 Classification of periodontal diseases

Gingival diseases

A. *Dental plaque-induced gingival diseases*
   1. Gingivitis associated with dental plaque only
   2. Gingival disease modified by systemic factors (e.g. puberty-associated gingivitis, pregnancy-associated gingivitis)
   3. Gingival disease modified by medications
   4. Gingival disease modified by malnutrition

B. *Non-plaque-induced gingival lesions*
   1. Specific bacterial origin (e.g. gonorrhoea)
   2. Viral origin (e.g. herpes)
   3. Fungal origin (e.g. linear gingival erythema)
   4. Genetic origin (e.g. hereditary gingival fibromatosis)
   5. Gingival manifestations of systemic conditions (e.g. allergic reactions)
   6. Traumatic lesions (factitious, iatrogenic, accidental) (e.g. chemical injury)

Periodontal diseases

A. *Chronic periodontitis*
   1. Localized
   2. Generalized
B. *Aggressive periodontitis*
   1. Localized
   2. Generalized
C. *Periodontitis as a manifestation of systemic disease*
   1. Associated with haematological disorders
      (i) Acquired neutropenia
      (ii) Leukaemias
      (iii) Others
   2. Associated with genetic disorders
      (i) Familial and cyclic neutropenia
      (ii) Down syndrome
      (iii) Many other rare conditions
   3. Associated with metabolic disorders
      (i) Diabetes mellitus
      (ii) Others
D. *Necrotizing periodontal diseases*
   1. Necrotizing ulcerative gingivitis (NUG)
   2. Necrotizing ulcerative periodontitis (NUP)

Ecology of the gingival crevice and the periodontal pocket

- The gingival crevice is **more anaerobic** than most locales of the mouth and is constantly bathed by the gingival **crevicular fluid** (GCF) and its **humoural** and cellular defence factors, including **polymorphs**.
- **Dramatic changes** ensue during the transition of the **crevice into a periodontal pocket**.
- 1- The oxygen tension falls further and becomes highly anaerobic and

- 2-the flow of GCF increases.
- 3-The mostly **proteolytic bacteria** living in the periodontal pocket raise the pH to **alkaline** levels (pH 7.4–7.8; compared with neutral values in health), which in turn **promotes** the growth of bacteria such as ***Porphyromonas gingivalis***.
- 4- The **exposed cemental surface** of the tooth is first colonized mainly by pioneer, including ***streptococci and Actinomyces*** spp. Secondary colonizers such as **Prevotella and Porphyromonas** spp. can adhere to this layer of cells by **coaggregation**. Others, such as ***Peptostreptococcus micros***, can adhere to the **crevicular epithelium**. Thus, the inhabitants and the ecology of a **deep periodontal pocket** are markedly different from that of the gingival crevice.

Aetiological factors

- The main aetiological agent of periodontal disease is **microflora** inhabiting **subgingival plaque biofilms**. However, the 1-**host tissues** and its 2-**specific and non-specific host defence mechanisms** play crucial modulating roles (i.e. modifying factors) in the disease process.
- **Host tissues**
- The periodontium comprises the gingivae, periodontal ligament, cementum and alveolar bone (Fig. 33.1). Although the **dentogingival junction** is perhaps the most vulnerable site for microbial attack, it is not breached as long as oral hygiene is satisfactory. However, when **plaque accumulates** close to the gingival margin, the host defences are overcome, and gingival inflammation (gingivitis) and subsequent periodontal inflammation with loss of attachment ensue (periodontitis).

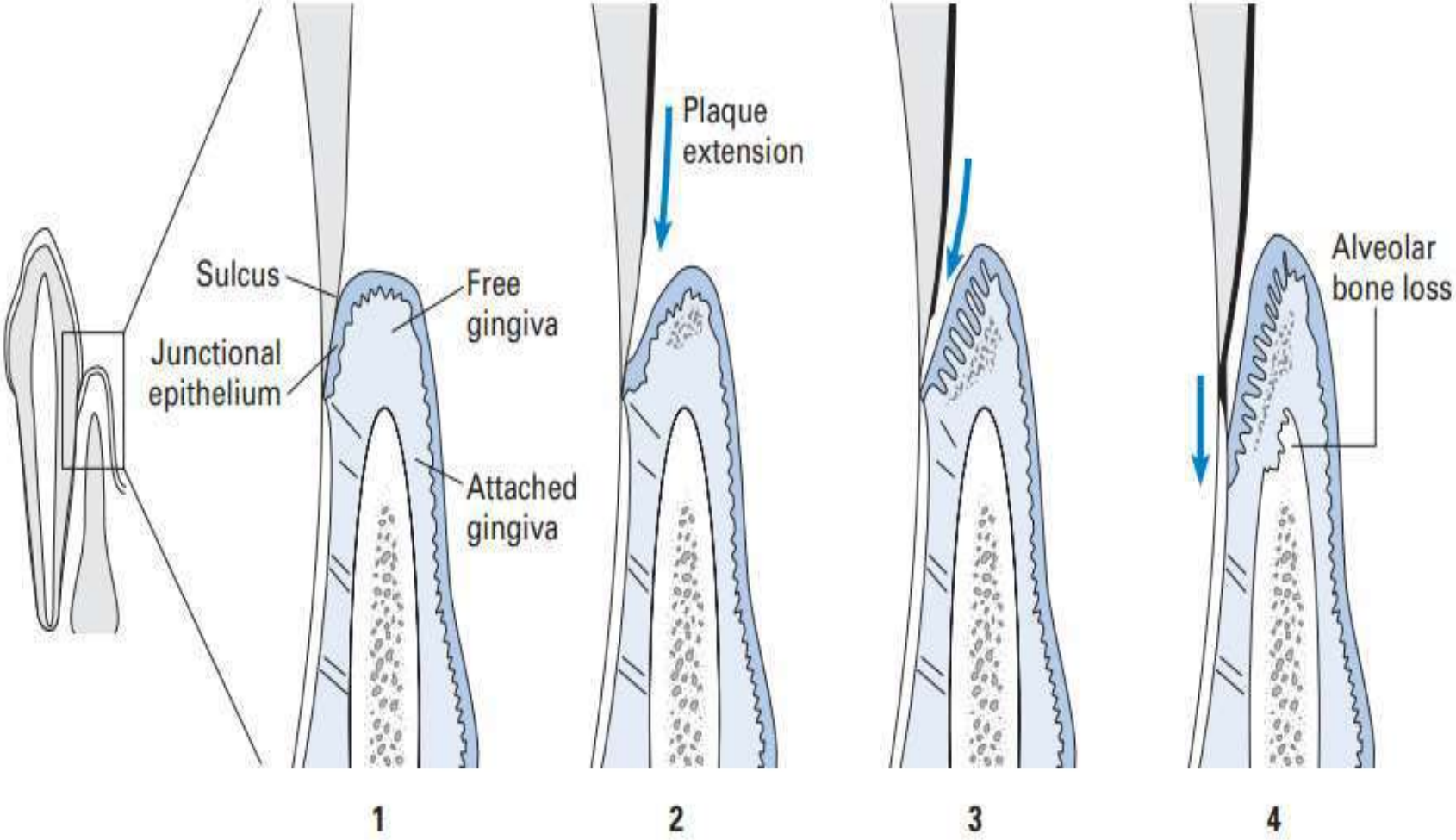
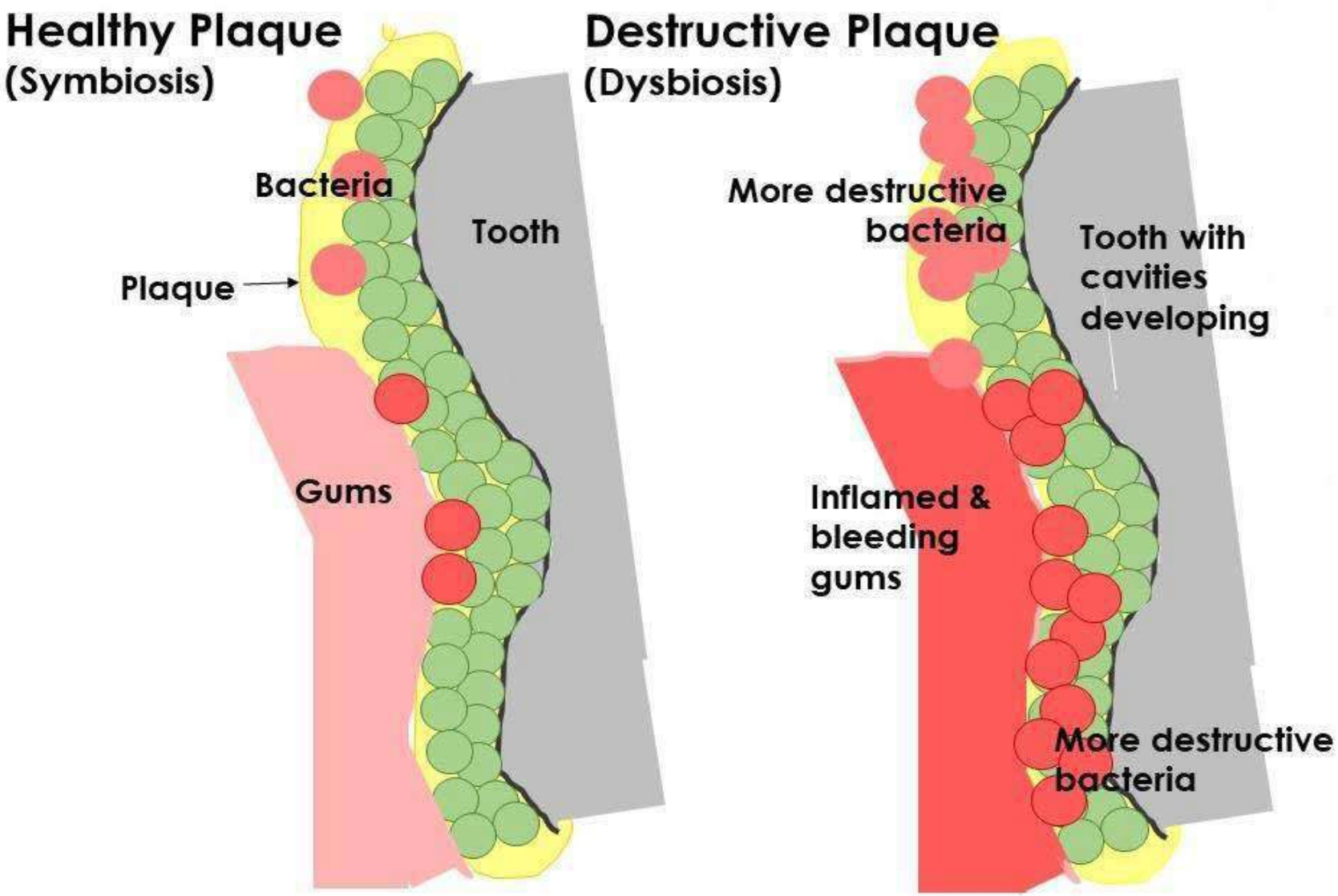


Fig. 33.1 The progression of a marginal periodontium from health to disease. (**1**) A healthy gingival sulcus with minimal supragingival plaque. (**2**) Established chronic gingivitis with minor inflammatory enlargement. (**3**) Long-standing chronic gingivitis with subgingival plaque extension into the pocket. (**4**) Chronic periodontitis with destruction of the periodontal membrane, alveolar bone loss and apical migration of the epithelial attachment.


Healthy Plaque
(Symbiosis)
Bacteria
Tooth
Plaque
Gums
Destructive Plaque
(Dysbiosis)
More destructive
bacteria
Tooth with
cavities
developing
Inflamed &
bleeding
gums
More destructive
bacteria

Host defence factors

- Both the specific and non-specific immune responses of the host to **subgingival plaque** are considered to play critical roles in the **initiation, progression and recovery** from **periodontal diseases**. One of the most important components of the host response is the GCF, which contains both specific and non-specific defence factors

Polymorphonuclear leukocytes

- Clinically healthy gingiva contain small numbers of polymorphonuclear leukocytes (**PMNLs**). Their numbers **increase** markedly during the onset of **gingivitis and periodontitis**. The PMNLs migrate from **venules and enter the gingival sulcus through the junctional epithelial cells**. When PMNLs encounter bacteria, phagocytosis ensues, and the ingested organisms are then killed with a combination of **proteolytic** and **hydrolytic** enzymes

- The interaction between PMNLs and plaque bacteria may result in:
- 1-death of the microorganism
- 2-• death of the leukocytes
- 3-• neutrophil **autolysis** and release of **lysosomal enzymes** (e.g. **hyaluronidase, collagenase, elastase, acid hydrolase**). Thus, PMNLs may have both a protective and a damaging effect on host tissues.

Antibody

- Locally derived specific antibodies (IgM, IgG and IgA) to **subgingival plaque organisms** are found in the **GCF**. An elevated titre of specific antibody to a **periodontopathogen** may be:
- • protective
- • involved in damaging hypersensitivity reactions to the host tissues
- • non-specific

Microorganisms in subgingival plaque biofilm

- That **dental plaque biofilm** is the essential **aetiological agent** of the common forms of **chronic gingivitis and periodontitis** is shown by the following:
- **Epidemiological data** indicate a **strong positive association** between **plaque levels** and the **prevalence and severity of periodontal diseases**

Specific and non-specific plaque hypotheses

- Although bacteria are definitive agents of periodontal diseases, there are **conflicting** views as to whether a **single or a limited number of species** are involved in the disease process – the **specific plaque hypothesis** – or disease is caused by any combination of a wider range of non-sp

The specific plaque hypothesis

- In certain disease states such as **necrotizing ulcerative gingivitis**, the **key aetiological agents** are **fusobacteria and spirochaetes**. Furthermore, this disease can be resolved by appropriate antibiotics active against anaerobes (e.g. metronidazole).
- Other studies have convincingly shown the direct involvement of ***Aggregatibacter actinomycetemcomitans*** in **aggressive (juvenile) periodontitis**, and disease resolution after therapy with tetracycline, which is active against this organism. These observations led to the theory of specific plaque hypothesis.

The non-specific plaque hypothesis

- This hypothesis proposes that **collective groups** or **consortia** of **different bacteria** have the total complement of **virulence factors** required for **periodontal tissue destruction** and that some bacteria can substitute for others absent from the **pathogenic consortium**.
- This hypothesis implies that plaque will cause disease irrespective of its composition, and it is supported by the clinical findings of numerous bacterial species in diseased periodontal pockets.

The ecological plaque hypothesis

- has also been proposed for the aetiology of periodontal disease. This postulates the following causative process:
- 1-The reaction of the host to natural plaque accumulation in the crevice is an inflammatory response.
- 2-The ensuing increased GCF flow
- 3-Periodonto aetiopathogenic organisms suppress the growth of species common in the healthy crevice and a **population shift** occurs in the resident flora.

Oral Microbiology

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Sterilization and Disinfection

Basic terms: The following terms are commonly employed in connection with antimicrobial agents and their uses .

- sterilization
- disinfection
- Antisepsis
- Bacteriostasis
- Asepsis

sterilization

The process of destroying all microbial forms.A sterile object is one free of all microbial forms, including bacterial spores.

- **Biocide**: A general term describing a chemical agent, usually broad-spectrum, that inactivates microbes.

disinfection

The reduction or elimination of pathogenic microorganisms in or on materials, so they are no longer a health hazard.

- Disinfectant: Product or biocide used to **kill**mic.**On inanimate objects or surfaces.**

Asepsis

No living microorganisms exists.

Antisepsis

The Use of chemical agents on skin or other living tissue to inhibit or eliminate microbes; no sporicidal action is implied.

- Antiseptic: a biocide or product that destroy or inhibit the growth of mic**. In or on living tissues.**

Bacteriostasis

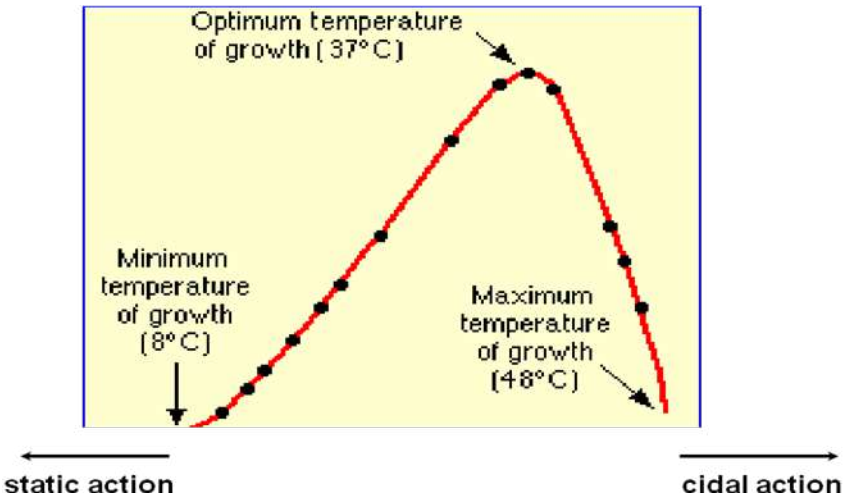
Inhibition the growth of microorganisms.

- **Bacteriostatic** : A specific term referring to the property by which a biocide is able **to inhibit** bacterial multiplication. (ex. Fungistatic, sporostatic).It is reversible.
- **Bactericidal** : A specific term referring to the property by which a biocide is able **to kill** bacteria. (ex. Fungicidal, sporicidal and virucidal).it is irreversible.

Controlling Microorganisms By Physical Agents

- High Temperature
- Radiation
- Filtration
- Low Temperature
- Desiccation

High Temperature



- **Dry heat：protein oxidation**
  - **Hot air sterilization: for sterilization of materials that must remain dry . Electric oven**
  - **Incineration**

Moist heat：denature proteins and melt lipids; more effective

- **Autoclaving:** 121℃,103.4kPa,20min

cidal for both vegetative organisms and endospores

- **Boiling water**
- **Pasteurization: to kill particular spoilage organisms or pathogens**
  - flash method: 71.6°C, 15s
  - holding method: 62.9°C, 30 min

fractional sterilization:

1) Steam heating to 100 °C for 30 min .Vegetative cells are destroyed but endospores survive

2) Incubate at 30 °C -37 °C over night .Most bacterial endospores germinate

3) Second heat treatment, 100 °C, 30 min .Germinated endospores are killed.

4) Second incubation at 30°C-37 °C overnight .Remaining endospores germinate

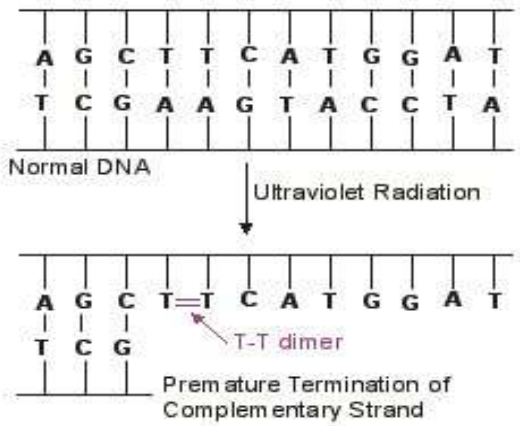
5) Third heat treatment, 100 °C, **60 min** .Last remaining germinated endospores are killed

Radiation

- **Ultraviolet Radiation**
- **Ionizing Radiation**

Ultraviolet Radiation

- microbicidal activity of ultraviolet (UV) light depends on:
  - ☐ length of exposure
  - ☐ wavelength of UV: 260 nm - 270 nm
- Mechanism: thymine-thymine dimmers (DNA damage)



Charactristics

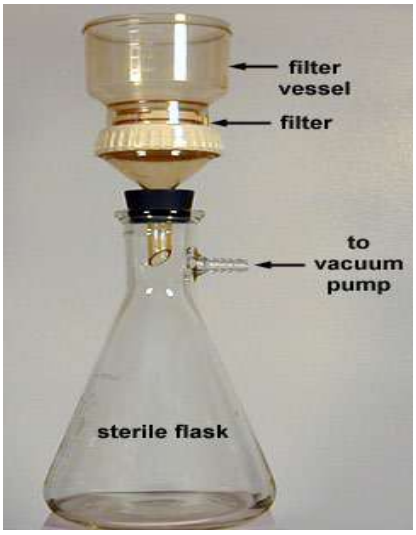
- very poor penetrating power
- damage the eyes, cause burns, and cause mutation in cells of the skin

Ionizing Radiation

- X-rays and gamma rays
- more energy and penetrating power than UV
- used to sterilize pharmaceuticals and disposable medical supplies such as syringes, surgical gloves, catheters, and sutures
- used to retard spoilage in seafoods, meats, poultry, and fruits

Filtration

- sterilize solutions that may be damaged or denatured by high temperatures or chemical agents



Low Temperature

- inhibits microbial growth by slowing down microbial metabolism

Desiccation

- has a static effect on microorganisms by inhibiting the action of microbial enzymes

Using Disinfectants and Antiseptics to Control Microorganisms

- **Antimicrobial modes of action for disinfectants and antiseptics**
- 1- damage the lipids so disruption of cell membrane
- 2- denaturation of proteins of the semipermeable cytoplasmic membrane of microorganisms(for both 1 and 2) resulting in leakage of cellular materials needed to sustain life
- 3- denature microbial enzymes and other proteins by disrupting the hydrogen and disulfide bonds. Removal of free SH group.
- 4- damage of DNA (ex. DNA-reactive chemicals.
- 5- Chemical antagonism.

Different categories of such chemical agents:

- **Phenol and phenol derivatives**
  - ☐ alter membrane permeability and denature proteins
  - ☐ chlorhexidine
  - ☐ ineffective against endospores
- **Soaps and detergents**
  - ☐ Anionic (negatively charged) detergents: mechanically remove microorganisms and other materials but are not very microbicidal.
  - ☐ Cationic (positively charged) detergents: alter membrane permeability and denature proteins; ineffective against endospores, *M. tuberculosis*, and *P. species*.
- **Alcohols**
  - ☐ denature membranes
  - ☐ 70% solutions of ethyl or isopropyl alcohol
  - ☐ ineffective against endospores and non-enveloped viruses
- **Acids and alkalies**

alter membrane permeability and denature proteins and other molecules

- Salts of organic acids: food preservatives
- Undecylenic acid: dermatophyte infections

- **Heavy metals**

denature proteins

- Mercury compounds (mercurochrome, merthiolate) : bacteriostatic, ineffective against endospores
- Silver nitrate (1%) : put in the eyes of newborns to prevent gonococcalophthalmia

- **Chlorine**

reacts with water to form hypochlorite ions, which in turn denature microbial enzymes

- **Iodine and iodophores**
  - denatures microbial proteins
  - effective against some endospores

- **Aldehydes**

denature microbial proteins

- Formalin (37% aqueous solution of formaldehyde gas)
- glutaraldehyde: kill vegetative bacteria in 10-30 minutes and endospores in about 4 hours

Factors Influencing Antimicrobial Activity:

- The concentration and kind of a chemical agent used;
- The intensity and nature of a physical agent used;

- The length of exposure to the agent;
- The temperature at which the agent is used;
- The number of microorganisms present;
- The species or strain of microorganism;
- The nature of the material bearing the microorganism;
- The presence of organic or other interfering substances.

Antimicrobial Chemotherapy

ا.م.د جتين عزالدين علي
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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 origon. (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

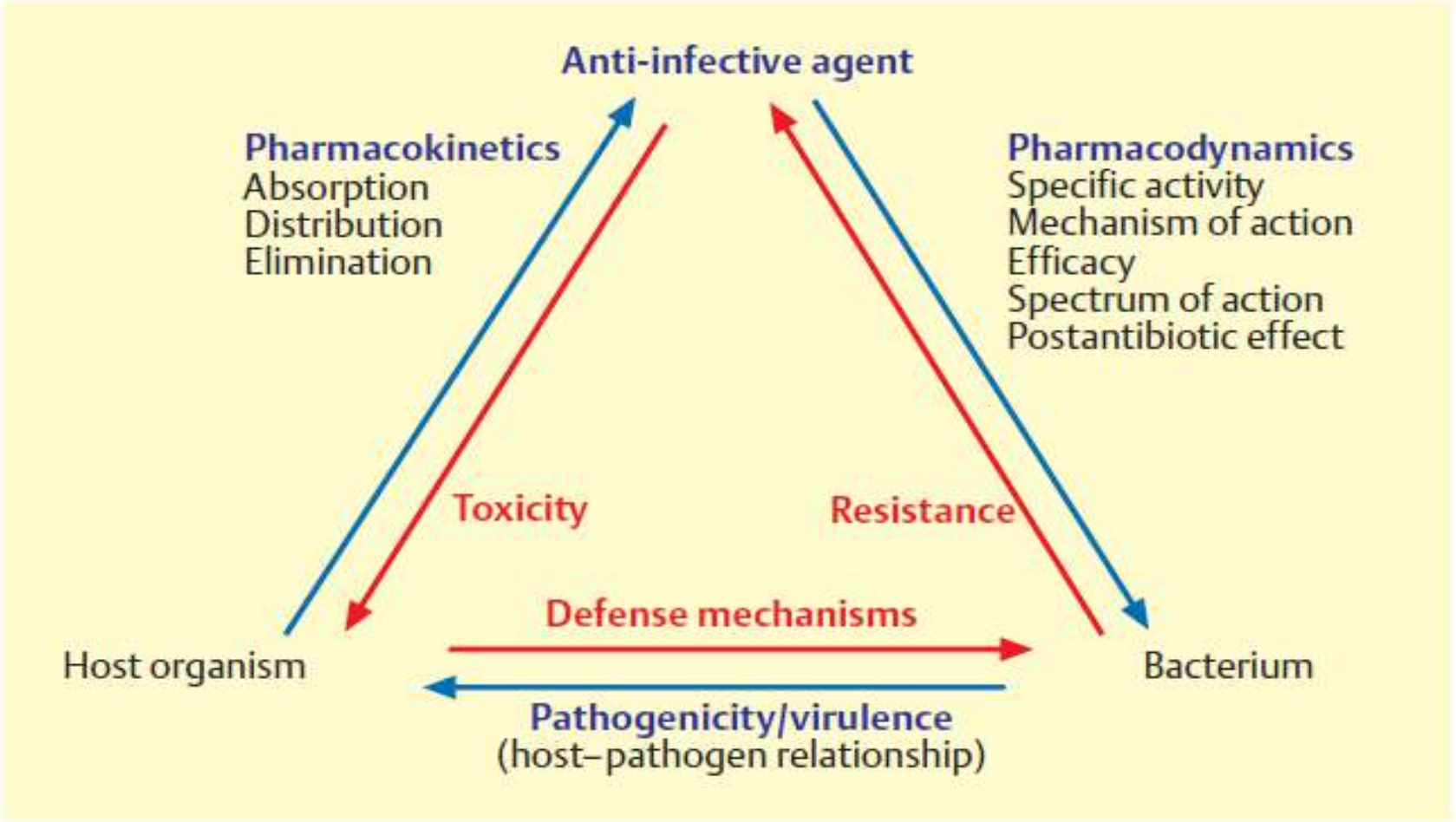


Fig. 3.**28** Interactions between the anti-infective agent and host organism are characterized by the terms pharmacokinetics and toxicity; interactions between the anti-infective agent and the bacterial pathogen are characterized in terms of pharmacodynamics and resistance.

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***
- Gentamyicin from ***Micromonospora purpurea***

b . Antibiotics from algae

c. Antibiotics from higher plants

d. Antibiotics from animals

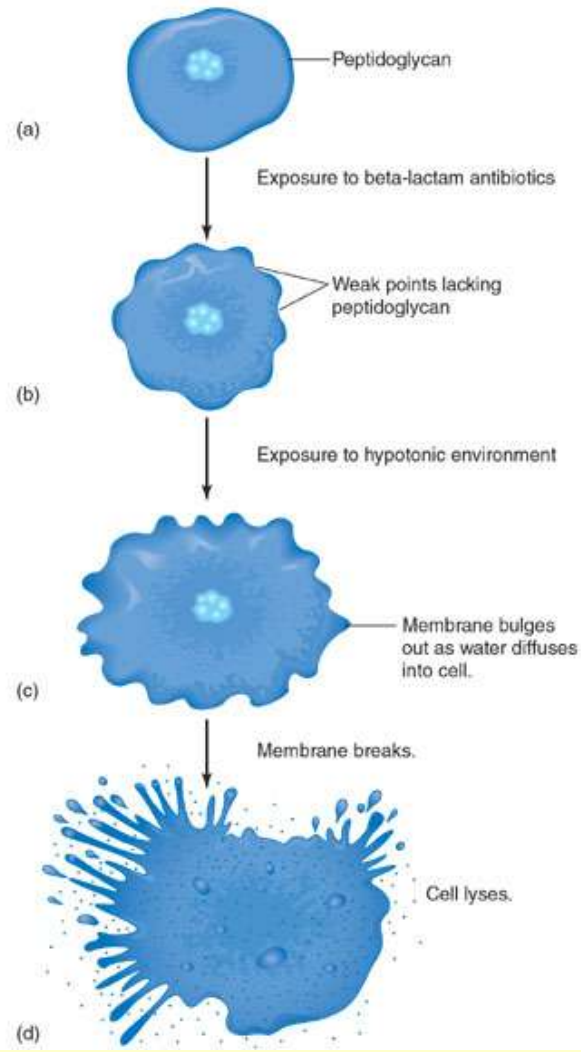
Selectively toxic

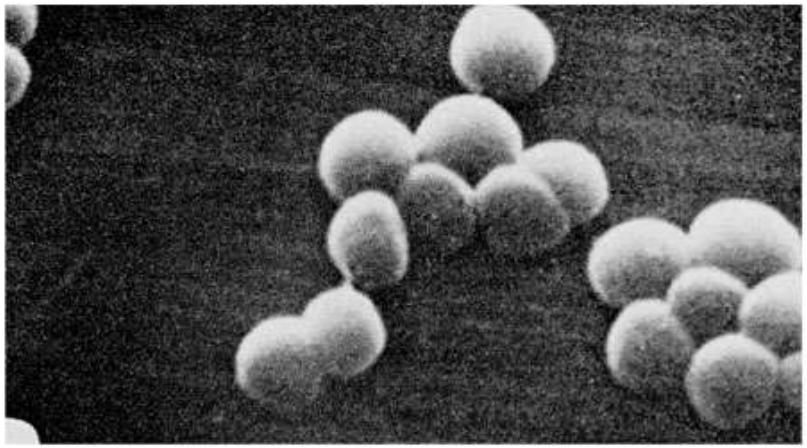
- Drugs should kill or inhibit microbial cells without simultaneously damaging host tissues.
- As the characteristics of the infectious agent become more similar to the vertebrate host cell, complete selective toxicity becomes more difficult to achieve & more side effects are seen.

Targets of antimicrobial drugs

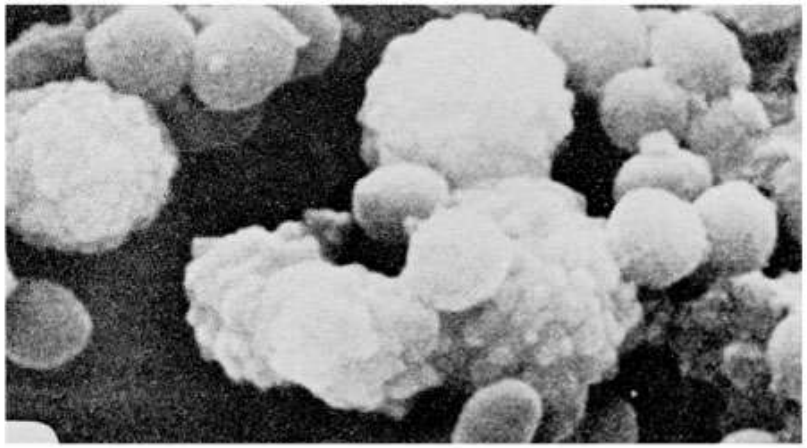
1. Inhibition of cell wall synthesis
2. Inhibition of nucleic acid synthesis, structure or function
3. Inhibition of protein synthesis
4. Disruption of cell membrane structure or function

Targets of antimicrobial drugs





(e)



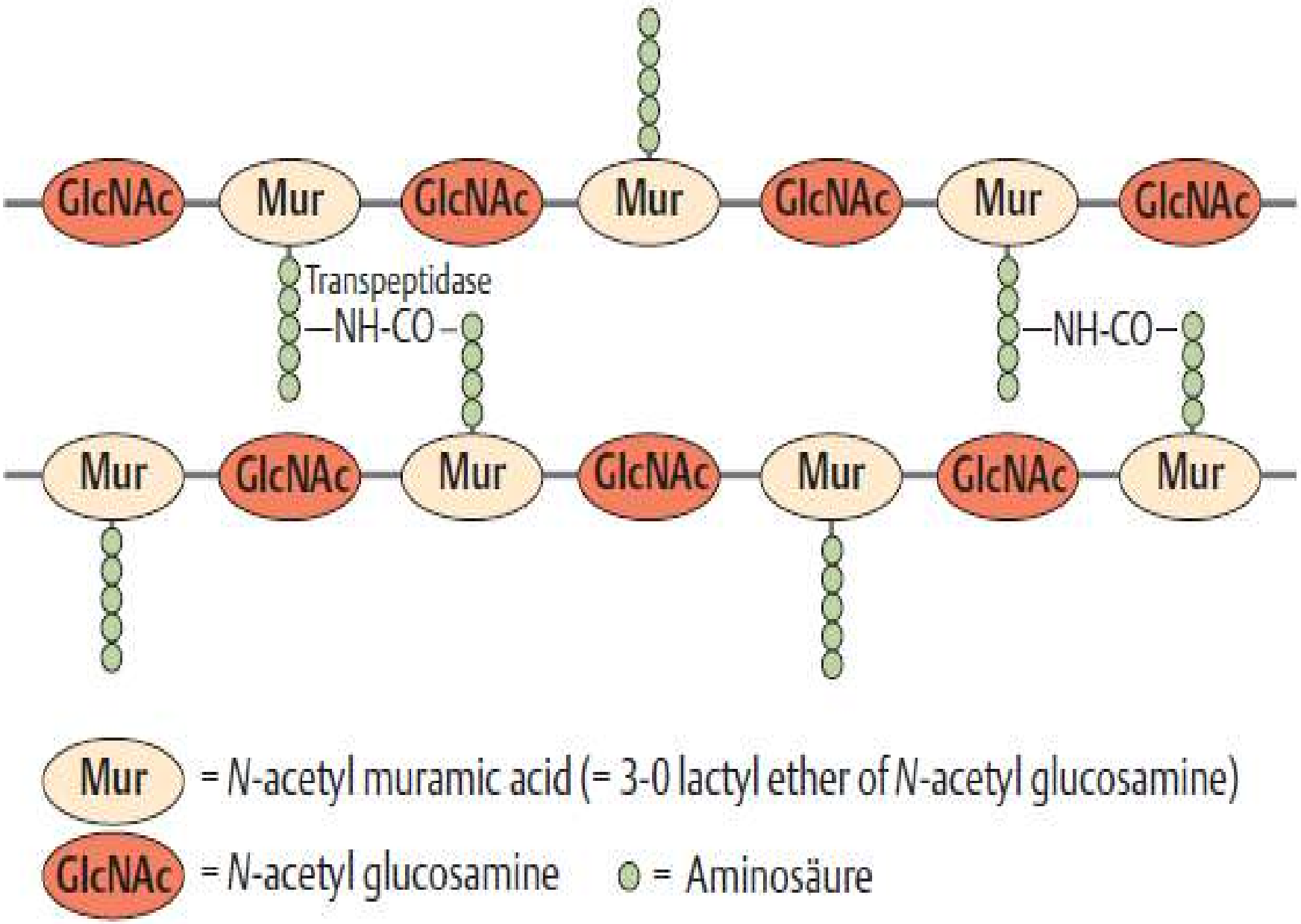
(f)

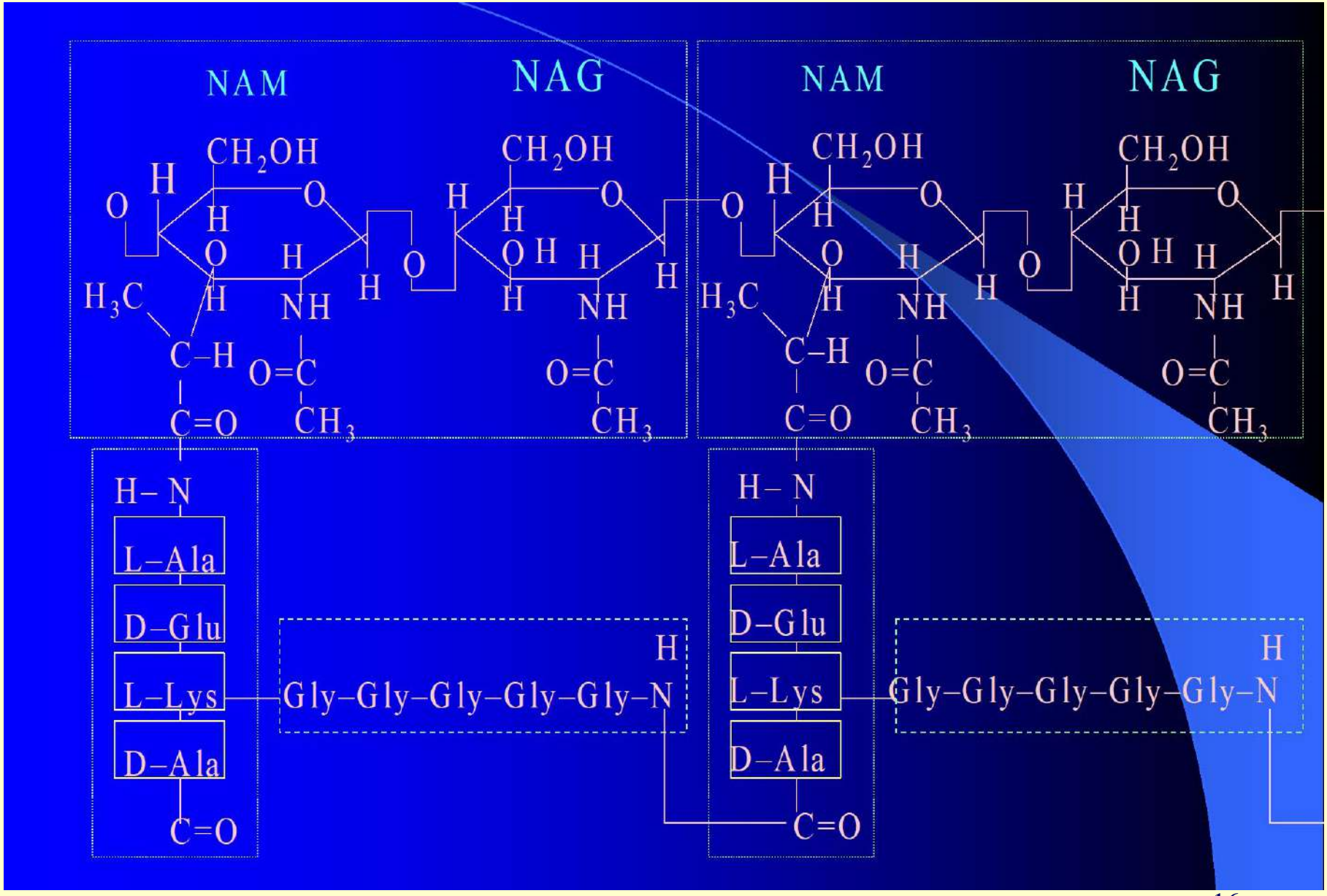
1. Drugs that affect the bacterial cell wall

- 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.

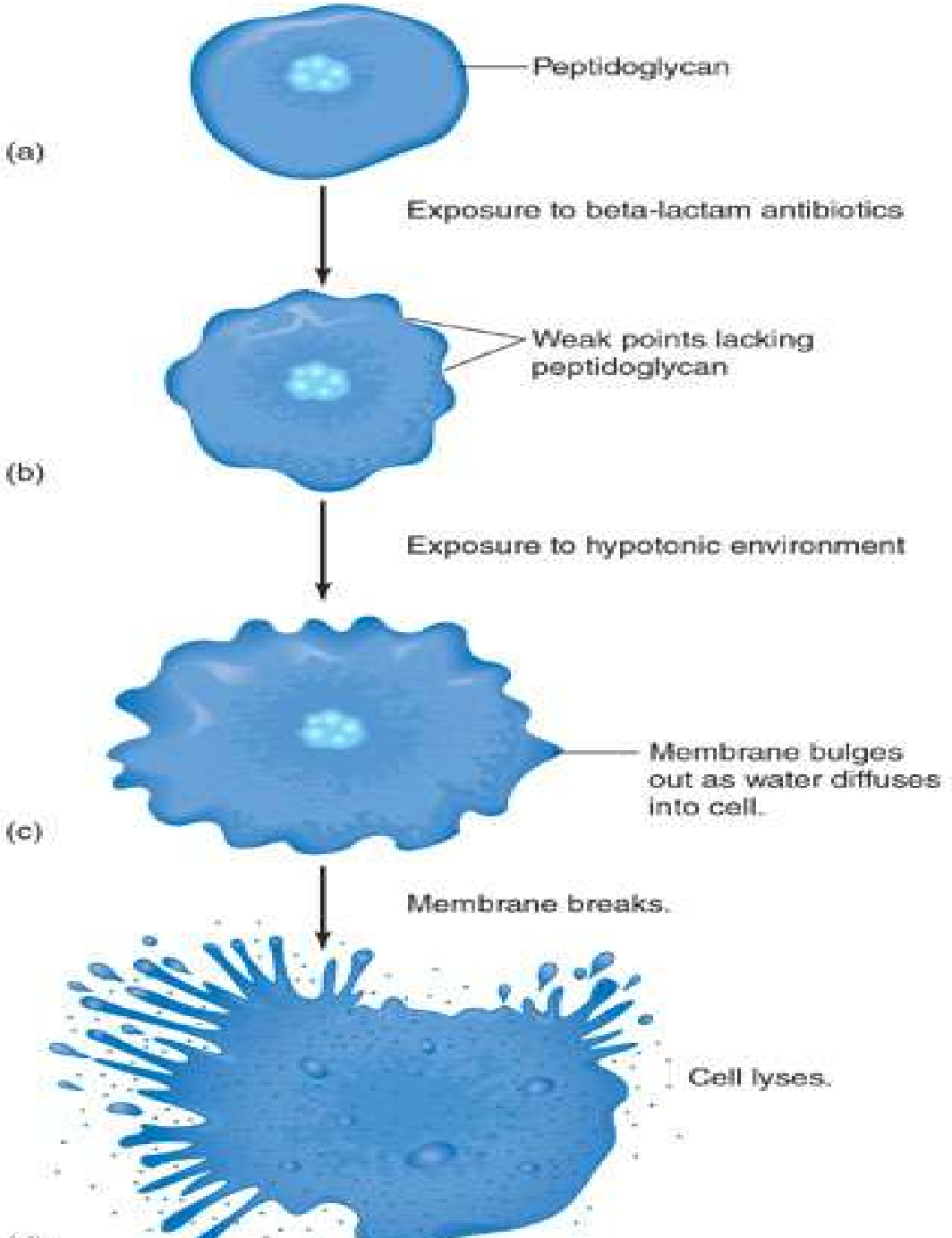
Betalactam antibiotics	Disturbance of murein biosynthesis: – Irreversible inhibition of DD-transpeptidase, which catalyzes the peptide crosslinkage in murein – Release of an inhibitor of autolytic murein enzymes – Enzymatic destruction of murein architecture with autolysins: "wrong place at the wrong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

The Structure of Murein

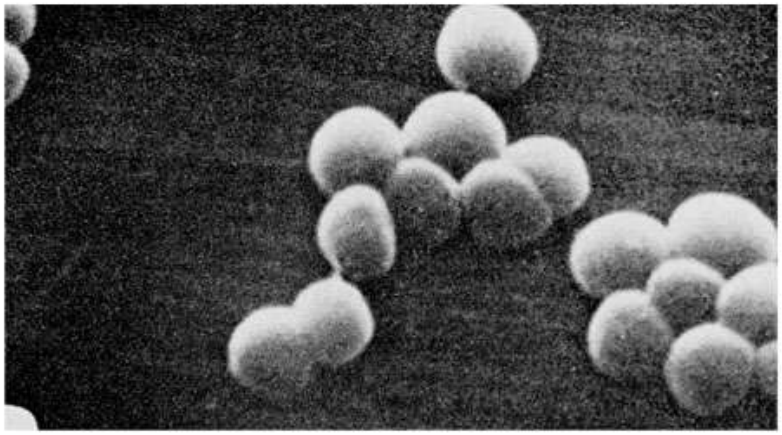



NAM
NAG
NAM
NAG
L–Ala
D–Glu
L–Lys
D–Ala
Gly–Gly–Gly–Gly–Gly–N
L–Ala
D–Glu
L–Lys
D–Ala
Gly–Gly–Gly–Gly–Gly–N

1. ll



1. Drugs that affect the bacterial cell wall



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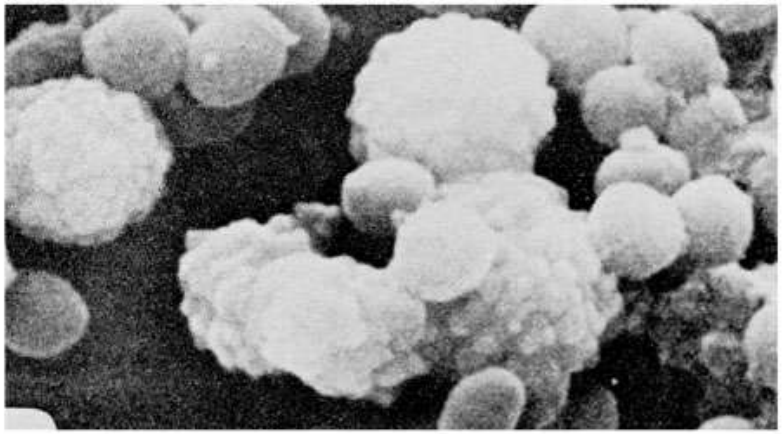


(f)

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

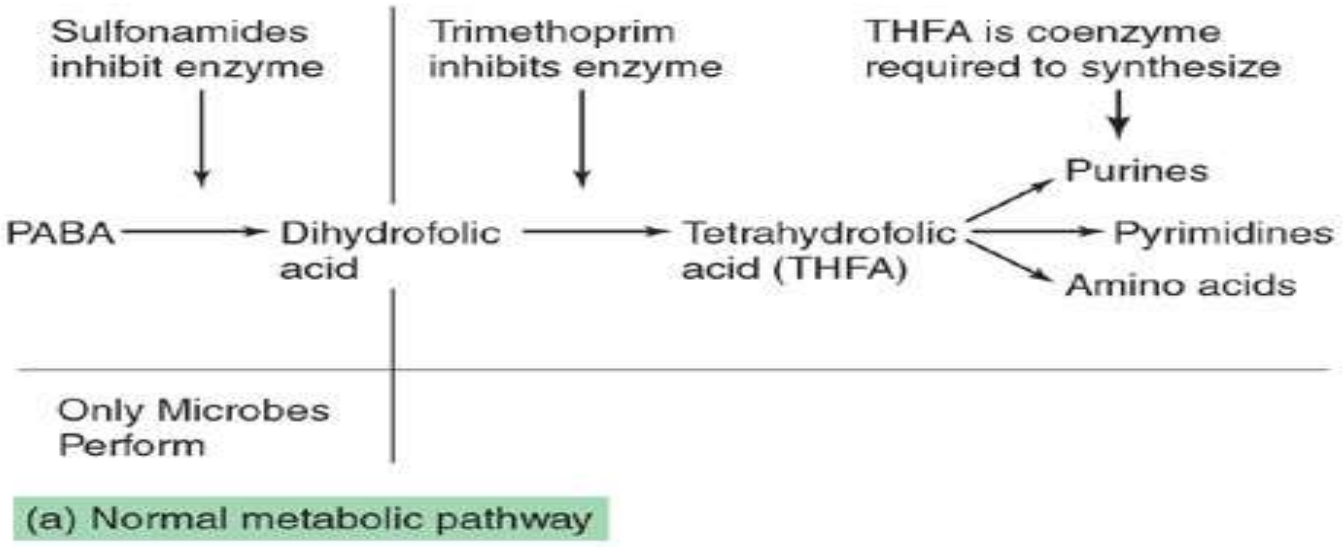
Rifamycin	Transcription: Blockage of DNA-dependent RNA polymerase

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

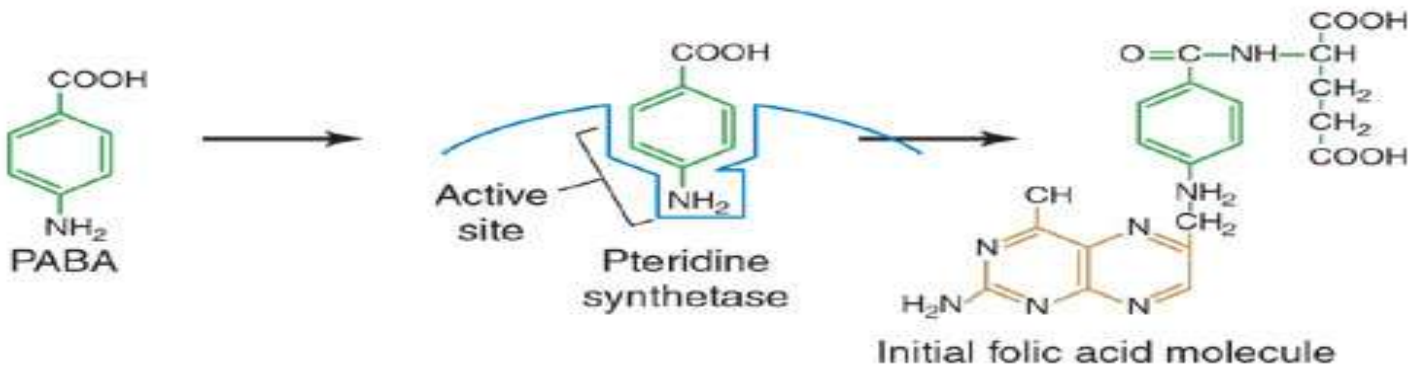
Sulfonamides	Competition with *p*-aminobenzoic acid as a substrate for dihydropteric acid synthetase, thus too little tetrahydrofolic acid
Trimethoprim	Inhibition of dihydrofolic acid reductase, thus too little tetrahydrofolic acid

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

2. Drugs that inhibit nucleic acid synthesis



(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

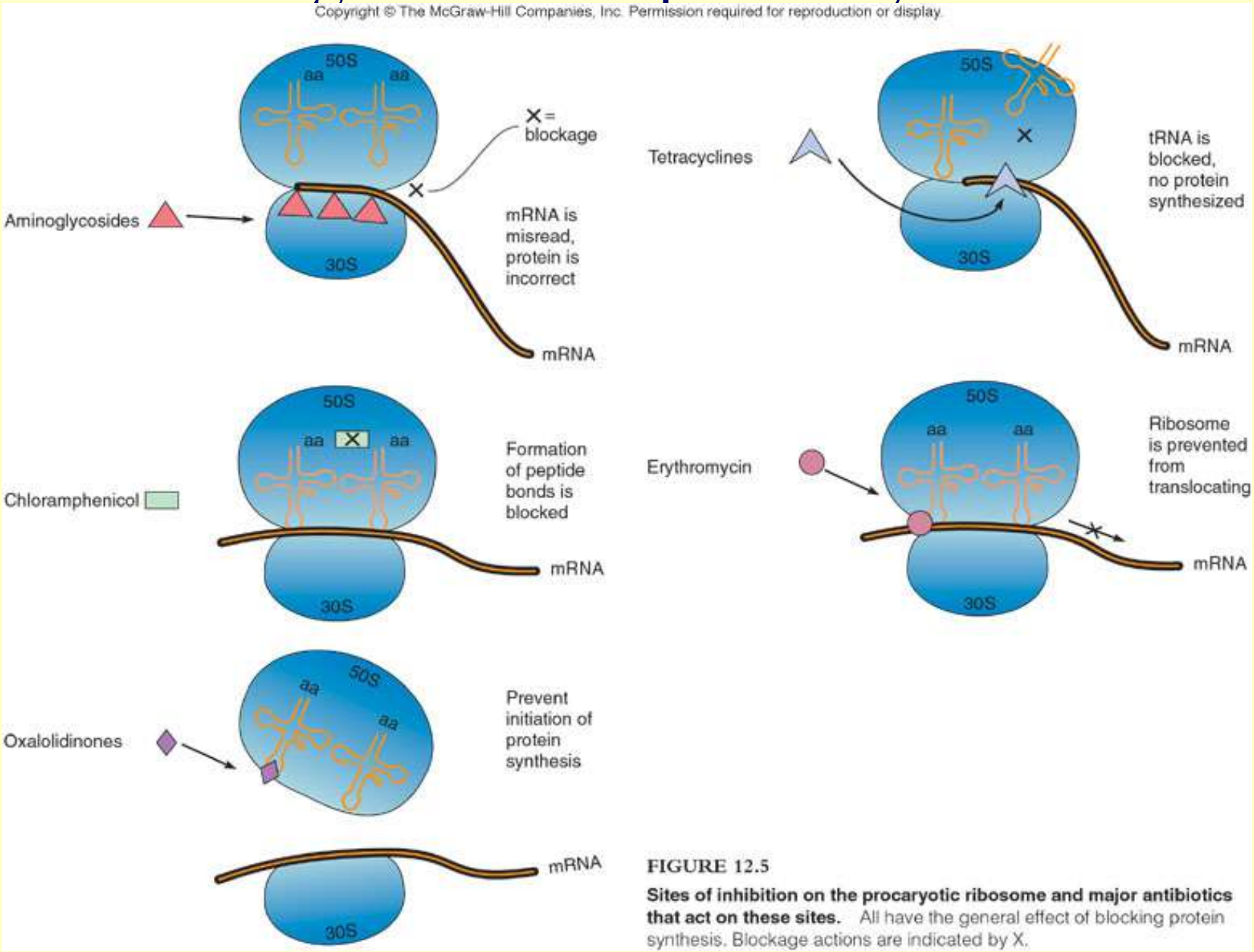
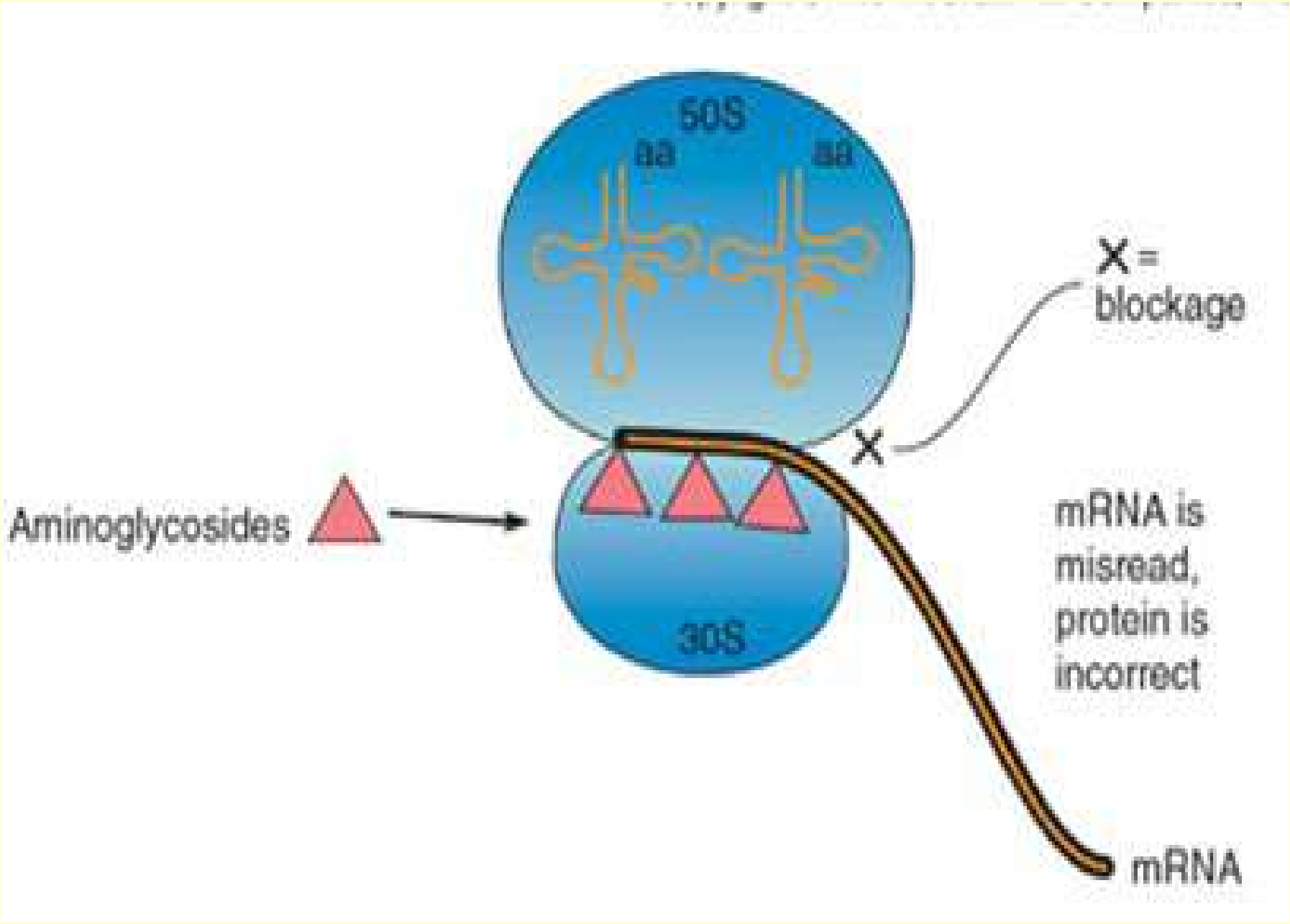
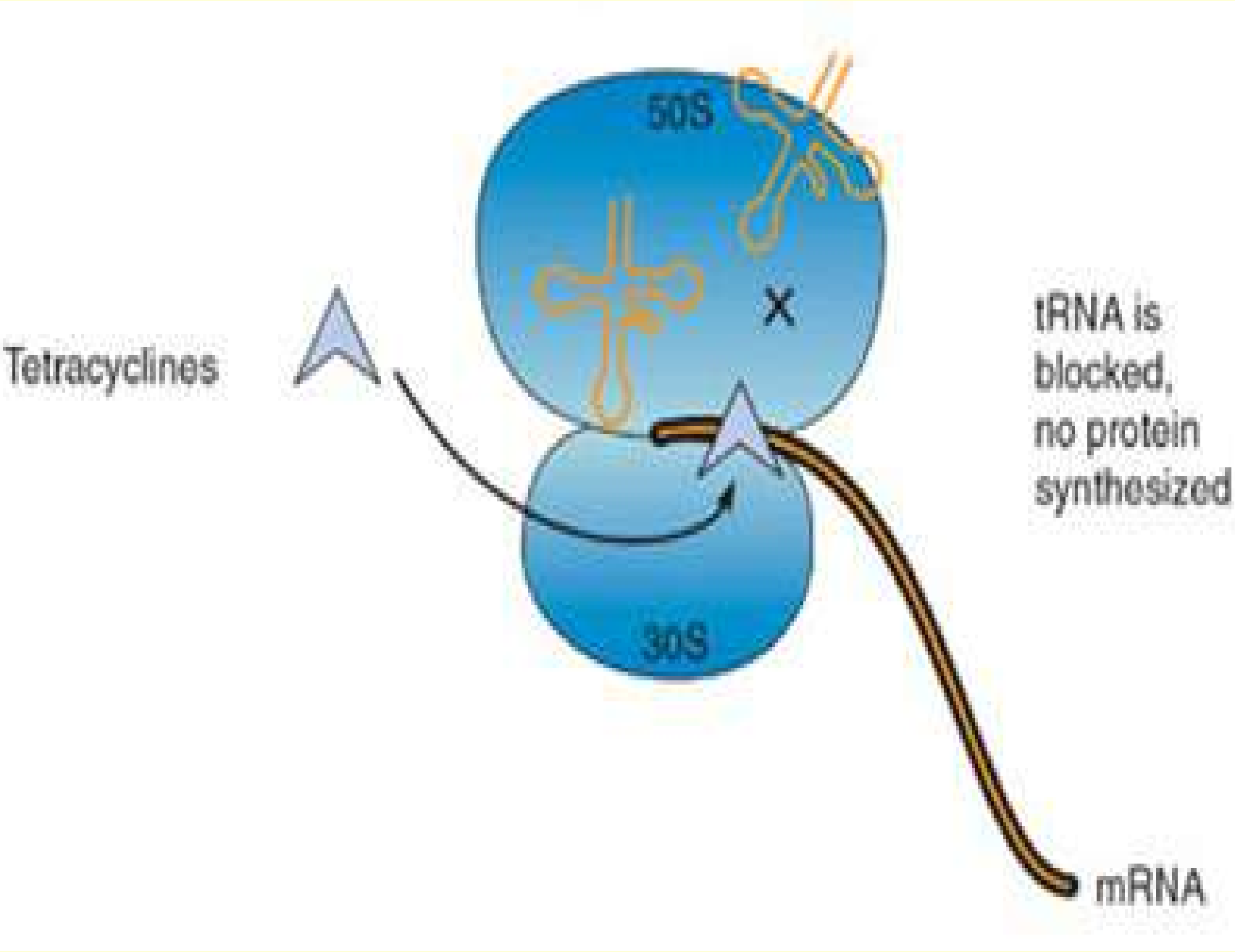
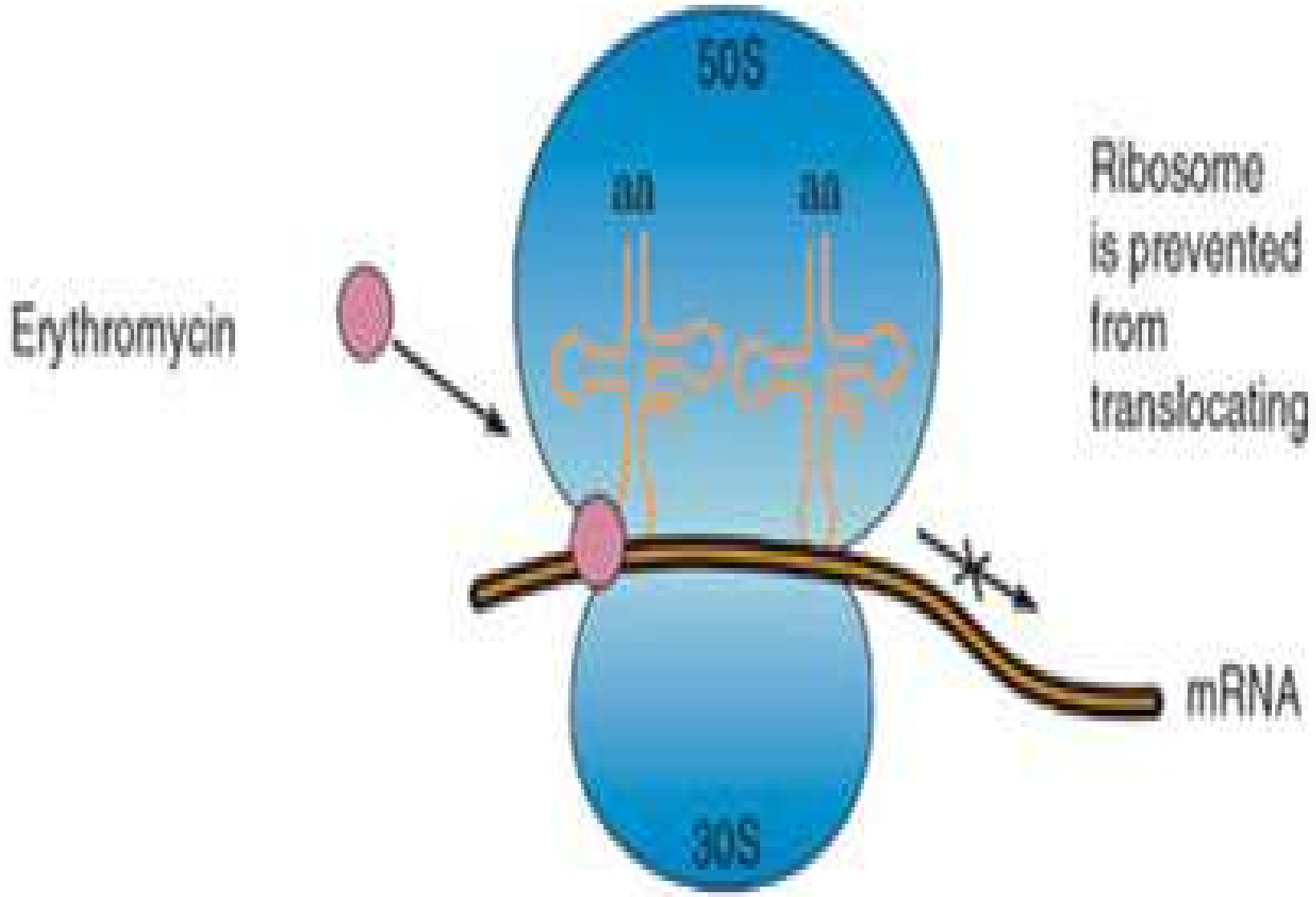


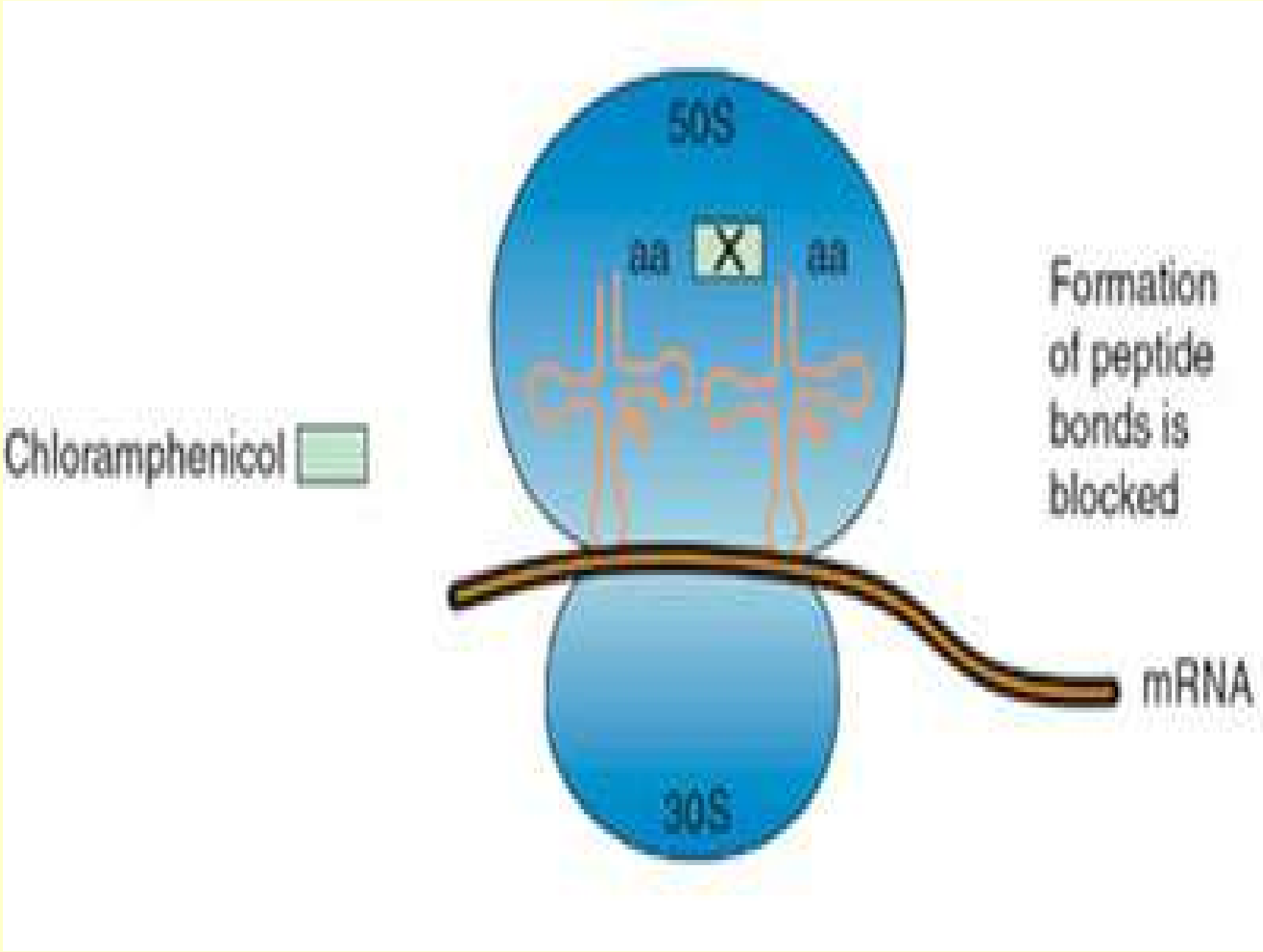
FIGURE 12.5

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


50S
aa
aa
X =
blockage
X
Aminoglycosides
mRNA is
misread,
protein is
incorrect
30S
mRNA


50S
X
Tetracyclines
tRNA is
blocked,
no protein
synthesized
30S
mRNA

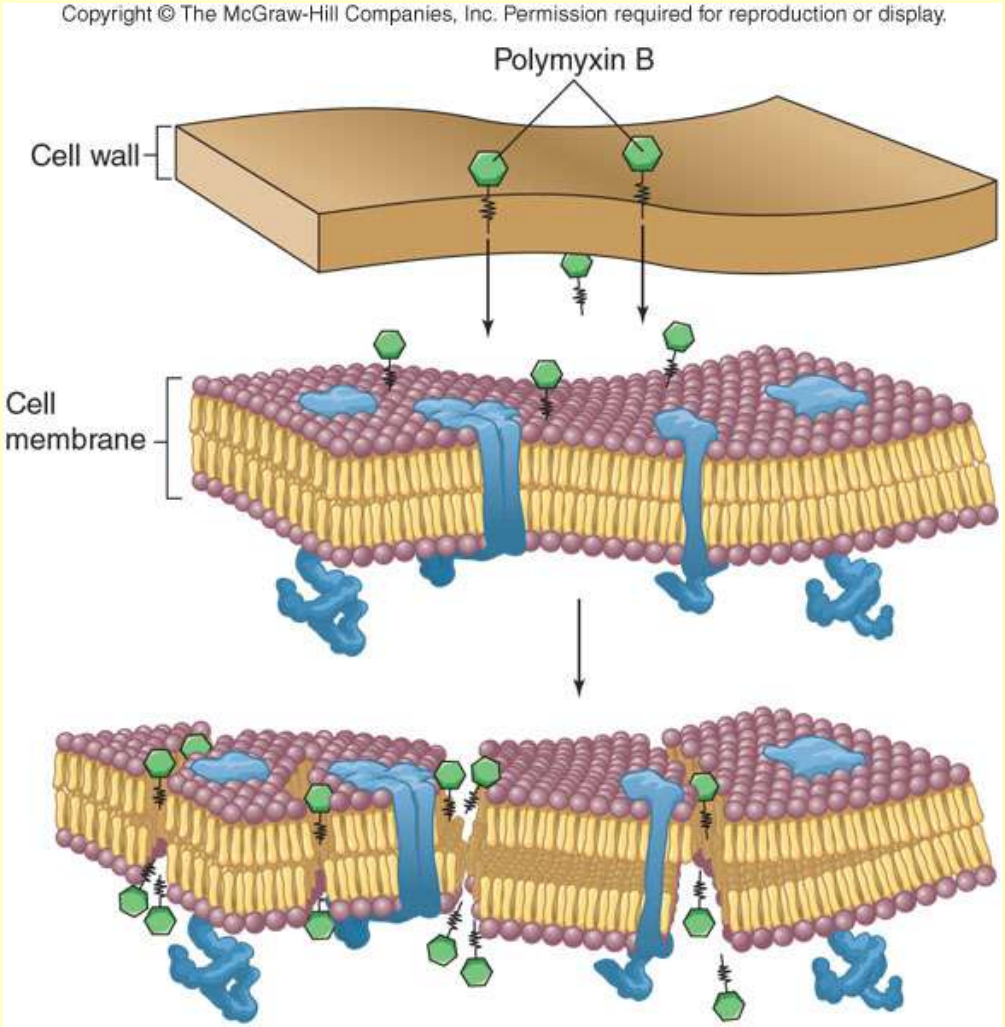

50S
aa
aa
Erythromycin
Ribosome
is prevented
from
translocating
mRNA
30S


50S
aa
X
aa
Formation of peptide bonds is blocked
Chloramphenicol
mRNA
30S

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.

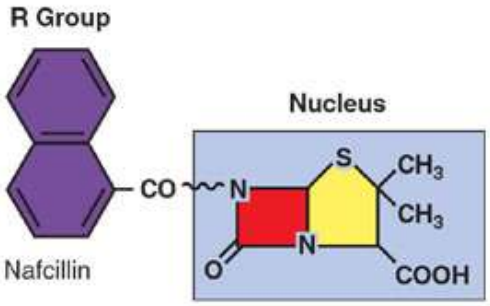
Antibacterial antibiotics

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

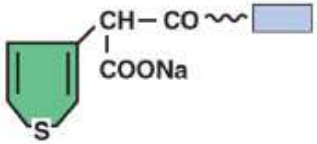
Penicillins

- 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

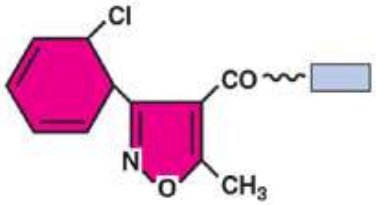
Penicillins



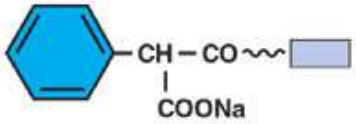
Nafcillin



Ticarcillin



Cloxacillin



Carbenicillin

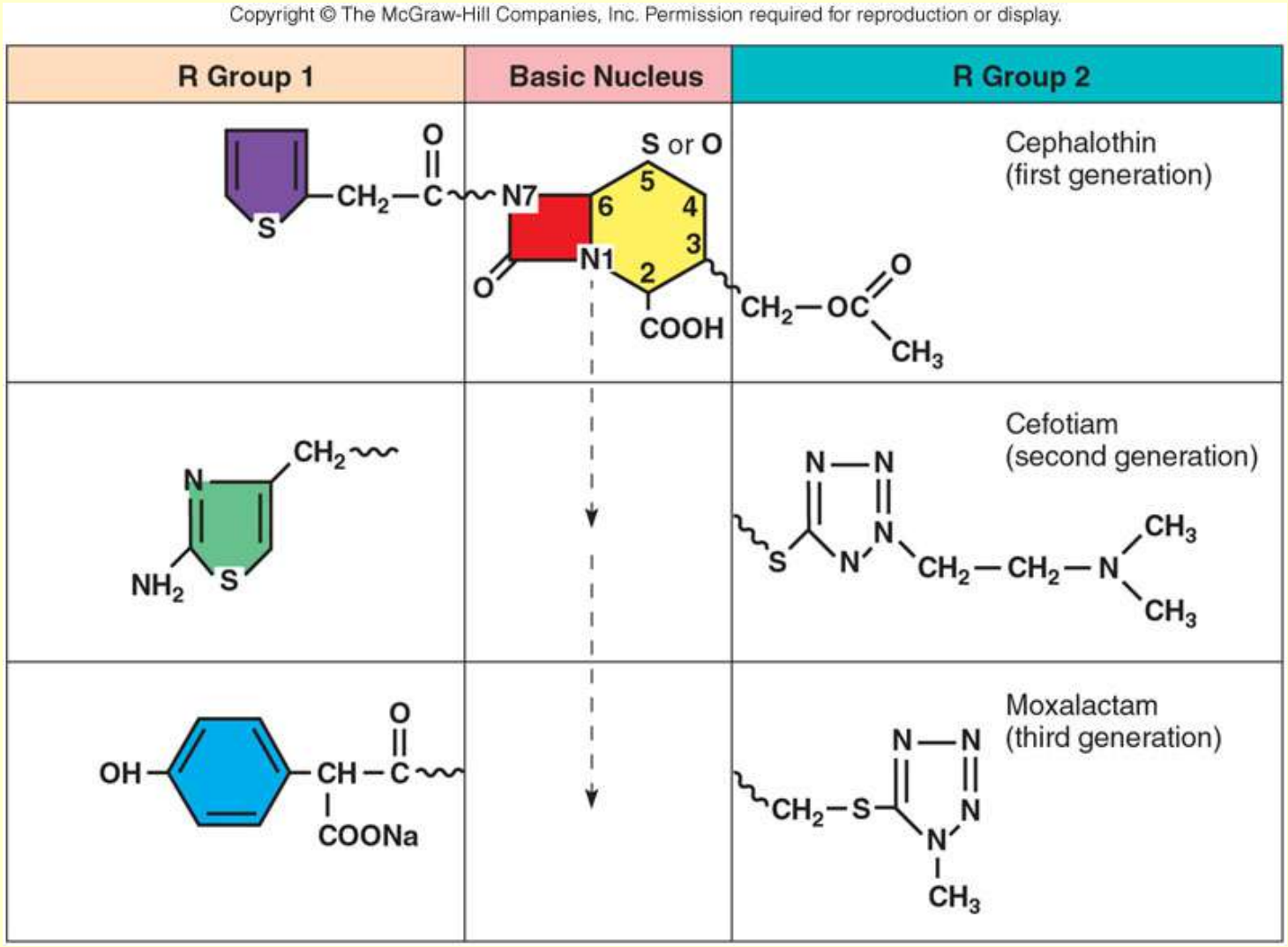
Penicillins

- 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



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

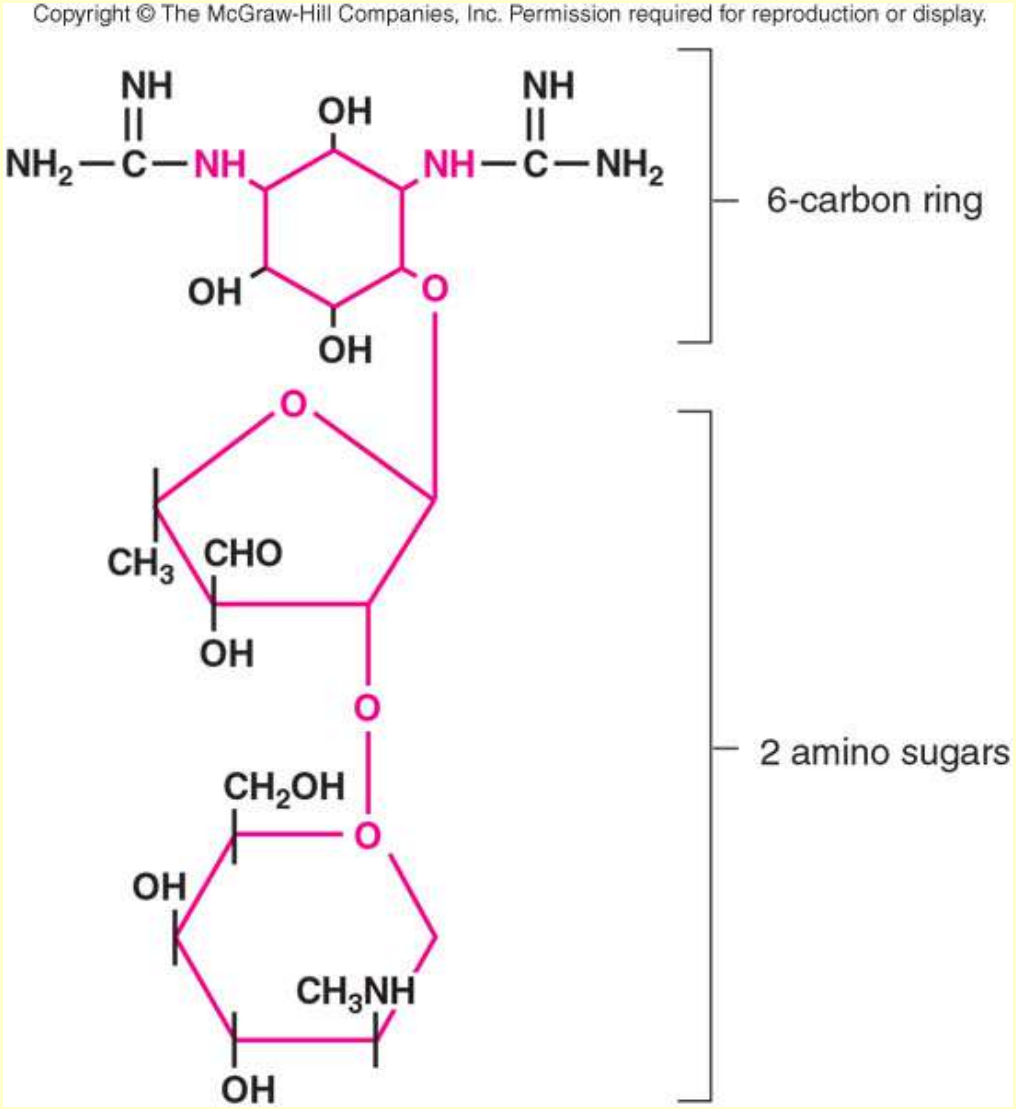
Other beta-lactam antibiotics

- 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

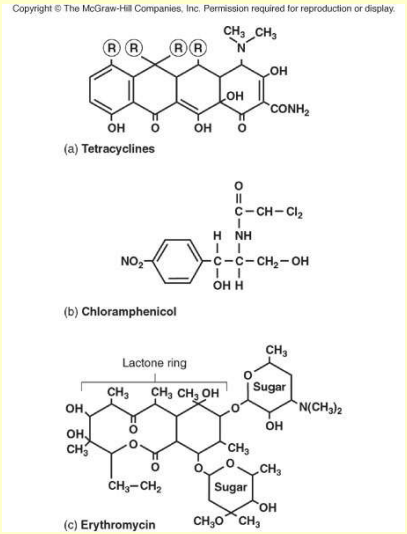
- composed of 2 or more amino sugars and an aminocyclitol (6C) ring
- products of various species of soil actinomycetes in genera *Streptomyces* & *Micromonospora*
- Broad-spectrum, inhibit protein synthesis, especially useful against aerobic gram-negative rods & certain gram-positive bacteria
  - Streptomycin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

Aminoglycosides



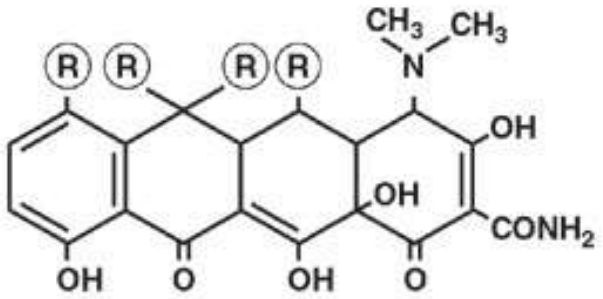
Tetracycline antibiotics

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

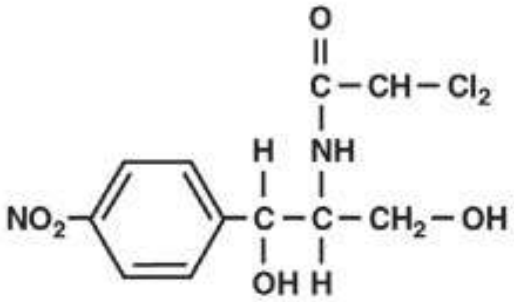


Chloramphenicol

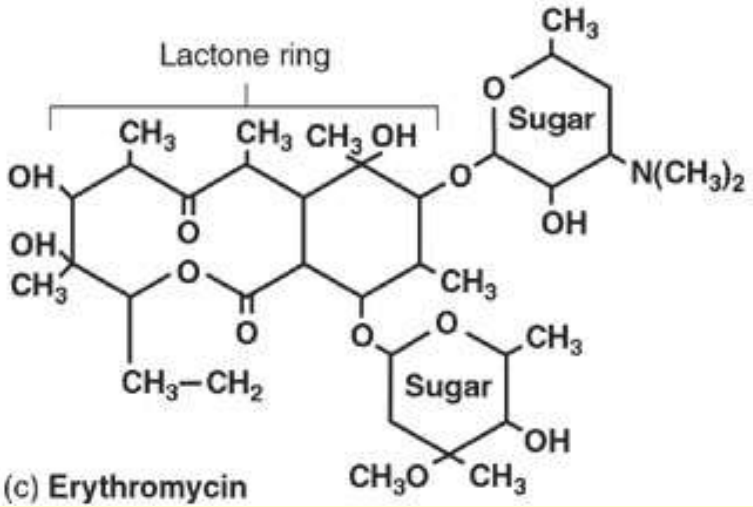
- Isolated from *Streptomyces venezuelae*
- Potent broad-spectrum drug with unique nitrobenzene structure
- Blocks peptide bond formation
- No longer derived from natural source
- Very toxic, restricted uses, can cause irreversible damage to bone marrow
- Typhoid fever, brain abscesses, rickettsial & 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, diptheria and as a prophylactic prior to intestinal surgery
- For penicillin-resistant – gonococci, syphilis, acne
- Newer semi-synthetic macrolides – clarithomycin, 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 pause 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

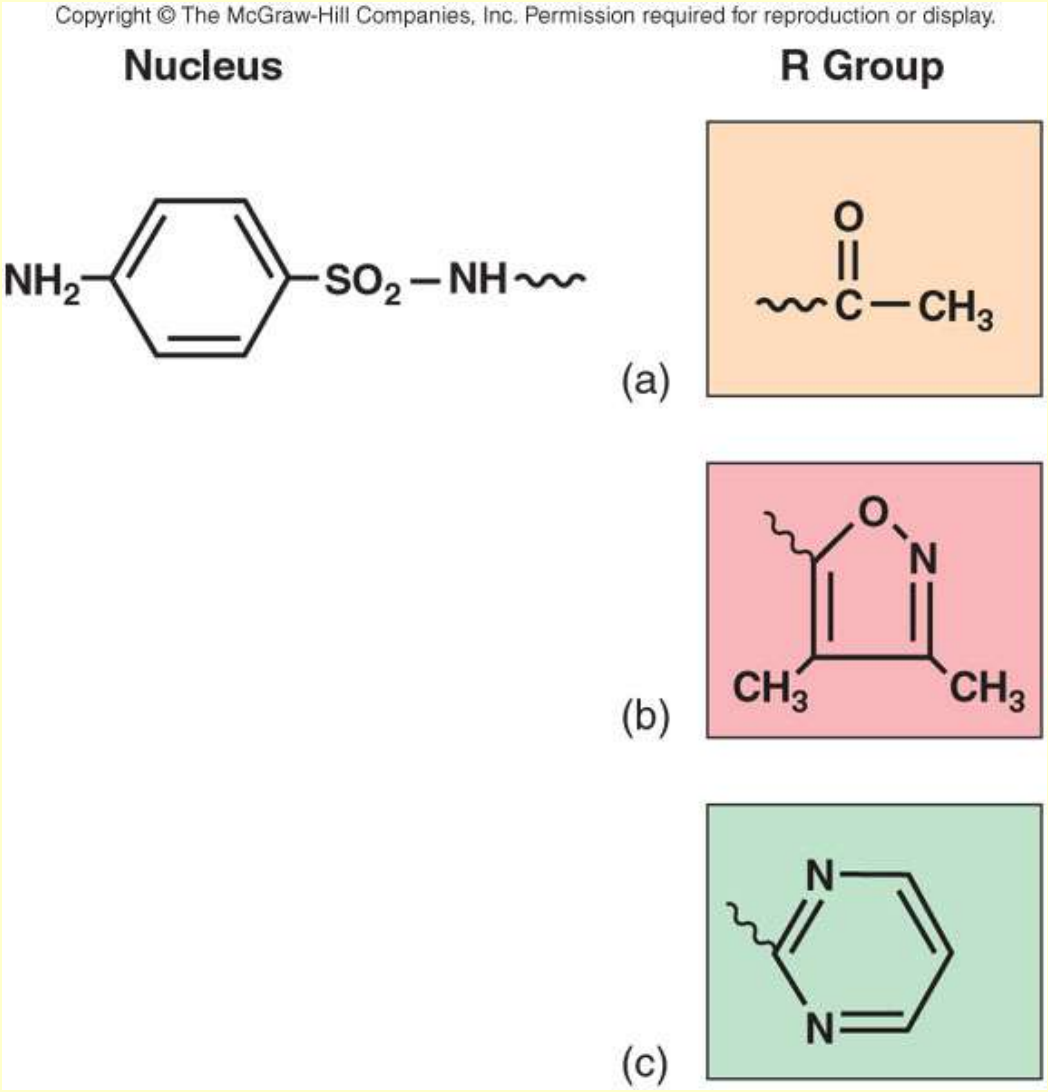
New classes of antibiotics

- Fosfomycin trimethamine – a phosporic 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 antimicrobic drugs
- Sulfisoxazole – shigellosis, UTI, protozoan infections
- Silver sulfadiazine –burns, eye infections
- Trimethoprim – given in combination with sulfamethoxazole – UTI, PCP

Sulfonamides



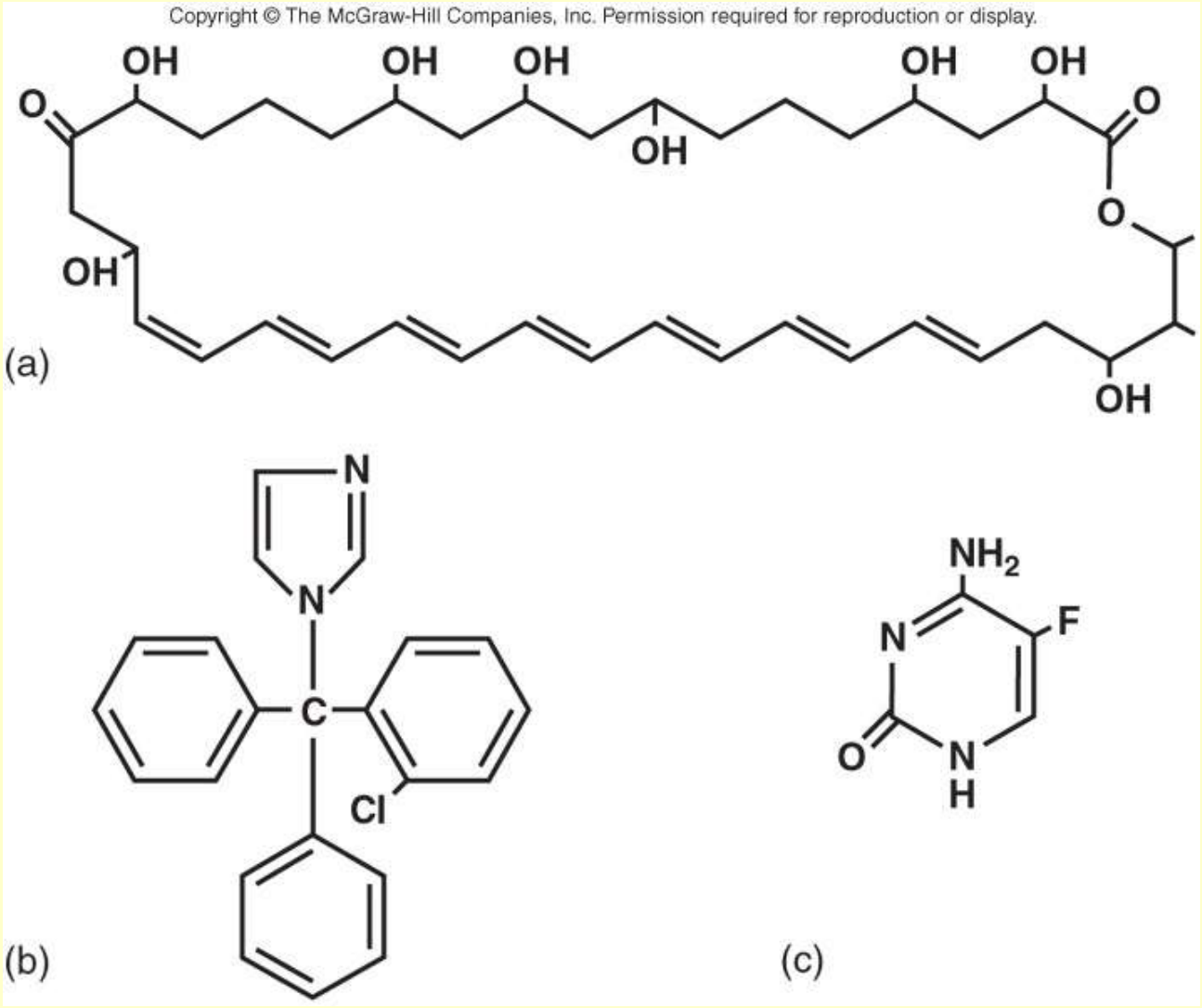
Miscellaneous antibacterial drugs

- 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, osteomyletitis, 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



Antiparasitic drugs

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

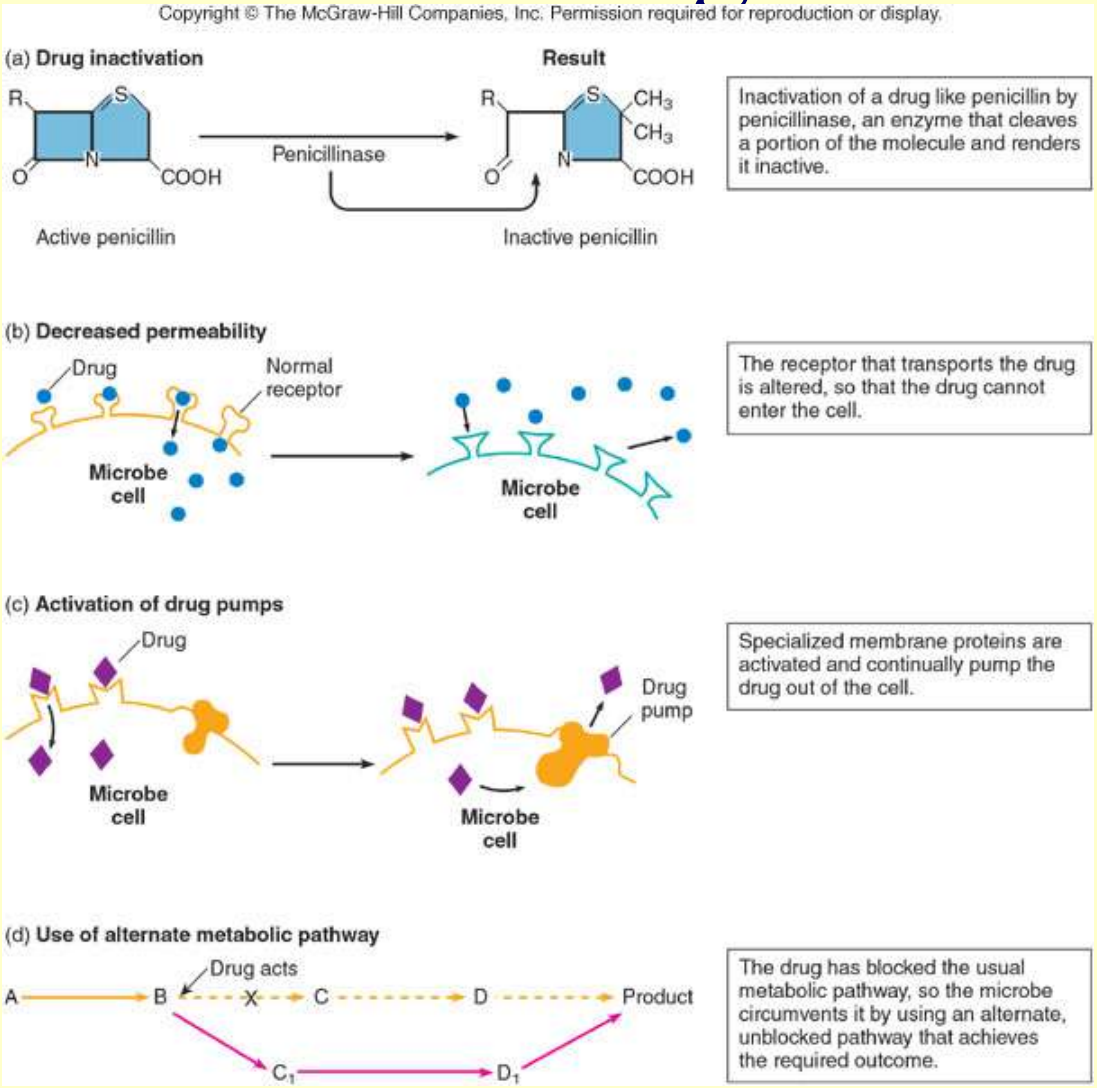
Antiviral drugs

- 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

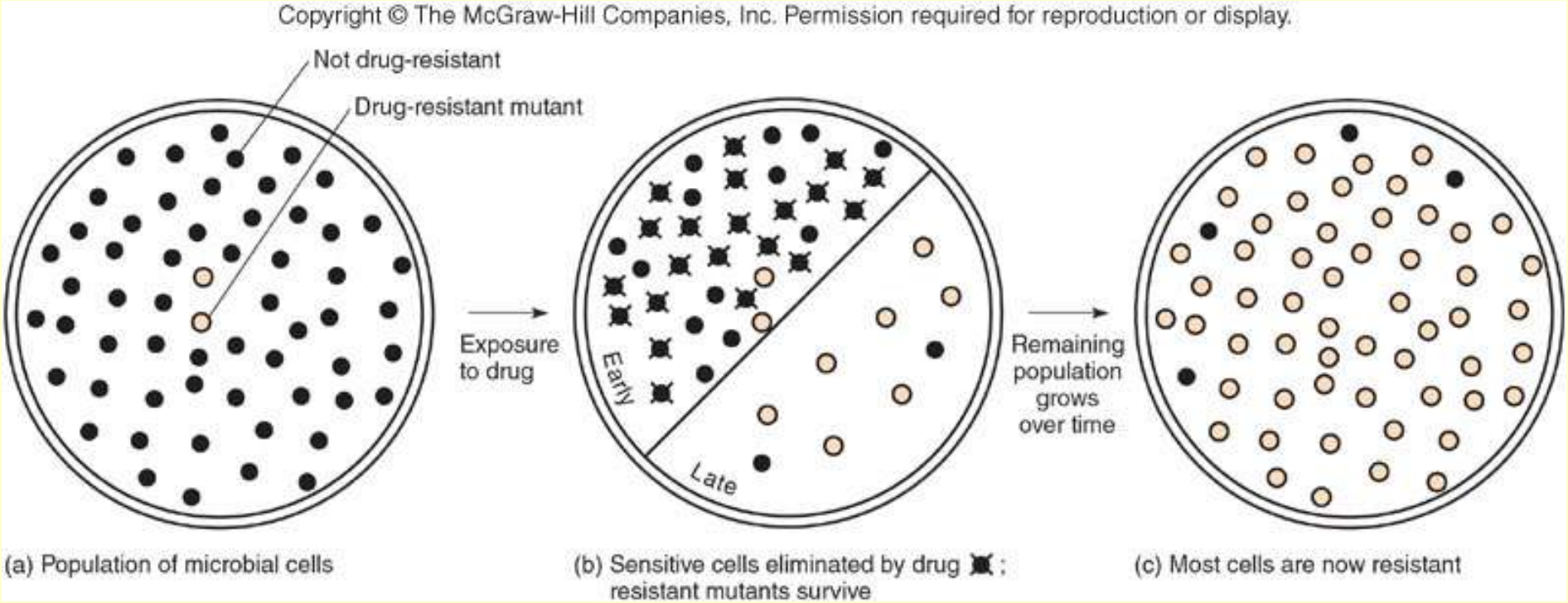
Mechanisms drug resistance

- 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

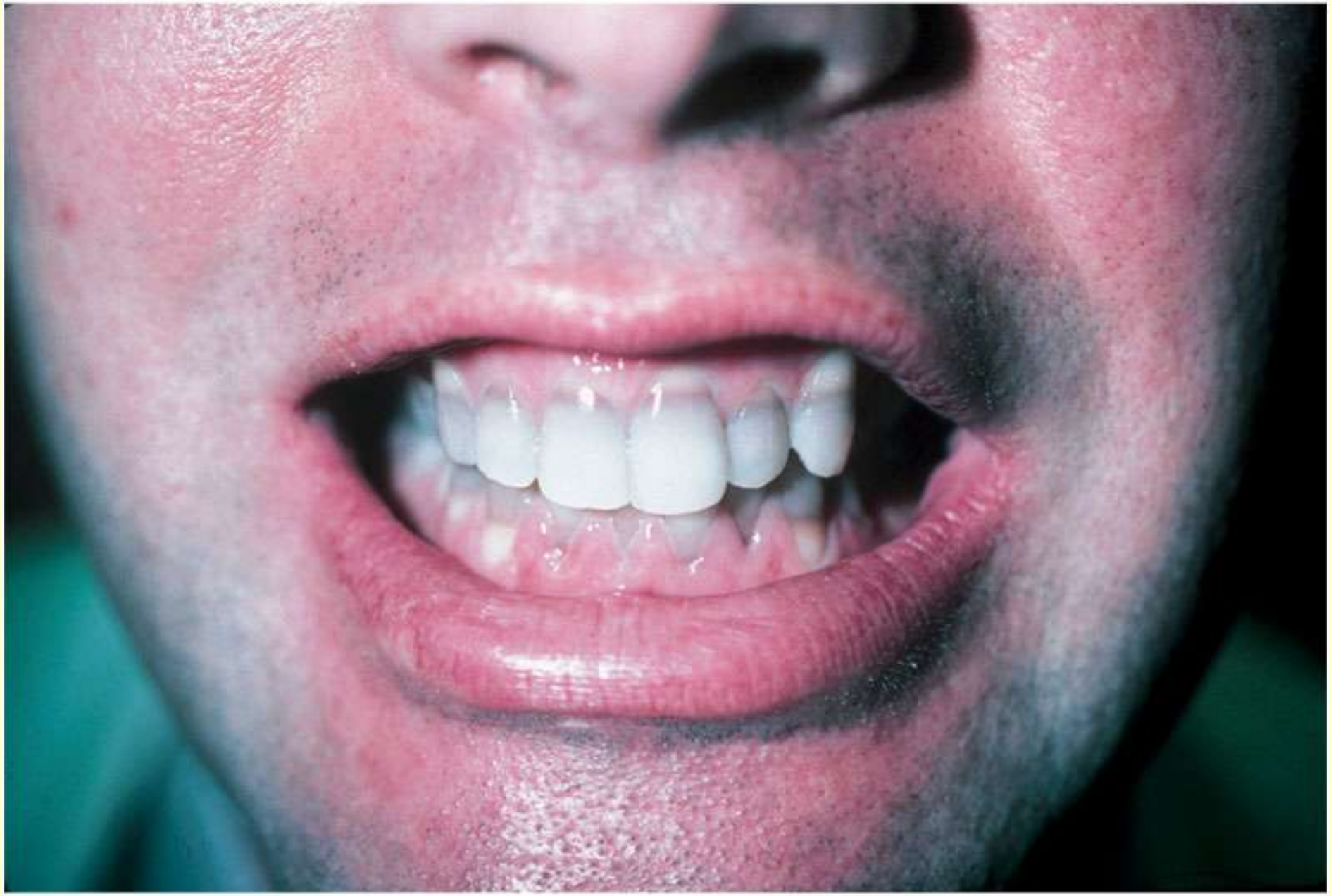


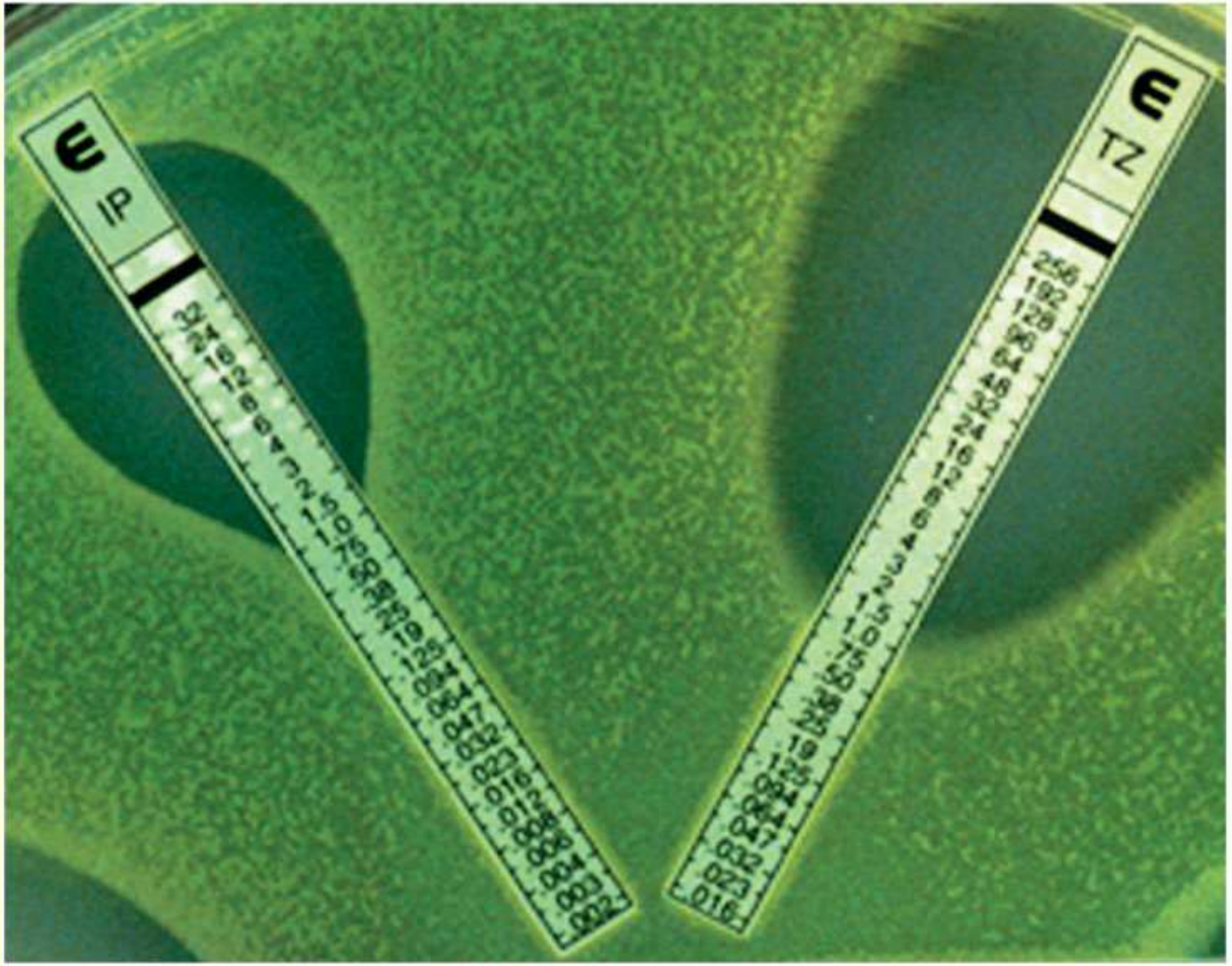
Selection for drug resistance



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, oligopeptidesand 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.

بسم اللة الرحمن الرحيم

Dr. chateen I Ali,
prof dr hadeel M.younis

Immune system : Introduction

Immunity : Ability of body to protect itself from foreign substances and cells, including disease-causing agents

So : the immune system exists to maintain the integrity of the body by removing the potentially pathogenic or threatening microorganisms

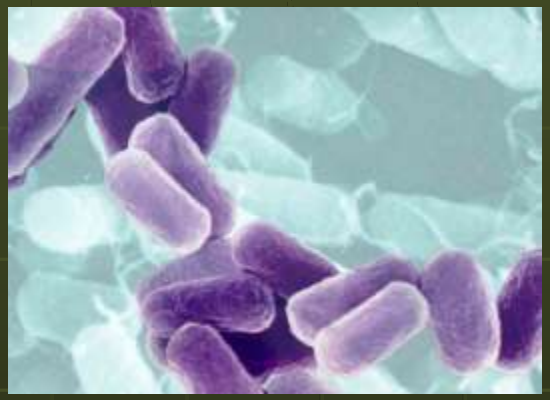
The immune system is:

Defense body mechanism

an interacting set of specialized cells and proteins designed to identify and destroy foreign invaders.

Pathogens

- = disease causing micro-organisms
  - bacteria
  - virus
  - fungi,
  - protozoa,
  - parasite,
  - prion



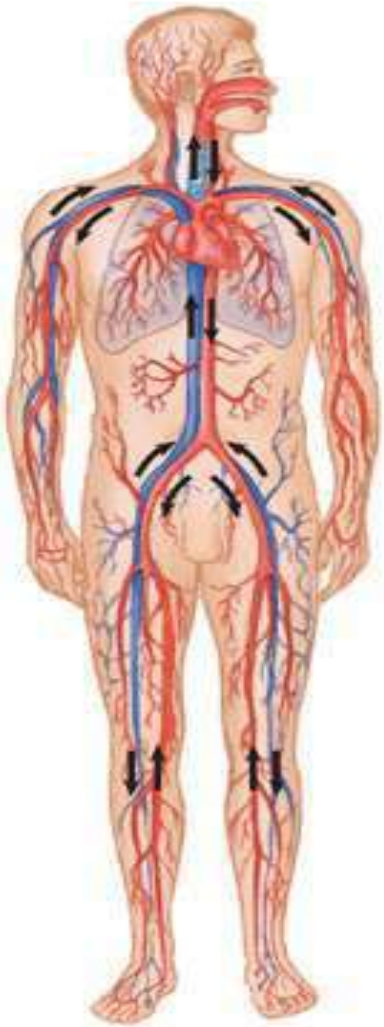
There are two kinds of immunological defence:

- 1. natural or innate immunity, pre-existing antigen-non-specific defences
- 2. adaptive or acquired immunity, during which the immune system responds in an antigen-specific manner to neutralize the threat efficiently with memory cells

Introduction

The immune system must be able to: differentiate between material that is a normal component of the body the body Ags (tolerance) ("self") and material that is not native to the body "nonself"

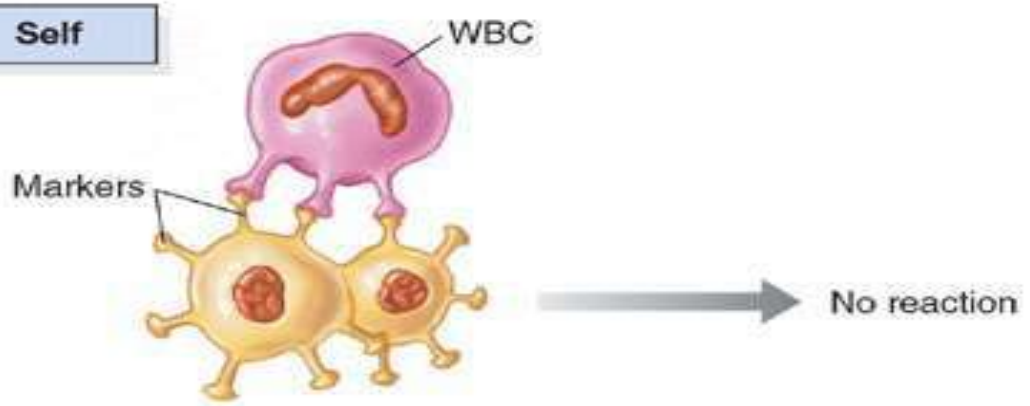
A highly specialized receptors present for discriminating between "self" and "nonself" body components



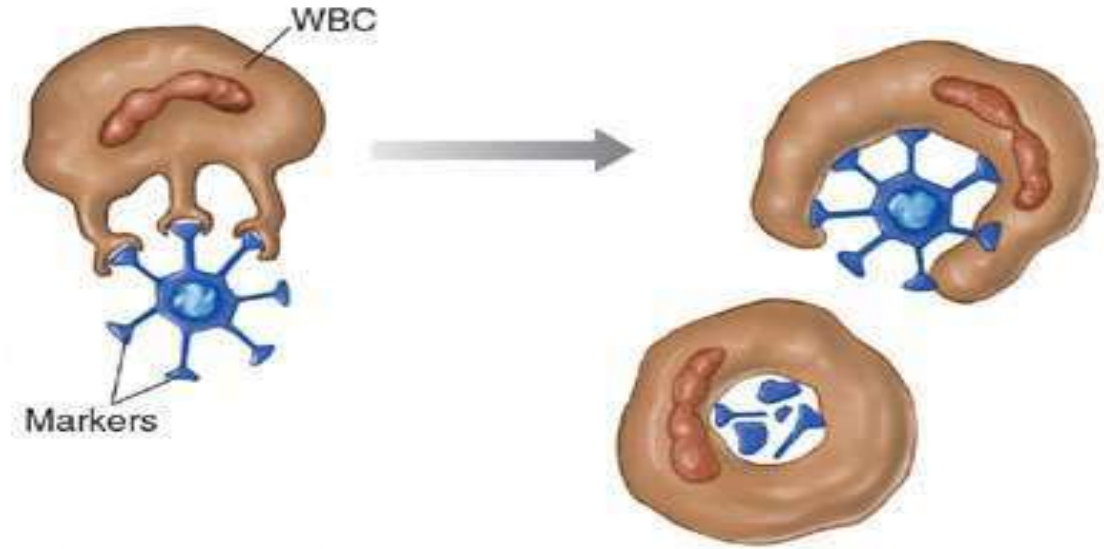
Surveillance

All body compartments are screened by circulating WBCs.

Self



Nonself



Detection and Recognition

Destruction

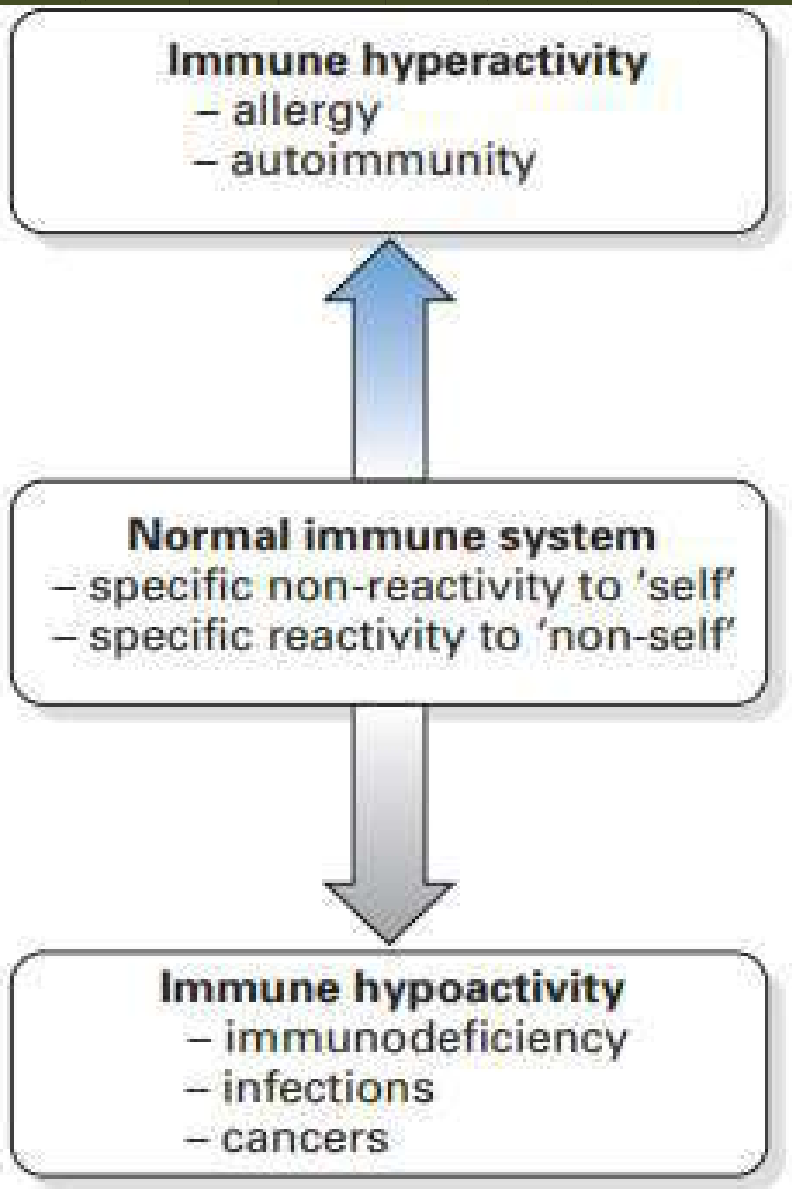


Fig. 8.1 Normal and aberrant immunity.

Introduction

*The discrimination between "self" and "non-self" and the subsequent destruction and removal of foreign material is accomplished by the two arms of the immune system

1) The innate (natural or nonspecific) immune system

2) The adaptive (acquired or specific) immune system

*These two systems perform many of their functions by cooperative interactions

Immunity

Innate immunity

Components

Macrophages
Granulocytes
Natural killer cells
Complement
Other chemicals: HCL, lysozyme

Characteristics

* Action is immediate
* Response is non-specific
* Response is not enhanced on repeated exposure to pathogen

Adaptive immunity

Humeral

Cell-mediated

Components

antigen presenting cells
T-cells
B-cells
Antibodies
Complement

Characteristics

* Action requires days to develop
* Response is specific
* Response is enhanced on repeated exposure to pathogen

Role of external body surfaces

* The skin consists of sheets of dry, cornified epithelial cells

  Intact skin act as barrier to bacteria and viruses

* Hair follicles and sebaceous glands produce: Antibacterial substances (fatty acids and enzymes)

* Normal microbial flora compete with: potential pathogens

Role of internal body surfaces

The normal movement of fluids and mucous act as mechanical factors for cleaning internal surfaces of:

Respiratory tract

Gastrointestinal tract

Genitourinary tract

Component of Innate Immunity

Innate Immune system

First line

1) Mechanical barriers
2) Chemical & biochemical inhibitors
3) Normal flora

Second line

A- cells
- 1- Natural killer
- 2- Phagocytes

B- Soluble factors

C- Inflammatory barriers

First line

1) Mechanical barriers

- Intact skin
- Mucous coat
- Mucous secretion
- Cilia of lower respiretory :

The movement of cilia can propel mucus-entrapped microorganisms from the tract (mucociliary escalator). -

-Blinking reflex and tears

- The hair at the nares
- Coughing and sneezing reflex

First line

2) Chemical & biochemical inhibitors

- Sweet and sebaceous secretion
- Hydrolytic enzymes in saliva
- HCl of the stomach
- Proteolytic enzyme in small intestine
- Lysozyme in tears
- Acidic pH in the adult vagina

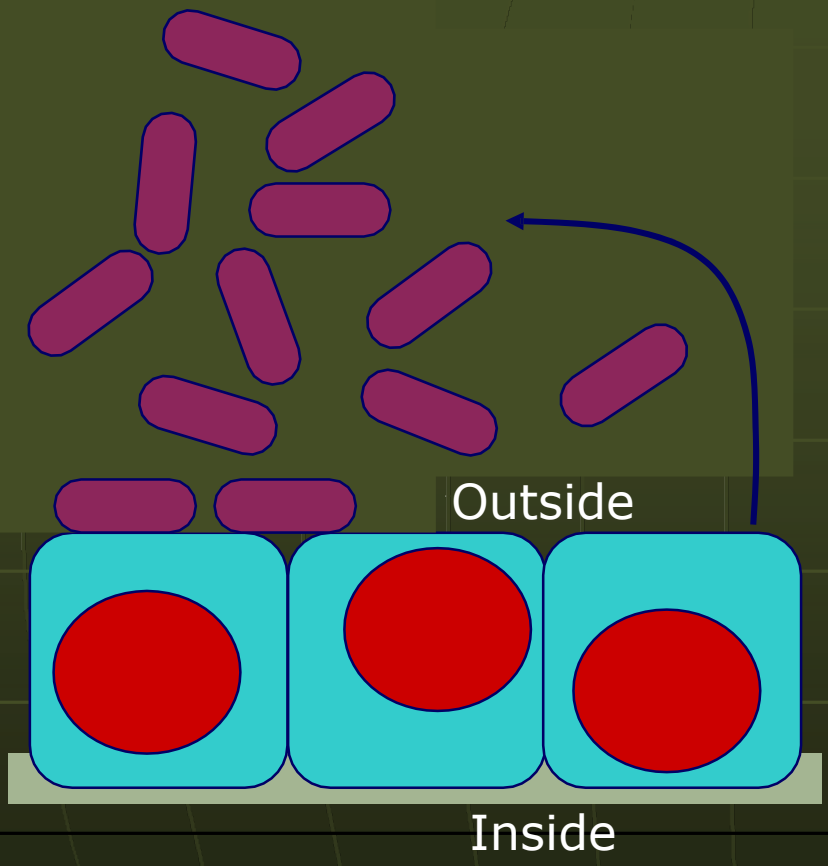
First line

3) Normal bacterial flora

- Competition for essential nutrients

- Production of inhibitory substances

First line of defence - Epithelial cells



Primary role is to block the entry of microorganisms

Functions within seconds of contacting a pathogen

A mechanical, selectively permeable barrier between the 'outside' and 'inside'

-Produce natural antibiotics - cationic antibacterial peptides - defensins

-May possess motile cilia in lower respiretory

Rapidly renewable

-Produce cytokines - proteins that alter the behaviour of other cells

-Produce chemokines - proteins that attract other cells

-produce mucins the

Transport antibodies from 'inside' to

Defensins and cathelicidins

- Defensins and cathelicidins are two major families of mammalian antimicrobial proteins.
- They contribute to host innate antimicrobial defences by disrupting the integrity of the bacterial cell membrane.
- Further, several members of defensins and cathelicidins have been shown recently to have chemotactic effects on host cells.

Table 8.1 Antigen-non-specific defence chemicals in oral secretions

Chemical	Antimicrobial function(s)	Major cell source(s)
Calprotectin	Divalent cation chelator, restricts microbe nutrition	Oral epithelial cells and neutrophils
Defensins (α and β types)	Membrane pore-forming peptides, cause osmotic lysis	Leukocytes and epithelial cells
Cathelicidins	Lysosomal antimicrobial polypeptides	Macrophages and neutrophils
Saliva	Ig, lysozyme, lactoferrin, peroxidases and GCF	Salivary acinar cells
Lysozyme	Muramidase activity, aggregates microbes and amphipathic sequences	Macrophages, epithelial cells and neutrophils
Peroxidase	Oxidizes bacterial enzymes in glycolytic pathways	Salivary acinar cells, neutrophils, eosinophils
Mucins	Aggregates bacteria, various effects, homotypic and heterotypic complexes	Salivary acinar cells

Second line

A) cells

1- Natural killer (NK)

Definition: Large granular lymphocytes
Innate cytotoxic lymphocytes

Source : Bon marrow precursors

Location : 10% or 15% of lymphocytes in peripheral blood
1% or 2% of lymphocytes in spleen

Function : Cytotoxic for
- Tumor cells
- Viral infected cells
- Bacterial, fungal, parasitic infection

Responsible for antibody–dependent cell mediated cytotoxicity (ADCC)

Second line

2- Phagocytes

Specialized cells for capture, Ingestion and destruction of invading microorganisms

* Polymorphoniclear leucocytes, mainly neutrophils: granulocytes circulate in blood

* Mononuclear cells (macrophages)

- Monocytes in blood

- Histocytes in connective tissues

- Fixed reticuloendothelial cells in liver spleen, lymph

Second line

B- Soluble factors

1- Acute phase protein (Plasma protein, CRP=C reactive protein, Fibrin.)

2- Complement (proteins in serum, body fluids)

2- Interferons (Proteins against viral infections)

3- Properdin (Complement activation)

5- Lactoferrrin,Transferrin (Iron binding protein)

6- Lactoperoxidase (Saliva & Milk)

7- Lysozyme (Hydrolyze cell wall)

Interferons

Proteins usually produced by virally infected cells

* Types of interferons:

1- Alpha interferon	Secreted by	Macrophages
	Induced by	Viruses
2- Beta interferon	Secreted by	Fibroblasts, Viruses
3- Gamma interferon	T- lymphocytes	

Interferons

Protective action of interferons:

1) Activate T-cells

2) Activate macrophages

3) Activate NK

Phagocytosis

The engulfment, digestion, and subsequent processing of microorganisms by macrophages and neutrophils

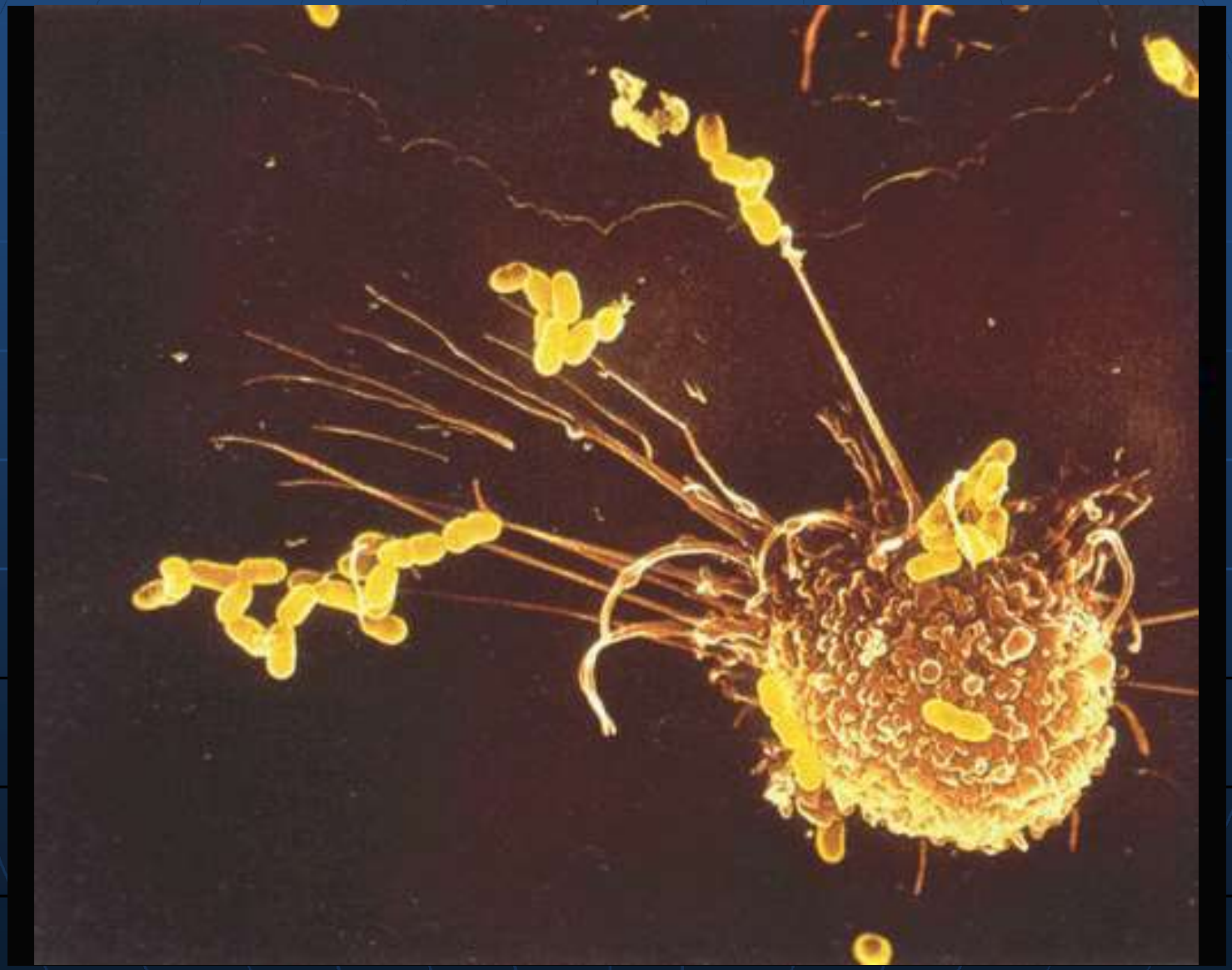
1) Chemotaxis & attachment:

a- Attraction by chemotactic substances (microbes, damaged tissues)

b- Attachment by receptors on surfaces of phagocytes

C-Opsinins and co-factors enhance phagocytosis

Phagocytes



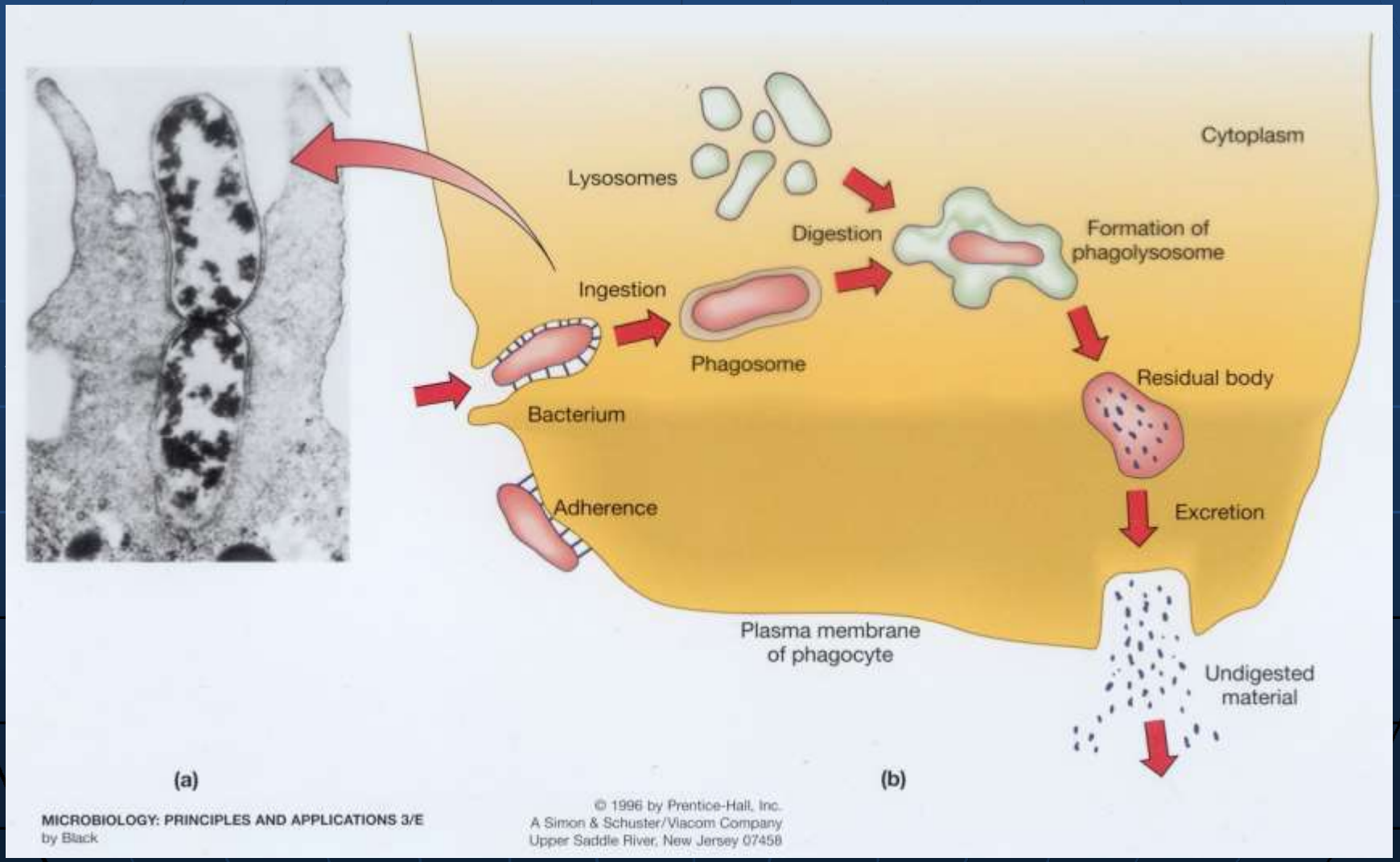
Phagocytosis

2) Ingestion:

* Phagocyte pseudopodia surround organism forming phagosom

* Fusion with phagocyte granules and release digestive, toxic contents

Phagocytosis



Phagocytosis

3- Killing (two microbicidal routes)

a- Oxygen depended system (powerful microbicidal agents)

Oxygen converted to superoxide, anion, hydrogen peroxide, activated oxygen and hydroxyl radicals.

b- Oxygen-independent system (anaerobic conditions)

Digestion and killing by lysozyme. Lactoferrin, low pH, cationic proteins and hydrolytic and proteolytic enzymes

C) Inflammatory Barriers

Acute Inflammation Resulting From Infection

Inflammation is a nonspecific response of living tissue to localize and eliminate the injurious agent

The injury may be:
physical, chemical or biological

Antigens

DEFINITIONS

- A. Immunogen : A substance that induces a specific immune response.
- B. Antigen (Ag) :A substance that reacts with the products of a specific immune response.
- C. Hapten : A substance that is non-immunogenic but which can react with the products of a specific immune response. - Haptens have the property of antigenicity but not immunogenicity.

- D. Epitope or Antigenic Determinant
  : That portion of an antigen that combines with the products of a specific immune response.
- E. Antibody (Ab): A specific protein which is produced in response to an immunogen and which reacts with an antigen.

- There are two main types of antigens, heteroantigens and autoantigens:
- **Heteroantigens** are substances that are foreign to your body and involve substances made by or found within:
  - viruses
  - bacteria
  - protozoa
  - blood and red blood cells from other people
  - snake venom
  - allergens such as pollen
  - certain proteins in foods
- **Autoantigens**, or self-antigens, are made by your body to fight your cells and are usually a sign of an illness such as an autoimmune condition.

Antibodies

- Antibodies, or immunoglobulins (Igs), are the secreted products of B lymphocytes, which have become activated following binding of antigen to their B cell receptors (BCRs). The formation of the antigen–antibody complex may result in:
- • neutralization of the antigen (e.g. soluble toxins, viruses)

- • removal of the complex by phagocytic cells
- • killing of antigen-bearing cells by the membrane attack complex of complement or by natural killer (NK) cells, monocyte/macrophages or granulocytes

- The basic Y-shaped, four-chain structure of the antibody molecule Antigen-binding specificity is provided by the combined variable (V) regions of heavy (H) and light (L) chains.

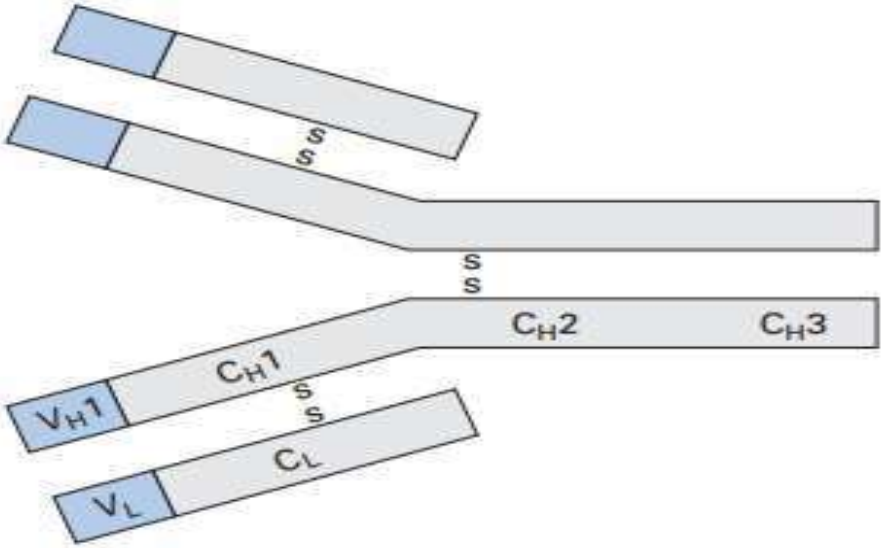


Fig. 9.1 Structure of the immunoglobulin molecule. C, constant region; H, heavy chain; L, light chain; C_H1, C_H2, C_H3 are globular domains with different biological properties; V, variable region.

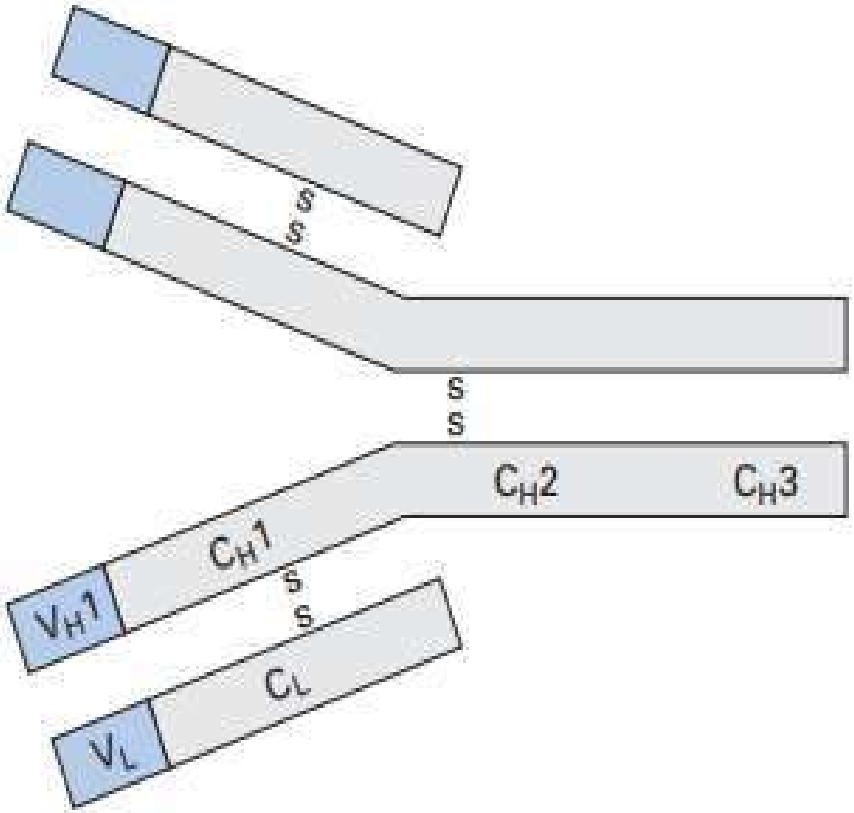


Fig. 9.1 Structure of the immunoglobulin molecule. C, constant region; H, heavy chain; L, light chain; C_H1, C_H2, C_H3 are globular domains with different biological properties; V, variable region.

- Since the basic Ig unit has two such pairings, the molecule can bind two identical epitopes; i.e. it is bivalent.

- The Ig heavy-chain constant region, particularly domains 2 and 3, which make up the Fc region, largely determines the biological activity of the molecule. There are five distinct classes of Ig (IgG, IgA, IgM, IgD, IgE), four subclasses of IgG (IgG1, IgG2, IgG3, IgG4) and two subclasses of IgA (IgA1, IgA2).

types of antibodies (immunoglobulins) include:

- **IgG.** These are the most abundant types of antibodies in your plasma. They detoxify harmful substances and provide long-term protection.
- **IgM.** These are the first antibodies made by B cells in response to antigens.
- **IgA.** These antibodies collect antigens and remove them from your body in your mucus or other body fluids.
- **IgE.** These antibodies trigger allergies and protect against parasites. Small amounts are in your skin, lungs, and mucosal membranes.
- **IgD.** These antibodies bind to B cells

Thanks

Cells Of Immune System

Cells involved in specific and nonspecific immune mechanisms are:

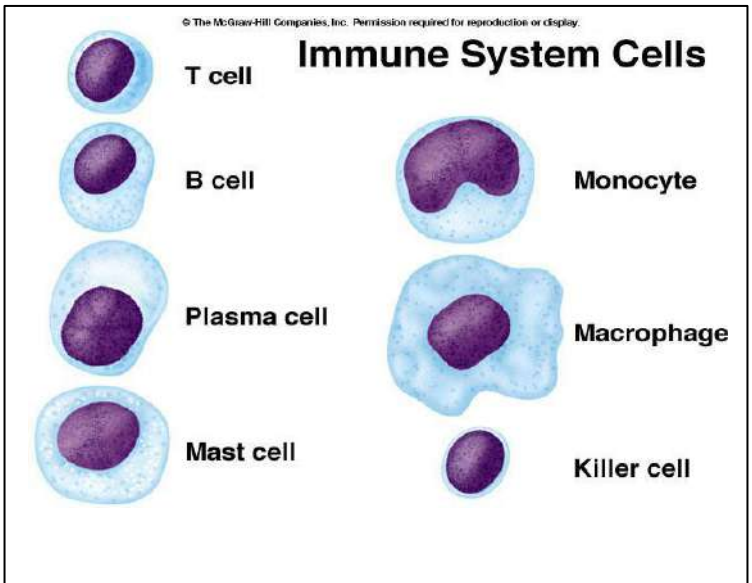
Innate immunity	Adaptive immunity
■ Granulocytes (i.e. neutrophils) ■ Macrophages ■ Dendritic cells ■ Natural killer (NK) cells	■ Lymphocyte ■ B cells ■ T cells ■ Cytotoxic T cells (CTLs) ■ Helper T cells (Th) ■ Memory cells

Hematopoitic leucocytes

The origin is the bone marrow : the immune cells include :

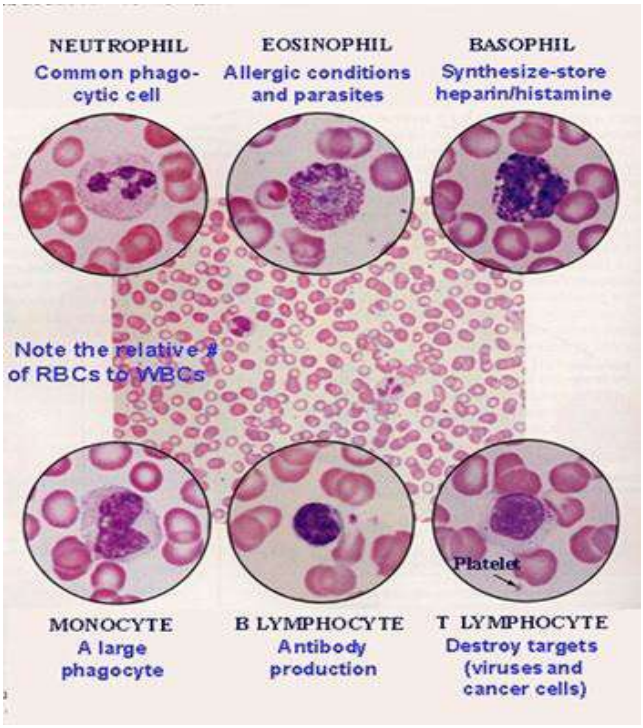
1- **Lymphoid** :with production of lymphocytes

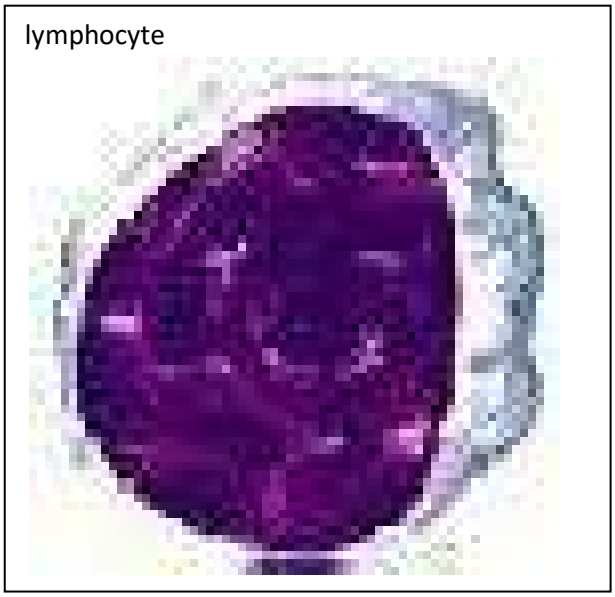
- Many types; important in both humoral and cell-mediated immunity
- B-cells produce antibodies
- T- cells
  - Cytotoxic T cells
  - Helper T cells
- Memory cells
- NK cells



* **T-lymphocytes**: characteristics :

- Antigen specific cells carrying CD3 complex, CD4, CD8
- Dominant blood lymphocytes (70%)
- Produce cytokines
- Activation of other cells (Th CD4)
- Suppressors for others (Ts CD8)





* **B-lymphocytes**:

- Antigen specific cells with surface receptor
- Less common lymphocytes (20%)
- Responsible for antibody production

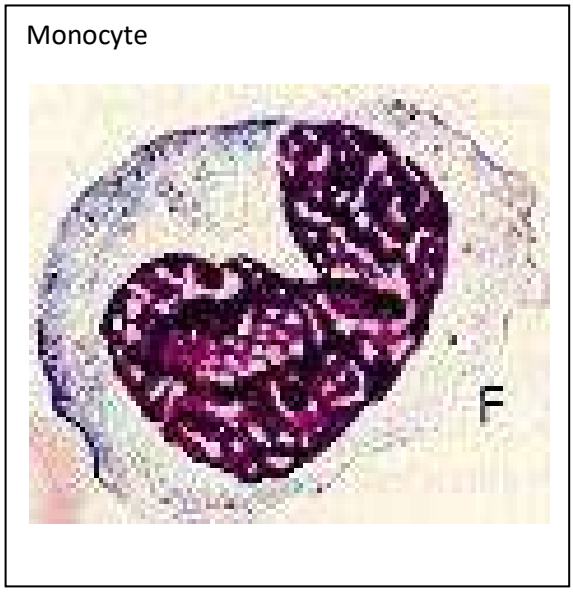
* **NK, K cells**:((Natural killer cell)

- NK cells do not require prior immunization or activation
- They attach to ‘target’ cells
- Cytotoxic granules are released onto surface of cell
- Effector proteins penetrate cell membrane and induce programmed cell death
- Not antigen specific
- Carry Fc receptors , NK-target cell receptor

2- **Monocytic myeloid**

a- **Monocyte-tissue macrophages**:

- Monocyte is a young macrophage
- There are tissue-specific macrophages
- MØ process antigen, are phagocytes and produce cytokines (esp., IL1 & IL6)
- Non specific
- Carry Fc receptors
- Phagocytic
- Antigen processing and presenting cells

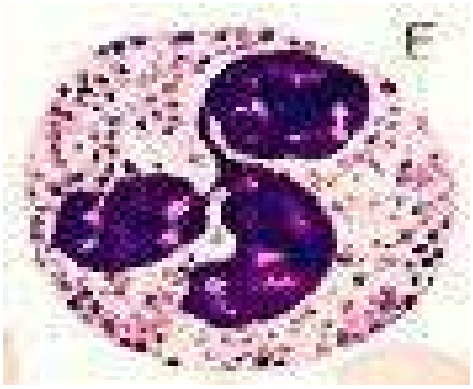


b- **Neutrophils**: First defense line

- Granulocyte
- Phagocytes

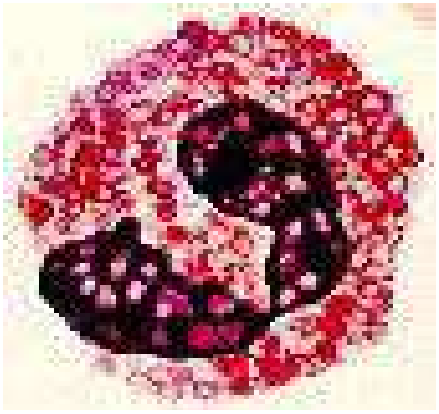
- Short life span (hours) production 10^{11} cells per day
- Very important at "clearing" bacterial infections
- Cytoplasmic granules
- Non specific
- Carrying Fc, complement molecules

Neutrophil



c- **Eosinophils**:

- A granulocyte
- A cell-killing cells
- Orange granules contain toxic compounds
- Produce allergic mediators and Important in parasitic infections
- Non specific
- Carrying Fc receptor



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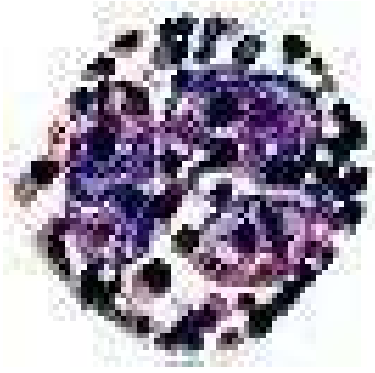
Basophil



d- **Basophils and Mast cells:**

- A granulocyte
- A cell-killing cells

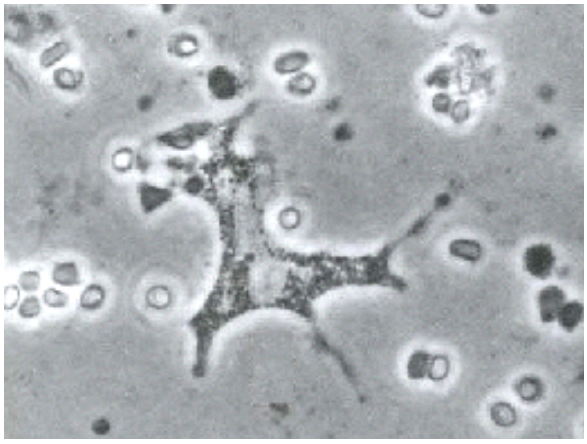
- Blue granules contain toxic and inflammatory compounds
- Produce allergic mediators so Important in allergic reactions
- Non specifc
- Carrying Fc receptors

.

e- - **Dentritic cells** :

- Found mainly in lymphoid tissue
- specialized APCs (professional APCs)Function as antigen presenting cells (APC)
- Most potent stimulator of T-cell response

APC : Antigen presenting (or processing) cells



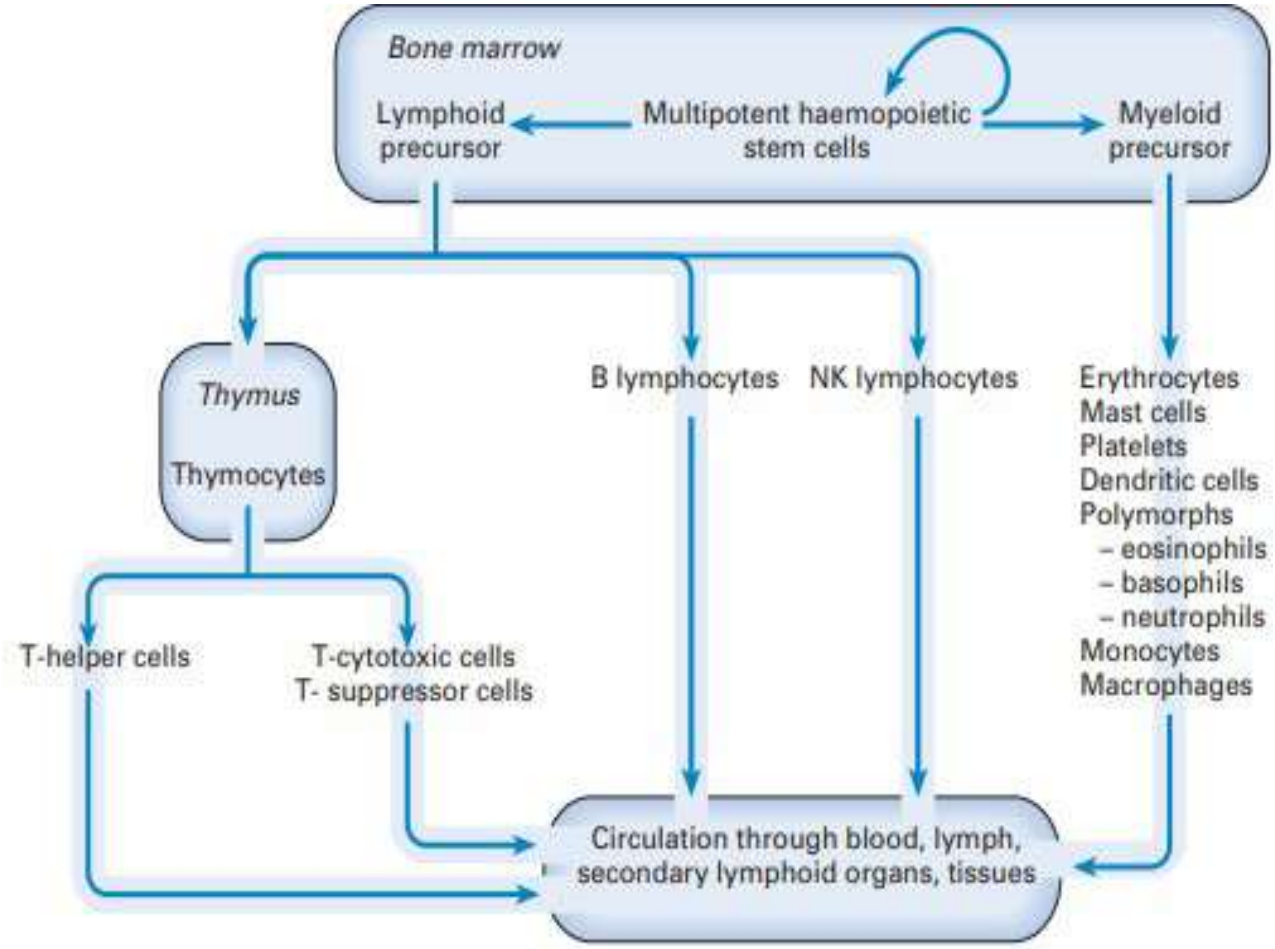


Fig. 8.8 Cells and organs of the immune system. NK, natural killer.

The lymphoid organs

-The primary sites of lymphocyte production are the bone marrow and thymus. Immature lymphocytes produced from stem cells in the **bone marrow** may continue their development within the bone marrow (B lymphocytes, NK cells)

-or migrate to the thymus and develop into T lymphocytes. 'Education' within the **primary lymphoid organs** ensures that emerging lymphocytes can discriminate self from non-self. They migrate through the blood and lymphatic systems to the **secondary lymphoid organs** – **spleen**, **lymph nodes** and **mucosa-associated lymphoid tissue (MALT)** of the GIT, respiratory and urogenital tracts. Here,

lymphocytes encounter foreign antigens and become activated effector cells of the immune response.

-The **spleen** acts as a filter for blood and is the major site for clearance of opsonized particles. It is an important site for production of antibodies against intravenous antigens.

-The lymph nodes form a network , which drain fluids from the tissues and concentrate foreign antigen on to APCs and subsequently to lymphocytes.

-Spleen and lymph nodes are encapsulated organs, whereas MALT is non-encapsulated dispersed aggregates of lymphoid cells positioned to protect the main passages by which microorganisms gain entry into the body.

-Gut-associated lymphoid tissue (GALT) includes **Peyer's patches** of the lower ileum, accumulations of lymphoid tissue in the lamina propria of the intestinal wall and the tonsils.

- Mature lymphoid cells continuously circulate between the blood, lymph, lymphoid organs and tissues until they encounter an antigen, which will cause them to become activated

Complement

The complement system is very much involved in the inflammatory response and is one of the **key effector mechanisms** of the immune system. It consists of at least **30 components – enzymes (protiens**), **regulators** and **membrane receptors** – which interact in an ordered and tightly regulated manner to : **phagocytosis or lysis of target cells**.

Tow types;

1- The alternative pathway
2- The classical pathway

Alternative activation

-Complement factor **C3** is the central component of both the classical and alternative pathways

- Products of C3 activation, C3b and inactivated C3b (iC3b) bind to microorganisms and are recognized by complement receptors (CRs) on phagocytes.

If any **C3b** molecules bind to a normal host cell surface, they can then bind the next component in the sequence, **factor B. Factor D** (**the only complement factor present in body fluids as an active enzyme**) splits off a small fragment, Ba, leaving an active **C3 convertase**, **C3bBb**, on the cell surface.

-the normal host cell is able actively to dissociate and **inactivate C3bBb**. This is achieved by action of **regulatory proteins :**

1- decay-accelerating factor (DAF),

2- membrane cofactor protein (MCP),

2- factor H

- When Factor H inactivated and split by bacterial endotoxin LPS

-The enzyme C3bBb converts C3 into C3a and C3b. The latter is incorporated, along with **properdin (factor P**), to form **PC3bBbC3b**. This is a stable enzyme whose substrates are C3 and **C5.** It amplifies C3b production and activates the membrane attack pathway

Classical activation

Classical pathway of complement activation is mainly initiated by **complexes of antigen with antibody**. Antibodies of the immunoglobulin (Ig) IgG1, IgG2, IgG3 and IgM classes, but not IgG4

-. The first component of the classical pathway, **C1**, is actually a complex of **C1q, C1r and C1s**. This complex can bind very weakly to **monomeric IgG,** but when IgG complexes with antigen in such a way that adjacent IgG molecules are close together, **C1q** binds firmly between the two molecules.

-The C1 complex can bind strongly to a single molecule of pentameric IgM, but only after the conformation of the latter has been altered by binding to antigen

-. Activated C1 reacts with C4 and C2, splitting off small peptides C4a and C2a. The resulting **C4b2b** is deposited on a surface and performs a similar job to **C3bBb** of the alternative pathway: it can **convert C3 into C3a and C3b**, and the latter can either **opsonize particles for phagocytosis** or bind to C4b2b. Cell-bound **C4b2b3b** is more stable than C4b2b, being somewhat protected from the regulatory proteins DAF . Like PC3bBbC3b, it activates the membrane attack pathway

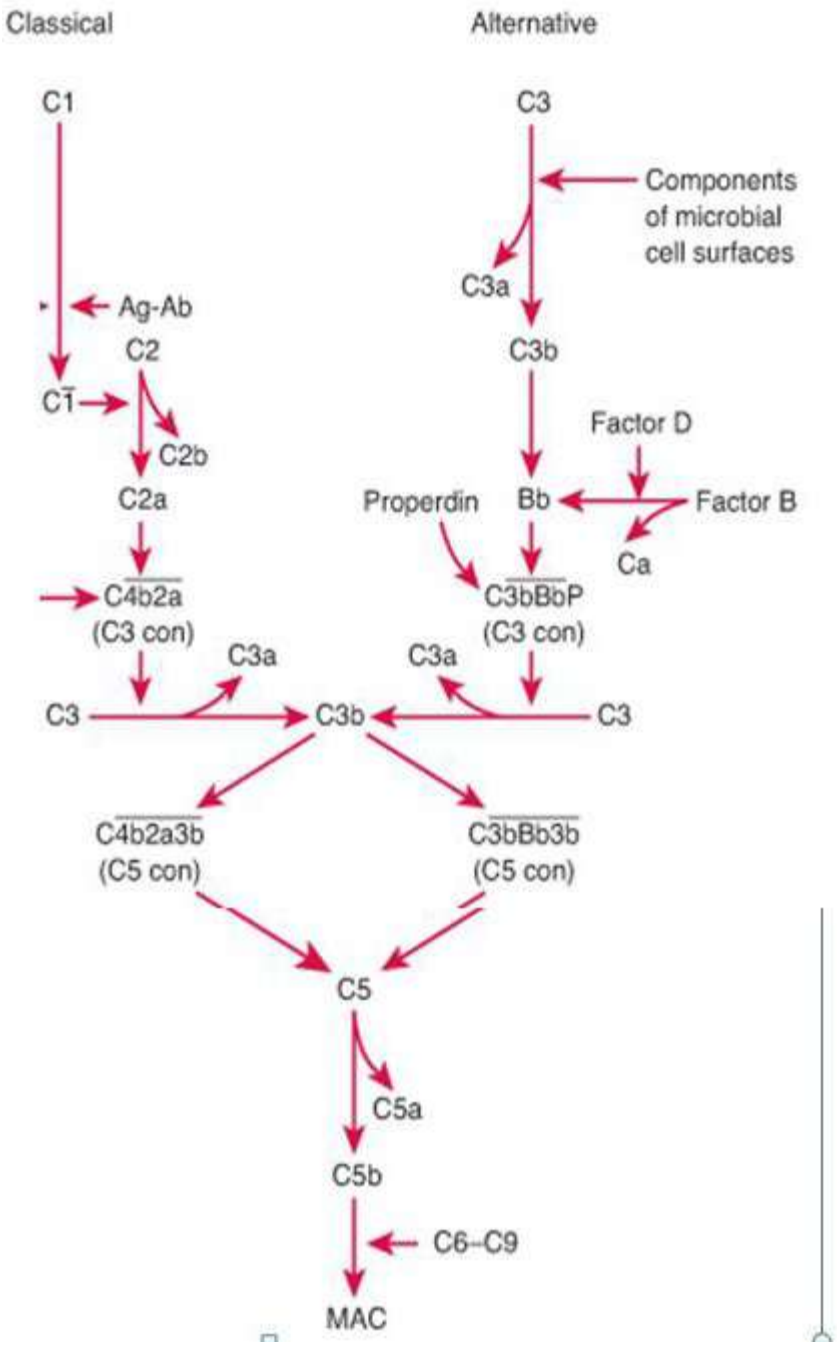


Fig. : Classical and Alternative Pathway

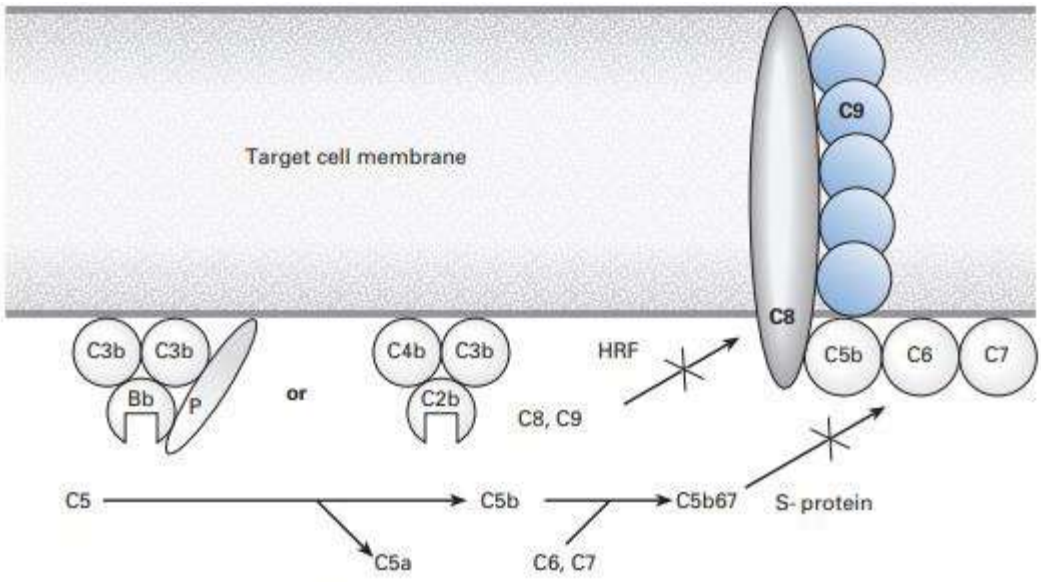


Fig. 8.6 Membrane attack pathway. HRF, homologous restriction factor; P, properdin.

Membrane attack Complex

The peptides Bb and C2b, bound into their respective alternative (PC3bBbC3b) and classical (C4b2b3b) pathway enzymatic complexes, initiate membrane attack (Fig. 8.6) by splitting a small peptide, C5a, from C5 to form C5b. This molecule binds C6 and C7. Cell-bound C5b67 acts as a template for the binding of one molecule of C8 and up to 18 molecules of C9. Normal cells in the body are largely protected from bystander lysis by homologous restriction factor (HRF), which intercepts C8 and C9 before they can be properly assembled into the membrane attack complex (MAC). The MAC forms **transmembrane channels**, which permit **osmotic influx** so that the target cell swells up and bursts.

Biological effects of complement activation

-Probably the most important function of the complement system is to **opsonize** antigen–antibody (immune) complexes, microorganisms and cell debris for phagocytosis by complement receptor CR1, CR3 and CR4

-The peptides C3a, C4a and C5a are **anaphylatoxins** that cause **mast cell degranulation** and **smooth-muscle contraction**. They increase vascular permeability, which permits cells and fluids to enter the tissues from the circulation.

Further important properties of C5a are:

• inducing adherence of blood phagocytes to vessel endothelium, following which they are able to migrate into the tissues during inflamation

• upregulating CR1, CR3 and CR4 • attracting phagocytes (chemotaxis) towards the site of complement activation.

Major histocompatibility complex

-In humans, products of the **highly polymorphic MHC genetic loci** on chromosome **6** are known as histocompatibility locus antigens (HLAs).

-Their function is to bind APC **processed short antigenic peptides** and present them on the APC surface to T cells.

HLA phenotype is responsible for tissue transplant rejection when the recipient and donor are not HLA-matched.

There are two classes of HLA molecules:

1. HLA-A, -B and -C (class I) are found on all nucleated cells in the body.

2. HLA-DQ, -DR and -DP (class II) molecules are usually only found on monocytes/macrophages, B cells, dendritic cells (i.e. APCs), some epithelial cells and activated T cells.

One **HLA-A, -B, -C**, and one -**DQ, -DR and -DP** antigen is inherited from each parent, so each individual expresses up to **six class I** and **six class II** antigens**. Each HLA molecule can bind a large number of different antigenic peptides**.

However, the complement of HLA antigens possessed by an individual will determine the range of antigenic peptides that can be presented by APCs. **Class I molecules present peptides to CD8+ T lymphocytes, while CD4+ T cells are restricted to MHC class II**

Cytokines

- Low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune system

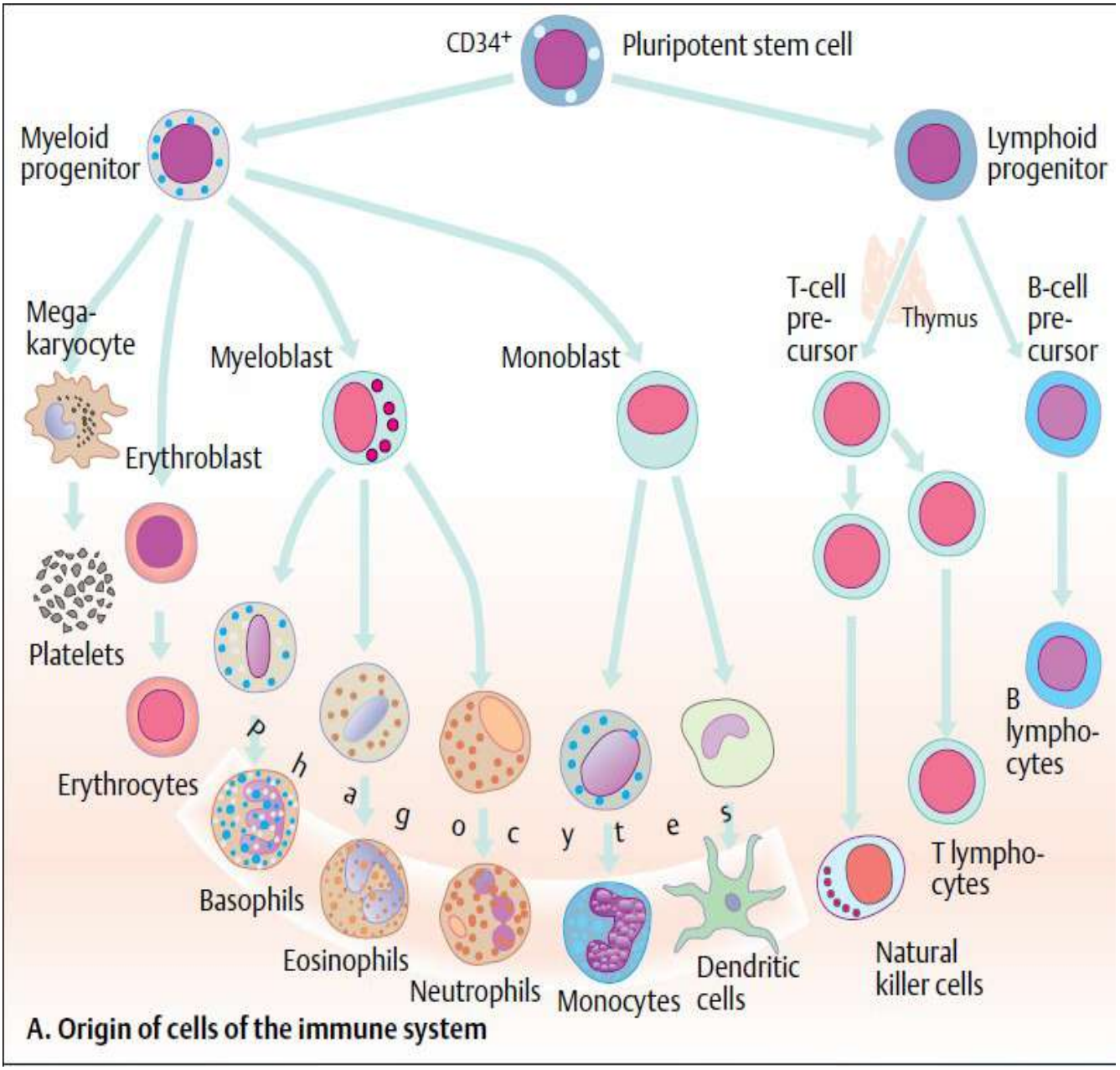
-Innate immune system

- Macrophages and Dendritic cells : produce
  - Tumor necrosis factor-alpha (TNF-α)
  - Interleukin-1 (IL-1)
  - Interleukin-12 (IL-12)

-Adaptive immune system

- T-lymphocytes: produce
  - Interleukin-2 (IL-2)
  - Interleukin-4 (IL-4)

The origin of Immune cells



IMMUNOLOGICAL TOLERANCE AND AUTOIMMUNITY

Dr. chateen I Ali,

prof dr hadeel M.younis

Tolerance

- **Our own bodies produce over 100,000 different proteins(self antigens), of immunology has been to understand how the immune system respond against foreign antigens(pathogens), while at the same time avoiding reacting to self antigens**
- **Immunotolerance**, describes a state of unresponsiveness of the immune system to substances or tissue that have the capacity to elicit an immune response. It contrasts with conventional immune-mediated elimination of foreign antigens

TOLERANCE

Introduction

- **Tolerance refers to the specific immunological non-reactivity to an antigen resulting from a previous exposure to the same antigen.**
- **While the most important form of tolerance is non-reactivity to self antigens, it is possible to induce tolerance to non-self antigens.**
- **When an antigen induces tolerance, it is termed tolerogen.**

TOLERANCE

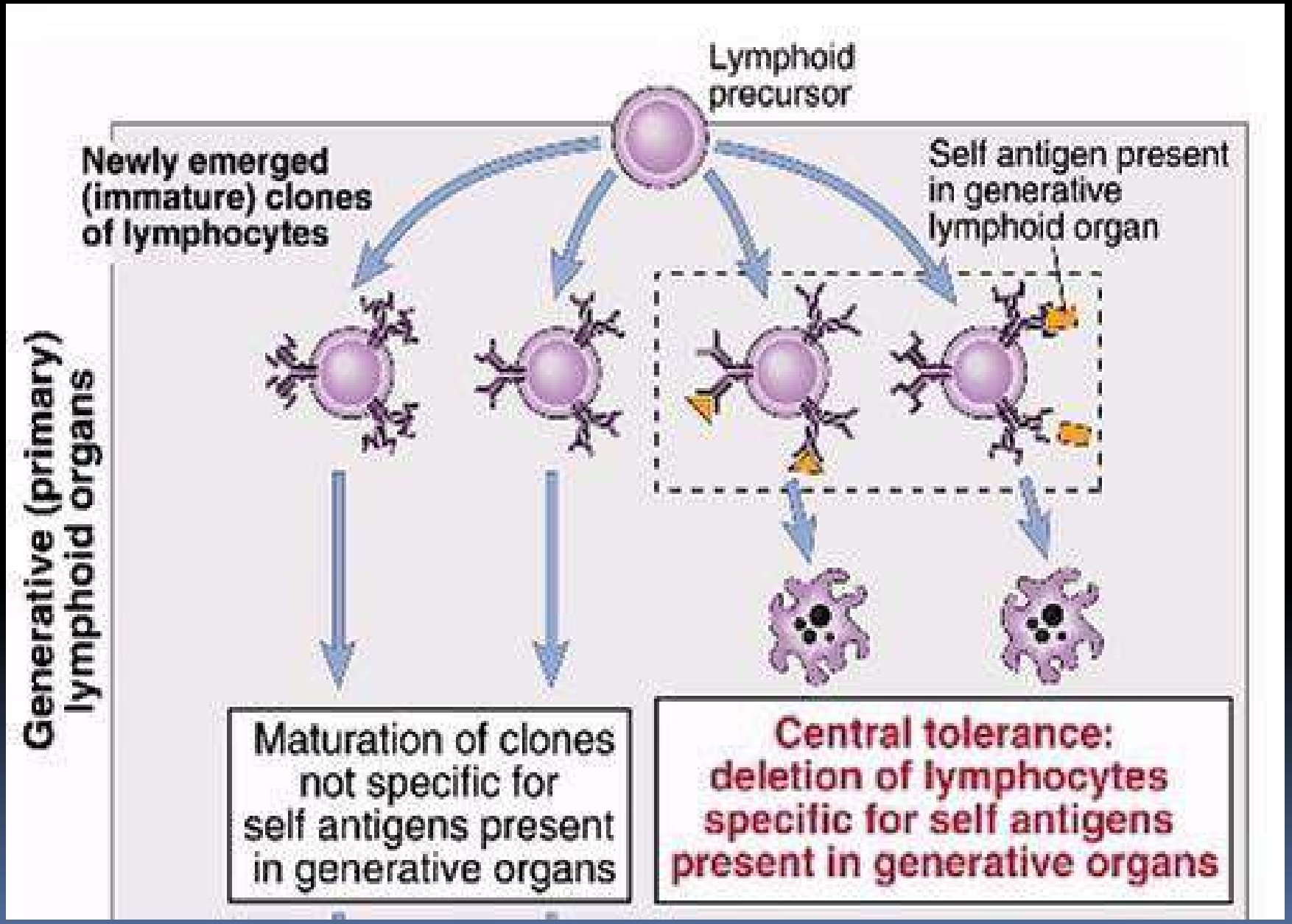
- Tolerance is different from immunodeficiency, Itis an active antigen-dependent process.
- Immunodeficiency : **The decreased ability of the body to fight infections and other diseases**.
- Tolerance is specific like immune response and immunological memory:
- it can exist in T-cells, B cells or both
- the tolerance at the T cell level is longer lasting than tolerance at the B cell level.

Tolerance

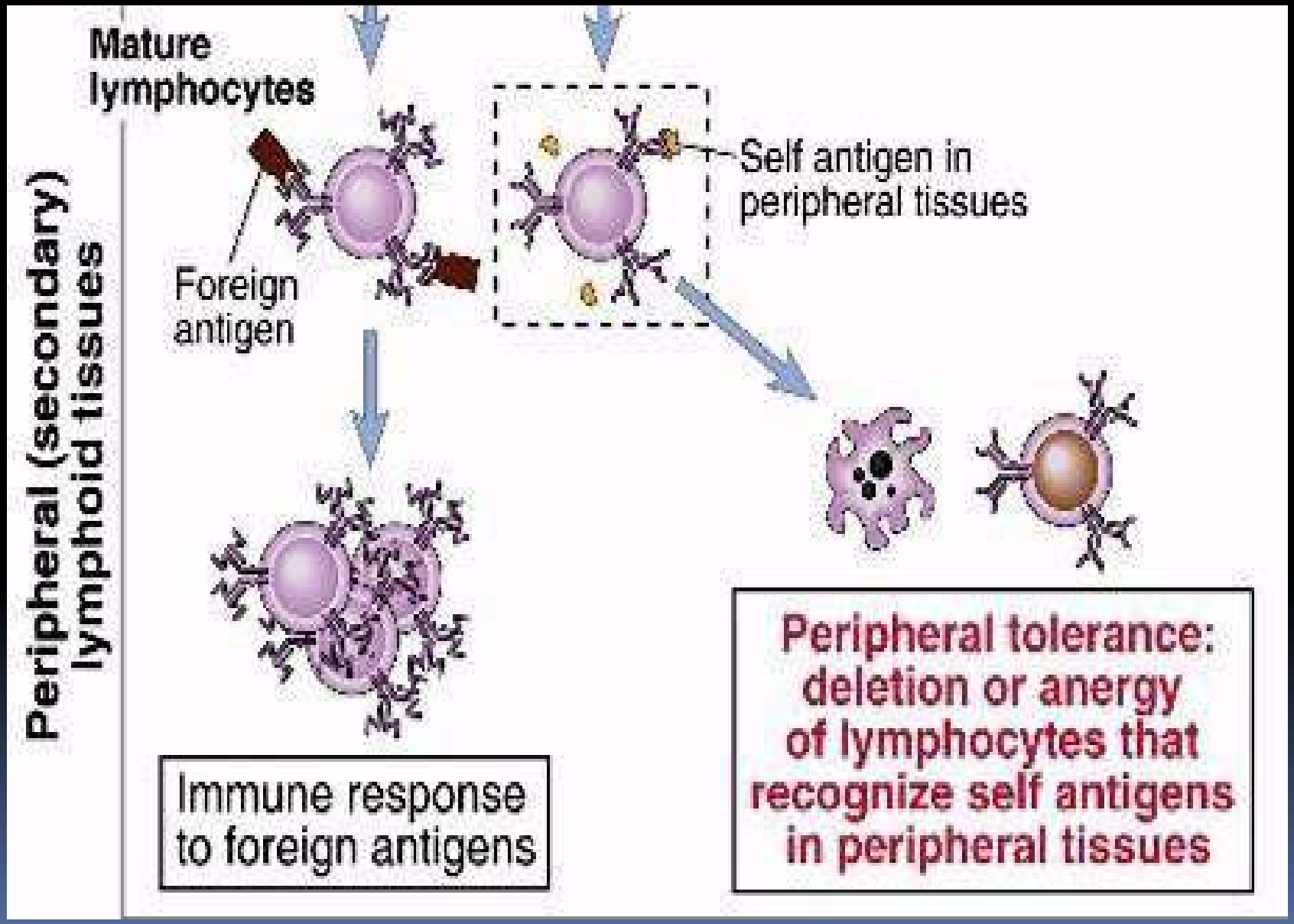
Tolerance is classified into:

- **Central Tolerance - this occurs during lymphocyte development *in the thymus and bone marrow***
- **Peripheral Tolerance - occurs after lymphocytes leave the primary lymphoid organs *in other lymphoid tissues and lymph nodes***
- Central tolerance is the main way for the immune system learns how to discriminate self from non-self. Peripheral tolerance is key for preventing over-reactivity of the immune system to various environmental entities (allergens, gut microbes, etc.)
- Defects in central or peripheral tolerance also cause autoimmune disease

Central Tolerance



Peripheral Tolerance



- **Mechanism of tolerance**
- **I. Clonal deletion (apoptotic cell death): During maturation of lymphocytes in the thymus for T cell or in the bone marrow for B maturation, immature lymphocytes that recognize and interact with self-antigen are deleted by negative selection.**
- **II. Clonal anergy: Functional inactivation without cell death of self reactive cells.** T cells require stimulation of additional receptors, termed co-stimulatory molecules, in order to be fully activated. CD28 is a major co-stimulatory receptor, whose ligands consist of B7-1 (CD80) and B7-2 (CD86) molecules expressed by APC

- Factors affecting the induction of tolerance
- 1) Immunologic maturity of the host: neonates are better than adults.
- 2) Structure and dose of tolerogen:
- a- Simple molecules induce tolerance better than complex ones.
- b- Very high and very low doses of tolerogen may result in tolerance.
- 3) Types of cells: T-cells become tolerant more and longer than B-cells.
- 4) The persistence of tolerogen.

Autoimmune Diseases

- Although the immune system has a perfect system of checks and balances to ensure self tolerance, occasionally this system breaks down .
- When the immune system attacks host components causing pathological changes, this situation is called autoimmunity.
- Both antibodies and effectors T cells can be involved in the damage in autoimmune diseases.

- Many people experience an autoimmune reaction during their lifetime. Mostly these are short-lived, self-resolving sequale of infection. However, in some individuals the reaction is chronic, debilitating and even life- threatening.

- Autoimmune disease form a spectrum ranging from organ specific , in which one organ is affected, to systemic diseases in which pathology is diffused throughout the body.

- ***Etiology of Autoimmunity***

- **The exact etiology of autoimmune diseases is not known. However, various theories have been offered, these include:-**
- **1- Sequestered antigen: release of antigens from some organs (e.g., testes, brain, eye, etc.) due to accidental traumatic injury or surgery can result in the stimulation of an immune response and initiation of an autoimmune disease.**
- **2-Bypass T-cell: normal immune system requires the activation of B-cells by T-cells before produce antibodies. This requirement of a T-cell can be bypassed in rare instances, such as infection by organisms producing super-antigens, which are capable of initiating polyclonal activation of T-cells and massive cytokine release.**

- 3-Molecular mimicry (Cross reaction): exposures to antigens that cross react with self antigens. An immune response to these antigens will result in immune attack against self antigens. Antibodies against M protein of *Streptococcus pyogens* may react with heart valves and cause rheumatic heart fever.
- 4-Lack of regulatory T cells: there are fewer regulatory T-cells in many autoimmune diseases.
- 5- Cytokines Imbalance.
- 6- Hormone disturbance (estrogens) e.g. systemic lupus erthymatosus (SLE) affects women 10 times more than men.
- 7-Genetic factors

- Effects of Autoimmunity

- 1) Tissue destruction:- In diabetes Type 1, cytotoxic T-cell (CTLs) destroy insulin-producing β-cells in pancreas.
- 2) Antibodies block normal function:- In myasthenia gravis, antibodies binds to acetylcholine receptors.

- 3) Antibodies stimulate inappropriate function:- In Graves' disease (thyrotoxicosis), antibodies binds thyroid stimulating hormone (TSH) receptor and mimics thyroid-stimulating hormone, then activates unregulated thyroid hormone production.
- 4) Antigen-antibody complexes affect function:- In rheumatoid arthritis: IgM specific for IgG produced, and lead to deposition IgM-IgG complexes in joints and cause inflammation.

- **Classification of Autoimmunity**
- Autoimmune diseases are generally classified on the basis of the organ or tissue involved.
- ***Organ specific autoimmune diseases*** **:** in which the immune response is directed against antigen associated with the target organ e.g., Diabetes mellitus, Coeliac disease and Thyroiditis.
- ***Systemic*** **(non-organ-specific)** ***autoimmune diseases*** **:** in which the immune response is directed against antigen not associated with the target organ e.g., SLE, Sjögren's syndrome and rheumatoid arthritis.

TABLE 16-1 Some autoimmune diseases in humans

Disease	Self antigen	Immune response
ORGAN SPECIFIC AUTOIMMUNE DISEASES		
Addisonis disns	Adrenal cells	Auto-antibodies
Autoimmune hemolytic anemia	RBC membrane proteins	Auto-antibodies
Goodpasture's syndrome	Renal and lung basement membranes	Auto-antibodies
Graves' disease	Thyroid stimulating hormone receptor	Auto-antibodies (stimulating)
Hashimoto's thyroiditis	thyroid proteins and cells	TH cells; auto-antibodies
Idiopathic thrombocytopenia purpura	Platelet membrane proteins	Auto-antibodies
Insulin-dependent diabetes mellitus	Pancreatic beta cells	TH cells; auto-antibodies
Myasthenia gravis	Acetylcholine receptors	Auto-antibody (blocking)
Myocardial infarction	Mean	Auto-antibodies
Pernicious anemia	Gastric parietal cells, intrinsic factor	Autoantibody
Poststreptococcal glomerulonephritis	Kidney	Antigen-antibody complexes
Spontaneous infertility	Sperm	Auto-antibodies
SYSTEMIC AUTOIMMUNE DISEASES		
Ankylosing spondylitis	Vertebrae	Immune complexes
Multiple sclerosis	Brain or white matter	TH cells and T_C cells, auto-antibodies
Rheumatoid arthritis	Connective tissue IgG	Auto-antibodies, immune complexes
Scleroderma	Nuclei, heart, lungs, stroma, intestinal tract, kidney	Auto-antibodies
Sjogren's syndrome	Sialovary, salivary gland, thyroid	Auto-antibodies
Systemic lupus erythematosus (SLE)	DNA, nuclear protein, RBC and platelet membranes	Auto-antibodies, immune complexes

Autoimmune Disease: Effects on Your Dental Health

Sjogren's Syndrome

Sjogren's Syndrome is one of the most common autoimmune diseases that affect dental health. It is a disease that weakens the salivary glands, the glands that produce saliva. Saliva is an important part of fighting off infection and how your mouth functions. With less saliva, you become prone to fungal infections, changes in taste, and you may see an increase in cavities.

Crohn's Disease

Crohn's disease is **a type of inflammatory bowel disease (IBD)**. Crohn's Disease can cause swelling in your mouth, particularly in your gums.

Hashimoto's Disease

Hashimoto's Disease doesn't affect the teeth or gums directly in most cases. It is more focused on the tongue and throat. It causes significant swelling in the esophagus, which makes swallowing difficult.

Scleroderma has a similar effect that can make it difficult to eat.

Lupus

Lupus affects your dental health by creating lesions and ulcers in your mouth.

Hypersensitivity:

- **refers to excessive, damaging and sometimes fatal reactions produced by the normal immune system**.
- Hypersensitivity reactions can *be divided into four types:* type I, type II, type III and type IV, based on the mechanisms involved and time taken for the reaction.

Type I Hypersensitivity:

- It is **also known as immediate or anaphylactic hypersensitivity**.
- The reaction may involve skin (urticaria and eczema), eyes (conjunctivitis), nasopharynx (rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis).
- The reaction may cause **a range of symptoms from minor inconvenience to death**.
- The reaction usually takes 15 - 30 minutes from the time of exposure to the antigen *(often called allergens*

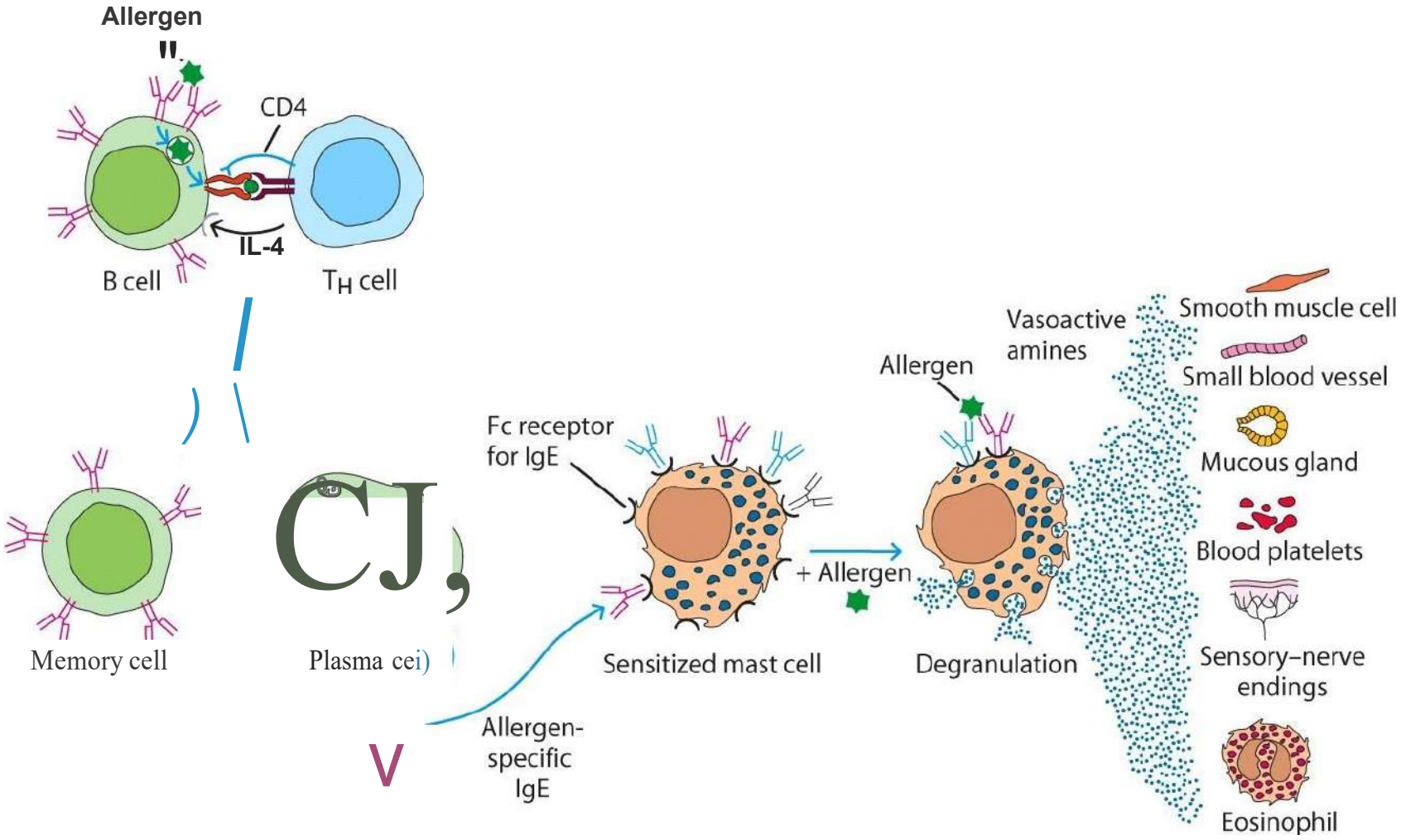
- *Allergens:* can be complete protein antigens or low–molecular-weight proteins capable of eliciting an IgE response.
- **Common allergens:** Plant pollens, house dust mite, fungi spores, foods (eggs, milk, peanuts and wheat), insect stings (bee and wasp), some drugs and chemicals.

- **Mechanism of Type I Hypersensitivity**
  Immediate hypersensitivity is mediated by IgE. The primary cellular component in this hypersensitivity is **the mast cell or basophile**.

- The mechanism of reaction involves production of IgE, in response to antigens. **IgE has very high affinity for its receptor (FcR) on mast cells and basophils.**
- **A subsequent (second) exposure** to the **same allergen** cross links the cell-bound IgE this will trigger mast cell degranulation and the release of various pharmacologically active mediators. These mediators cause *smooth muscle contaction, vascular permeability and mucous secreation*.

- ***Mediators of Immediate Hypersensitivity***
- **A-** Preformed mediators in granules
- -Histamine
- -Eosinophilic chemotactic factor

- -Tryptase
- - Kininogenase
- **B-**Newly formed mediator
- -Leukotriene
- -Prostaglandins
- -Platelet activating factor **(**PAF)


Allergen
CD4
IL-4
B cell
TH cell
Memory cell
Plasma cei)
Allergen-
specific
IgE
Fc receptor
for IgE
Sensitized mast cell
+ Allergen
Allergen
Degranulation
Vasoactive
amines
Smooth muscle cell
Small blood vessel
Mucous gland
Blood platelets
Sensory–nerve
endings
Eosinophil

- **Type II Hypersensitivity:**
- Type II hypersensitivity is **also known as cytotoxic hypersensitivity** it may affect a variety of organs and tissues.
- The antigens are **normally endogenous**, although exogenous chemicals **(haptens**) which can attach to cell membranes **can also lead to type II hypersensitivity**, (*drug-induced hemolytic anemia, granulocytopenia and thrombocytopenia are such examples).*

Haptens are small molecules that elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself.

- The reaction time is minutes to hours and is **primarily mediated by antibodies of the IgM and\or IgG classes.** *Complement, phagocytes and NK cells may also play a role*.
- Cytotoxic reactions involve primarily either the combination of IgG or IgM antibodies with antigen on cell surface or tissue **or** the adsorption of antigens or haptens to tissue or cell

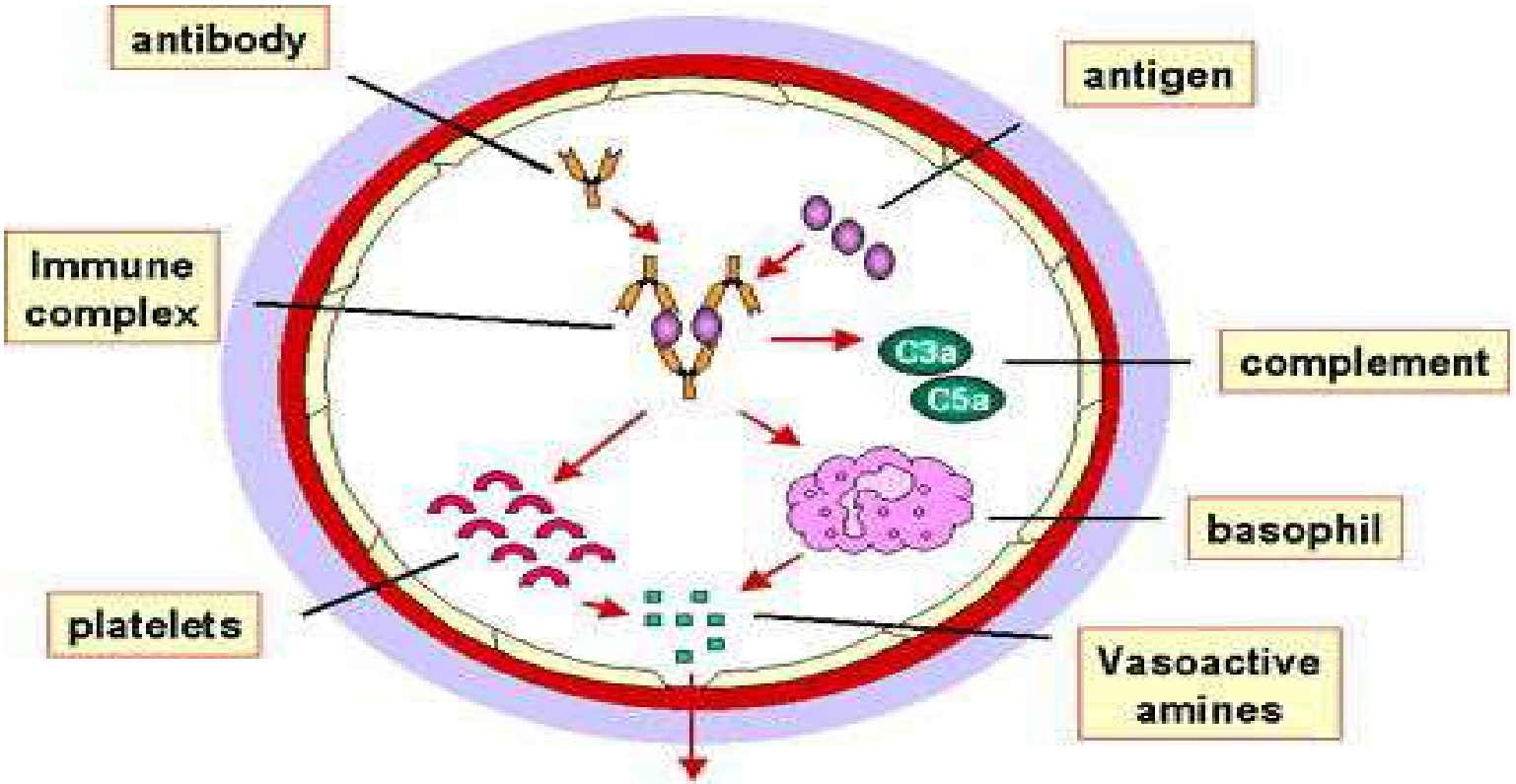
membrane, with subsequent attachment of antibodies to the adsorbed antigens.

- **Mechanism may lead to one of the following destructive processes.**
- 1. Activation of complement, with lysis or inactivation of target cells.
- 2. Phagocytosis of target cells, with or without complement activation.
- 3. Lysis or inactivation of target cells via NK cells (ADCC).

- **Type III Hypersensitivity:**
  - Type III hypersensitivity is **also known as immune complex hypersensitivity.** The reaction may be **general** (e.g., *serum sickness*) or **may involve individual organs** including *skin* (e.g., *systemic lupus erythematosus), lungs (e.g., aspergillosis), joints (e.g., rheumatoid arthritis).*
  - The reaction may take 3 - 10 hours after exposure to the antigen
  - It **is mediated by soluble immune complexes. They are mostly of the IgG class, although IgM may also be involved**.
  - **The antigen** may be exogenous (chronic bacterial, viral or parasitic infections) **or endogenous**. The antigen is soluble and not attached to the organ involved. **Primary components are soluble immune**

complexes and complement (C3a, 4a and 5a). The damage is caused by platelets and neutrophils.

The most common diseases involving a type III hypersensitivity reaction are serum sickness, post-streptococcal glomerulonephritis, systemic lupus erythematosus, , and rheumatoid arthritis. The principle feature that separates type III reactions from other hypersensitivity reactions is that in type III reactions, the antigen-antibody complexes are pre-formed in the circulation before their deposition in tissues.


antibody
antigen
immune complex
C3a
C5a
complement
basophil
platelets
Vasoactive amines

RCH

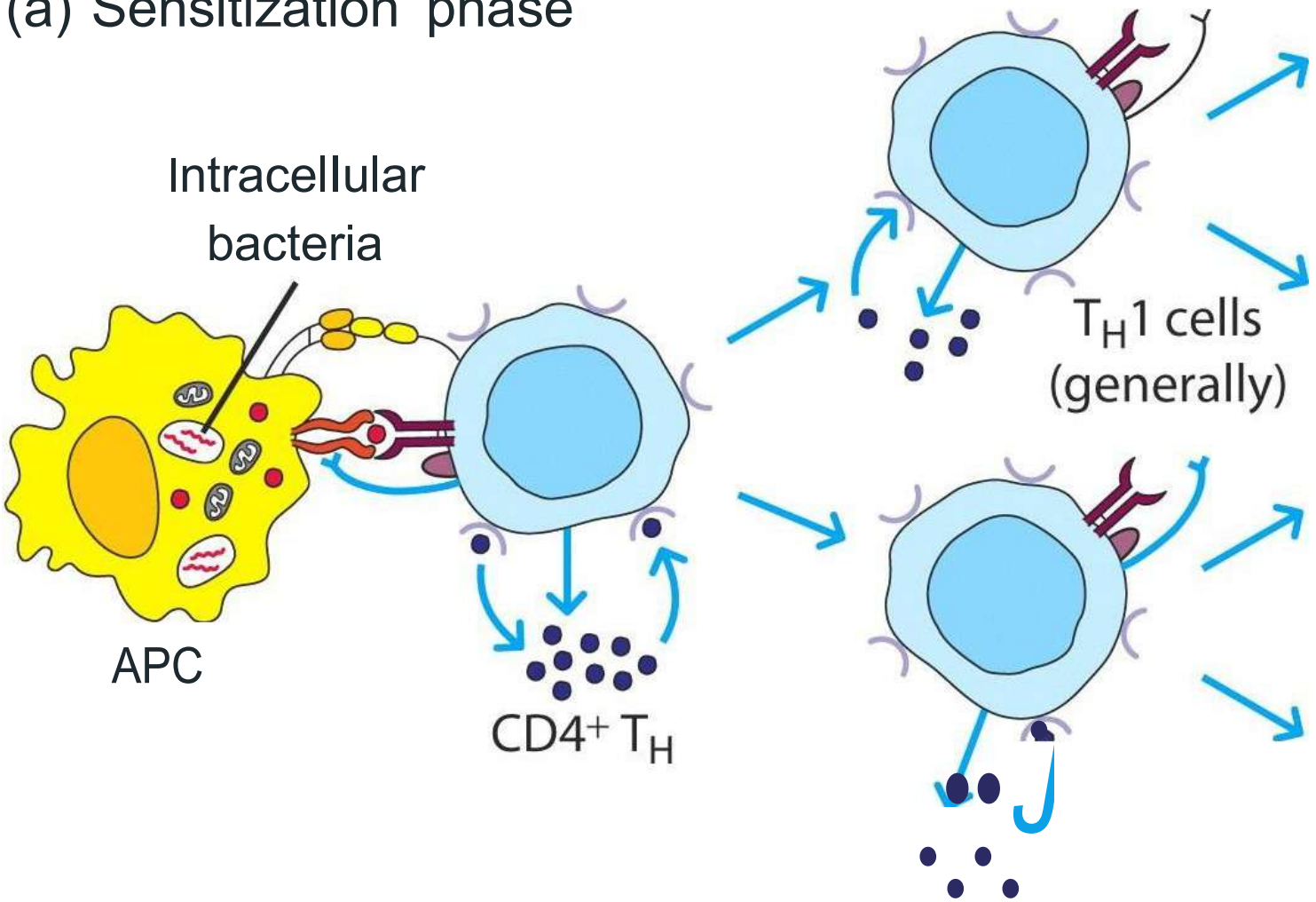
- **Type IV Hypersensitivity:**
- Type IV hypersensitivity is also known as cell mediated or **delayed type hypersensitivity**. The classical example of this hypersensitivity is tuberculin reaction which peaks 48 hours after the injection of antigen (tuberculin skin test). **The lesion is characterized by induration and erythema**.
- Type IV hypersensitivity **is involved in the pathogenesis of many autoimmune and infectious diseases** (*tuberculosis, leprosy,, histoplasmosis, toxoplasmosis, leishmaniasis,*

etc.) and granulomas due to infections and foreign antigens.

- **Mechanisms of damage in delayed hypersensitivity include**:
- ***T lymphocytes and monocytes and/or macrophages***.
- Cytotoxic T cells (CD+8) cause direct damage whereas helper T (CD+4) cells secrete cytokines which activate cytotoxic T cells and recruit and activate monocytes and macrophages, which cause the bulk of the damage. Cytokines involved in delayed

hypersensitivity reaction *include* ***monocyte chemotactic factor, interleukin-2, interferon-gamma, TNF alpha/beta.***

(a) Sensitization phase



Antigen-presenting eells:
Macrophages
Langerhans cells

DTH-mediating cells: TH1 cells generally CDS cells occasionally

In Oral Cavity :

The most common clinical entities associated with oral hypersensitivity reactions are oral lichenoid reactions and allergic contact cheilitis.

Oral Lichenoid reactions

Oral lichenoid contact reactions have been described and are **associated with a variety of topical agents including dental materials and flavoring agents** drugs etc.

Allergic contact cheilitis result from allergy to chemicals in lip balms and lip glosses, lipsticks, and sunscreens.

Defense Mechanism Of Oral Cavity

Dr. Chateen I Ali Pambuk
Prf.dr.Hadeel mizher younis

The oral mucosal epithelium

The oral mucosa is an **anatomical barrier** that prevents entry of potentially harmful microbes. Oral health depends on the integrity of the mucosal barrier, which also provides a habitat for normal oral flora. Continuous sloughing (**desquamation**) of the oral mucosal epithelium continuously removes microbes that colonize the mucosa, and this **minimizes the microbial biomass** in the oral cavity.

- **The immune system is essential for the maintenance of periodontal health and is central to periodontal pathogens.**
- However, if the immune response is dysregulated, inappropriate, persistent or excessive, then damaging chronic inflammatory responses may result.
- The oral cavity is loaded with normal **commensal microorganisms,** aerobes and anaerobes. These organisms becomes pathogenic if the host defense is compromised.
- Hence an effective defense mechanism is necessary to safeguard the oral cavity from these attacks.

Two major mechanisms of innate immunity in the oral cavity are

immune exclusion and **inflammation.** Immune exclusion refers to the inactivation and clearance of microbes from the oral mucosal epithelium and enamel surfaces.

So it includes :

anatomical barriers

- mechanical removal
- antigen-non-specific defence chemicals
- microbial antagonism
- defence cells and their activation
- phagocytosis

: Inflammation occurs when there is a need to remove infectious agents at sites of mucosal penetration

inflammation

- fever
- the acute-phase response
- complement.

- **Defense mechanisms of oral cavity can be broadly classified into**:

- Saliva
- Sulcular fluid
- Epithelial keratinocytes


SALIVA

- The major salivary glands are:
- Parotid, sublingual and submandibular glands, there basic functional units are cluster of cells called an acini.
- **The composition of saliva**:
- Water- 99% or more and the other 1% include:
- Antibacterial factors
- Salivary antibodies
- Enzymes
- Salivary buffers and coagulation factors

Functions of saliva:

- It provide protection by its continuous flow and lubrication
- Buffering action: regulate pH via phosphate and bicarbonate
- Digestion: digest starch and lipids by amylases and lipases
- Facilitation of taste
- Defensive action against microbes: through antibodies, lysozymes, lactoferrin and secretary IgA.

- **Antibacterial Factors in Saliva:**

It contains numerous inorganic and organic factors that influence bacteria and their products in the oral environment.

Inorganic factors include: bicarbonate, sodium, potassium, phosphates, calcium, fluoride….

Organic components include:

- Lysozymes
- Lactoferrin
- Myeloperoxidase
- Lactoperoxidase
- Agglutinins(glycoprotein, mucins, fibronectin)

- Lysozyme is a hydrolytic enzymes that cleaves the linkage between structural components of the cell wall of certain bacteria(both gram-negative and –positive) leading to cell lysis,its targets include Veilonella spp.and Actinobacillus actinomycetemcomitans.
- Lactoferrin it binds the free iron in saliva causing bactericidal or bacteriostatic effects on various organisms requiring iron for their survival.
- It also provides fungicidal, antiviral, anti-inflammatory and immunomodulatory functions.

- **Peroxidases:**
- **The Lactoperoxidase-thiocyanate** system in saliva is bactericidal to some strains of Lactobacillus and Streptococcus by preventing the accumilation of lysine and glutamic acids essential for bacterial growth, also it is effective against Actinobacillus speceis.
- **Myeloperoxidase**: an enzyme similar to salivary peroxidase. It is released by **leukocytes** and is bactericidal for Actinobacillus.

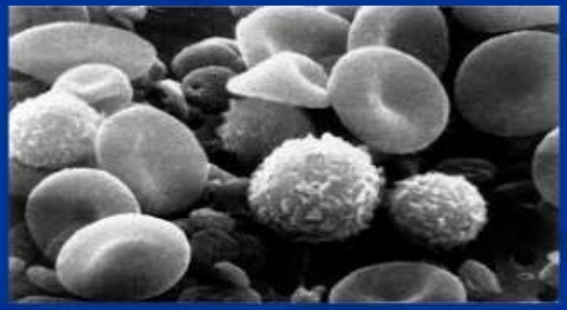
- **The histatins**: characteristics:
- 1- a family of histidine-rich peptides
- 2- have antimicrobial activity against some strains of *Streptococcus mutans* and
- 3-inhibit some enzymes ofperiodontopathogen *P. gingivalis*
- 4- Neutralize lipopolysaccarides of G-ve bacteria.
- 5- Potent inhibitors of Candida albicans

- **Salivary Antibodies**
- Salivary immunoglobulins include sIgA which is important defense substance in saliva, it inhibits bacterial adherance
- in addition to small amounts of IgM and IgG
- **Enzymes**
- The major enzyme is parotid amylase.
- Binds to bacteria promotes adhesion lead to either surface immune exclusion or adhesion of cariogenic or periodontopathogenic bacteria.
- Proteolytic enzymes in saliva are generated by both the host and oral bacteria and has been recognized as contributors to the initiation & progression of periodontal diseases

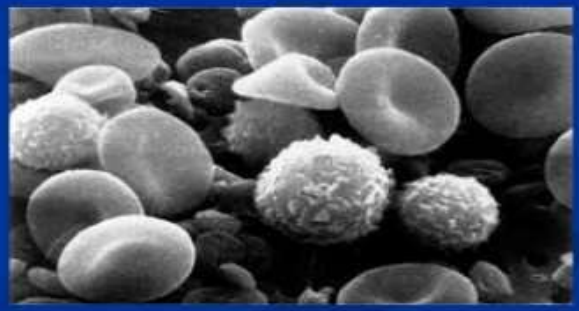
- To combat with these enzymes, saliva contains antiproteases that inhibit cystein proteases as cathepsins and antileukoproteases that inhibit elastase.
- **Salivary Buffers & Coagulation factors**
- The most important buffer is bicarbonate-carbonic acid system which maintain the physiologic hydrogen ion concentration(pH) at the mucosal epithelial cells and the tooth surfaces
- The coagulation factors include plasma thromboplastin antecedent(PTA); Hageman factor which hasten blood coagulation and protect wounds from bacterial invasion.
- An active fibrinolytic enzyme may also be present.

LEUKOCYTES

- In addition to desquamated epithelial cells, the saliva contains all forms of leukocytes of which the principal cells are PMNs.
- The number of PMNs varies from person to person at different times of the day and is increased in gingivitis.



- PMNs reach the oral cavity by migrating through the lining of the gingival sulcus.
- Living PMNs in saliva are sometimes called **orogranulocytes**, and their rate of migration into the oral cavity is termed the **orogranulocytic migratory rate.**

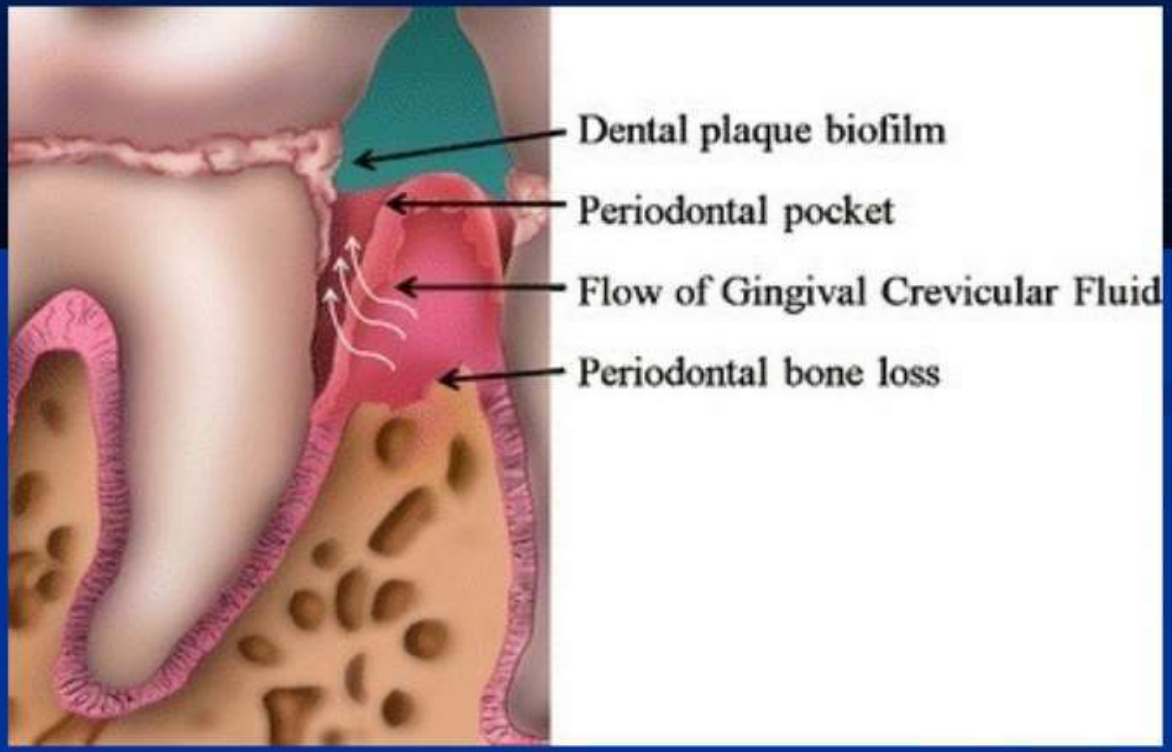


2.SULCULAR FLUID

- Sulcular fluid or gingival crevicular fluid (GCF) is serum exudate secreted by sulcular epithelium in the gingival sulcus.

- The presence of this fluid has been known since the 19th century, but its composition and possible role in oral defense mechanism was elucidated by the pioneering work of *Waerhaug* and *Brill* and *Krasse* in the 1950s.

GENERATION OF GCF



COMPOSITION OF GCF

- Cellular elements
- Electrolytes
- Organic compounds
- Metabolic and bacterial prod1-1cts
- Enzymes and enzyme inhibitors

- **Cellular elements**
  - bacteria
  - desquamated epithelial cells
  - leukocytes (PMNs, lymphocytes, monocytes /macrophages)

- **Electrolytes**
  - Potassium
  - sodium
  - calcium

METABOLIC & BACTERIAL PRODUCTS

- Lactic acid
- Hydroxy proline
- Prostaglandins
- Urea
- Endotoxins
- Cytotoxic substances
- Antibacterial factors

CELLULAR & HUMORAL ACTIVITY IN GCF

- Analysis of GCF has identified cell and humoral responses in both healthy and those with periodontal diseases
- The cellular immune response include the appearance of cytokines in GCF, but there is no clear evidence of a relationship between cytokines and disease.

- However, interleukin-1 alpha and IL-1 beta are known to increase the binding of PMNs and monocytes to endothelial cells, stimulate the production of prostagladinE_2 and release of lysosomal enzymes & stimulate bone resorption.

- Interferon alpha present in GCF have a protective role in periodontal disease because of its ability to inhibit bone resorption activity of IL-1 beta.

- Differential counts of leukocytes from clinically healthy gingival sulci have shown 91.2-91.5% PMNs and 8.5-8.8% mononuclear cells.

- Mononuclear cells were identified as 58% B lymphocytes, 24% T lymphocytes & 18% mononuclear phagocytes.

- The ratio of T lymphocytes to B lymphocytes was found to be reversed from normal ratio
- 1:3 in GCF

CLINICAL SIGNIFICANCE

- GCF is an inflammatory exudate.
- The amount of GCF is greater when inflammation is present.
- Factors which influence the amount of GCF are
  - Circadian periodicity
  - Sex hormones
  - mechanical stimulation
  - Smoking
  - Periodontal therapy

3- Epithelial cells

They plays an important role in **innate host defense by responding to bacterial infections**.

This epithelium protect the deep structures and allow a selective interchange with the oral environment by its proliferation and differentiation

The **principle cell type** of gingival epith. is the keratinocytes

The role of their defense is by the degree of keratinization

Other cells are nonkeratinocytes which include the langerhans cells, merkel cells and melanocytes.

- Epithelial cells stimulated with bacterial components and cytokines directly produce MMPs, which contribute to loss of connective tissue.
- Epithelial cells also secrete a range of cytokines in response to periodontal bacteria (*P.gingivalis, A.actinomycetemcomitans, F.nucleatum, P.intermedia)*, which signal immune responses.
- cytokines IL-1beta, TNF-alpha & IL-6 ,which serve to signal neutrophils and monocytes migration from the vasculature into periodontal tissues.

- There are several factors which may prevent penetration of the intact oral mucosa by microorganisms as protective barrier include:

- 1-Saliva
- 2-Keratin
- 3-Granular layer
- 4-Basement membrane
- 5-leukocytes
- 6-Antibody

- Antigen-non-specific defence chemicals in oral secretions
- Various antigen-non-specific defence chemicals promote innate immune defence in the oral cavity. These include **calprotectin**, **defensins**, **saliva** (and the enamel pellicle), **gingival crevicular** fluid (**GCF**) and **mucins**. Non-cellular mediators of antimicrobial defence help to protect the oral mucosa through potent **antibacterial, antiviral, and antifungal** activities, which can affect oral microbes in several ways:

: • they can aggregate or agglutinate microbes,

- they can promote or inhibit microbial adhesion,
- they can directly kill or inhibit the growth of microbes,

and/or • they can contribute to microbial nutrition.

- **Calprotectin** : is a calcium- and zinc-chelating antimicrobial peptide produced by **non-keratinized oral epithelial cells**.. Calprotectin is present in neutrophils, monocytes, macrophages and probably GCF

Defensins, in contrast, are a class of pore-forming peptides that insert into the phospholipid bilayer of bacterial membranes causing osmotic instability and cell lysis.

Defensins are divided into **α-** and **β-**defensins

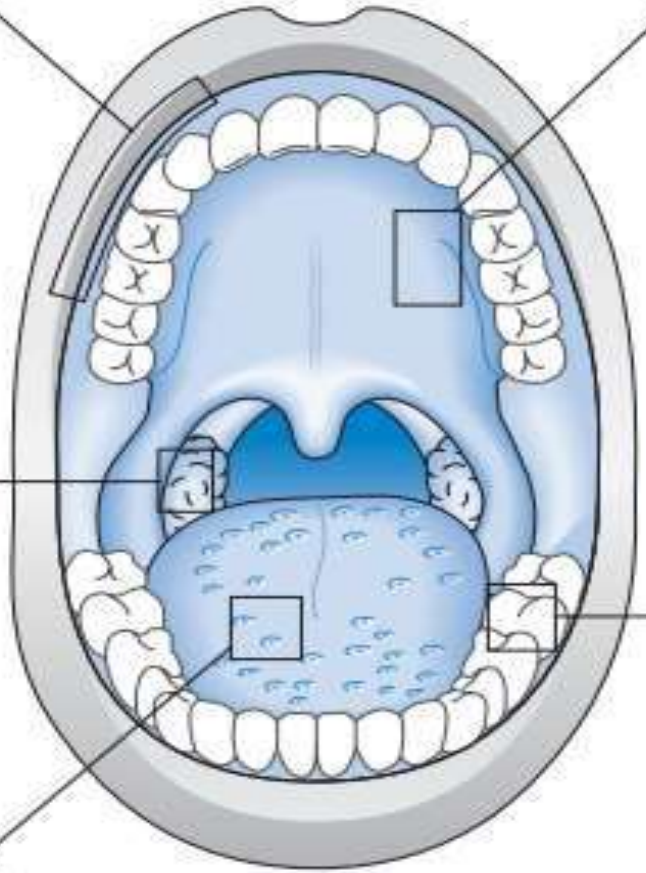
Defensins in saliva , besides **bacteria**, are also active against **fungi** and **enveloped viruses**; cause degranulation of mast cells; and are chemotactic for neutrophils, dendritic cells and memory T cells. Eukaryotic cells resist the lytic action of defensins due to lower phospholipid content in the membranes of these cells.

- **Cathelicidins** :are a family of antimicrobial polypeptides found in lysosomes in macrophages and neutrophils that provide innate immune defence against bacteria.

Table 8.3 Non-specific host defence factors of the mouth

Defence factors	Main function
Epithelial desquamation	Physical removal of microbes
Saliva flow	Physical removal of microbes
Mucin/agglutinins	Physical removal of microbes
Lysozyme	Cell lysis (bactericidal, fungicidal)
Lactoferrin	Iron sequestration (bactericidal, fungicidal)
Apolactoferrin	Iron sequestration (bactericidal, fungicidal)
Sialoperoxidase system	Hypothiocyanite production (neutral pH); hypocyanous acid production (low pH)
Histidine-rich peptides	Antibacterial and antifungal activity
Salivary leukocyte protease inhibitor (SLPI)	Blocks cell surface receptors needed for entry of HIV
Intraepithelial lymphocytes and Langerhans cells	Cellular barrier to penetrating bacteria and/or antigens
Secretory IgA	Prevents microbial adhesion and metabolism
IgG, IgA, IgM	Prevent microbial adhesion; opsonins; complement activators
Complement	Activates neutrophils
Neutrophils/macrophages	Phagocytosis

Fig. 8.9 A diagrammatic representation of the natural defence mechanisms of the oral cavity. TLRs, Toll-like



Oral mucosa
TLRs
SIgA
β-defensins
Calprotectin
Mucus coat
Intraepithelial lymphocytes
Lamina propria lymphocytes
Microbial antagonism

Saliva and salivary glands
SIgA
Histatins, cystatins
Lactoferrin
Lysozyme
Thrombospondin-1
Peroxidase activity
Mucins
Agglutinins
SLPI

Nasopharynx ass. lymphoid tissue (tonsils)
SIgA
CD4 cells
B cells
TGF-beta
Th17 response

Tooth plaque fluid and adjacent GCF
Calprotectin
Thrombospondin-1
PMNL and lysosomal contents
Complement
Enamel pellicle
IgM, IgG
Peroxidase activity
Lysozyme

Tongue
Circumvallate papillae
Microbial antagonism
von Ebner's protein
von Ebner's glands
Normal flora

Lec 10 oral microbial

Streptococci

Dr. chateen I Ali,

prof dr hadeel M.younis

Genus *Streptococcus*

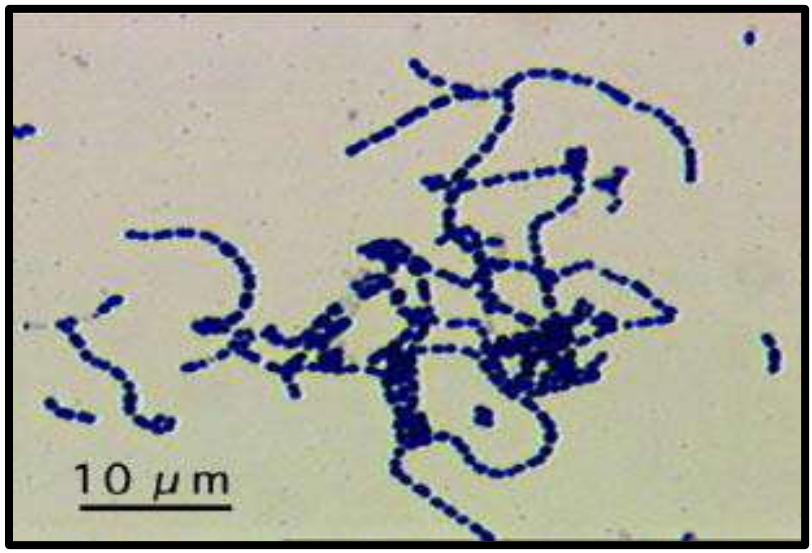
Commensals or **Parasites** of man & animals or **Saprophytes** of decaying matter

-So many medically important in this genus

Morphology and Characteristics

-Gram-Positive Cocci in **Pairs or Chains**

-These cocci grow well on blood agar



-Facultative Anaerobes

-Catalase Negative ($2H_2O_2$ ---> $O_2 + 2H_2O$)

Separation of streptococci from staphylococci

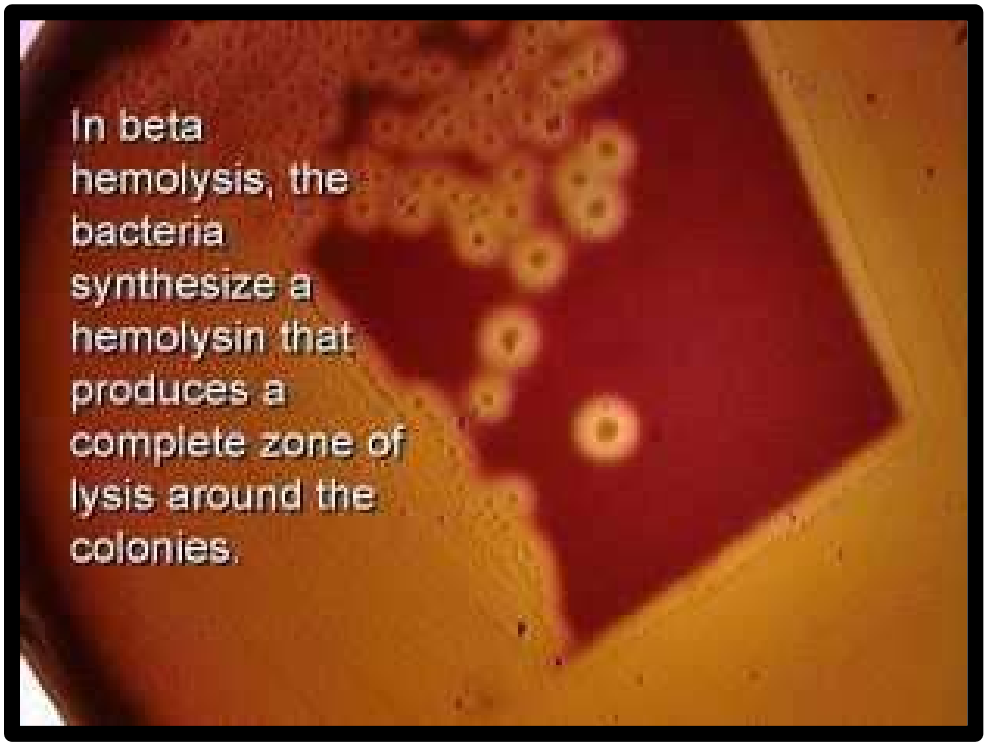
-Oxidase Negative (oxidoreductase oxidizes substrate w/ O_2)

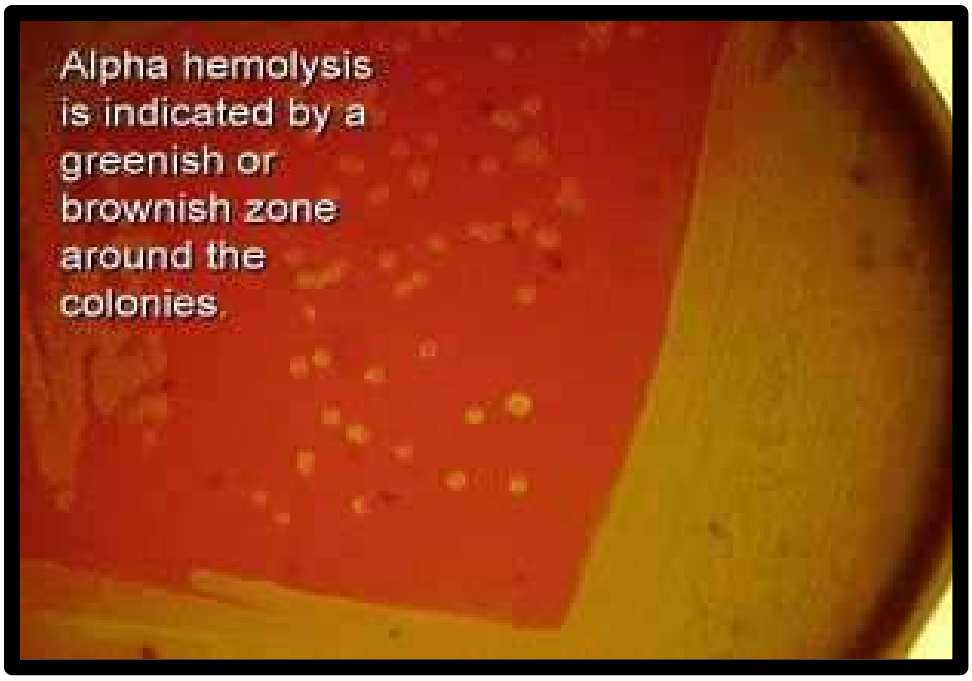
-Beta, **Alpha**, or **Gamma Hemolysis** on blood agar

Types of Hemolysis

One of the most important characteristic for identification of streptococci is the type of of hemolysis. **Typical haemolytic reactions are produced on blood agar**

- 1- Alpha-hemolytic
- 2- Beta-hemolytic
- 3-non-hemolytic





Grouping *Streptococcus*

Rebecca Lancefield Developed useful **serogrouping** system

- Classification of beta-hemolytic streptococci by **group-specific cell wall carbohydrate (CHO) antigen** . A large number of Serogroups A to H and K to V

1- Groups A, B, C, D, and G are most

comonly associated with **human disease**

- **group A** includes the important human pathogen

Streptococcus pyogenes

• **group B** contains one species, Streptococcus agalactiae,

an inhabitant of the female genital tract; it causes

infection in neonates

• **group C** mainly causes diseases in animals

• **group D** includes the enterococci (Enterococcus faecalis,

etc.) and ranks next to group A in causing human

disease.

2- Non groupable : Viridans streptococci and

Streptococcus pneumoniae have

no group-specific antigen

Antigenic Structure

Streptococcus pyogenes **(Group A)**

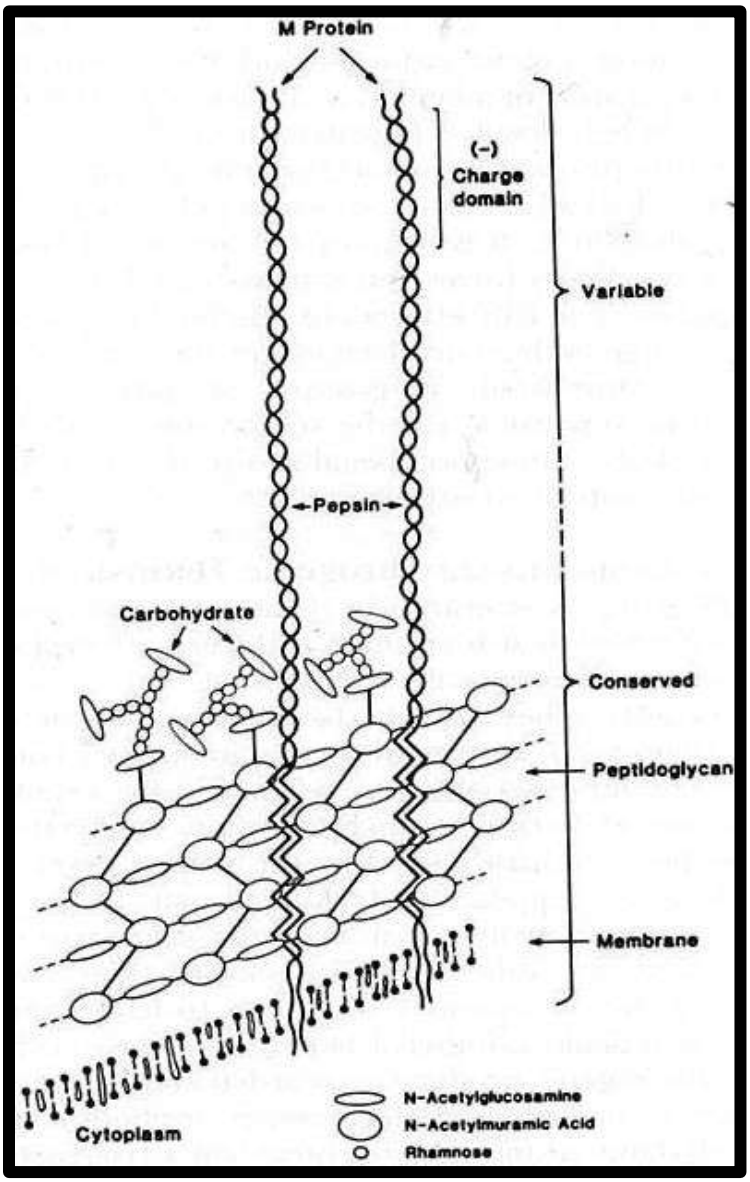
a- Lancefield Group-specific antigen (C polysaccharide)

Complex polysaccharide in cell wall

b- Proteins: the major classes, M **: M-Protein**: Type-specific antigen

- Fimbriae-like, hairy extensions

Specific adherence by lipoteichoic acid and M-protein (**LTA-M**) complexes



c- Capsular Polysaccharide:

Hyaluronic acid

-Not present in all strains

-Same as host hyaluronic acid (cartilage,skin etc)

-Nonimmunogenic

-Antiphagocytic

Lancefield Serogroup Classification **of Beta-Hemolytic Streptococci Important in Human Disease**

1- Group A Streptococci: *Streptococcus pyogenes*

One of Most Important Human Pathogens

1- Suppurative Diseases: causing

- Pharyngitis; Scarlet Fever;
- Cutaneous & Soft Tissue Infections
- Systemic Disease

2- Non-Suppurative (poststreptococcal) :ARF,AG

Non-Lancefield Group Streptococci

1- Viridans Streptococci

Dental Caries: *Streptococcus mutans*

Streptococcus sanguis; *Streptococcus salivarius; Streptococcus mitis*

2- Streptococcus pneumoniae

Major Human Diseases of Beta-Hemolytic Streptococci

Group A *Streptococcus* (*S. pyogenes*):

Diverse group of **acute suppurative** (pus-forming) & nonsuppurative diseases

Pathogenicity

Suppurative Streptococcal Diseases

-Pharyngitis (& **tonsilitis**):

-Scarlet fever: Complication of streptococcal pharyngitis when infecting strain is lysogenized

Cutaneous & Soft Tissue Infections.

1- **Pyoderma (Impetigo**: contagious pyoderma with superficial yellow weeping lesions)
2- **Erysipelas**: **Acute superficial cellulitis of skin** with lymphatic involvement; face and lower extremities, skin and subcutaneous tissues
3- **Cellulitis**: **Involvement of deeper subcutaneous tissues**; Deeper invasion with systemic symptoms
4- **Necrotizing fasciitis:** (a.k.a., "**flesh-eating bacteria**"): Infection deep in subcutaneous tissues that spreads along fascial planes, destroying muscle and fat; , gangrene, systemic toxicity, multiorgan failure and mortality in more than 50% of patients
5- **Wound Infections**

Other Suppurative Diseases

- Lymphangitis: Inflammation of lymphatic vessel(s)

-Pneumonia

Systemic Disease

1-Streptococcal Toxic Shock Syndrome (TSS):

Multisystem toxicity following soft tissue infection progressing to shock and organ failure (not to be confused with **Staphylococcal Toxic Shock Syndrome** where hyperabsorbent tampons have been identified as an important risk factor)

2-Bacteremia

Nonsuppurative D.

-Post-infection complications of Group A streptococcal disease; Serious complications;

1- **Acute rheumatic fever** (ARF):

Inflammation of heart, joints, blood vessels, sub-cutaneous tissues

2-Rheumatic heart disease (RHD):

Chronic, progressive heart valve damage

3- Acute glomerulonephritis (AG):

Acute inflammation of renal (kidney) glomeruli

Epidemiology of Acute Streptococcal Infection

- Predilection for **upper respiratory tract** or **skin**
- Group A commonly colonize **oropharynx of healthy children**
- **M-types** of strains colonizing throat differ from those on skin
- Rapidly killed after phagocytosis, but cell walls not digested and may lead to chronic inflammatory lesions

Pharyngitis transmitted by droplets from respiratory secretions

- Crowding increases risk (e.g., classrooms, day care facilities)

Determinants of Pathogenicity

<u>Cellular Virulence Factors</u>

1- Capsule

-Antiphagocytic; Nonspecific adherence

-Hyaluronic acid (polysaccharide) mimics animal tissue

2- Lipoteichoic Acid

-Adherence: Complexes with M protein (LTA-M) and binds to fibronectin on epithelial cells

3- M-Protein

-LTA-M protein is **adhesin**

-Antiphagocytic

-Inhibits alternate C' pathway and opsonization

Extracellular Virulence Factors

Exotoxins:

1- **Streptolysin O (SLO):**

Hemolytic and **Cytolytic**

Prototype of **oxygen-labile**

Causes **sub-surface hemolysis** on BAP (blood agar plates) why ?

Stimulate release of lysosomal enzymes

SLO titer indicates recent infection (300-500 in pediatric populations)

2- **Streptolysin S (SLS):**

Hemolytic and **Cytolytic**

Oxygen stable, non-antigenic

Lytic for red and white blood cells

Lysogeny: Lysogenized bacteriophages play key role in directing synthesis of various Group A streptococcal enzymes and toxins

- **Pyrogenic Exotoxin** (erythrogenic toxin)

3- **Pyrogenic (Erythrogenic) Exotoxins (Types A, B &C)**

Produced by more than 90% of Grp A strep

Lysogeny: Structural gene is carried by bacteriophage, as is the case with diphtheria toxin

-Mediate pyrogenicity (fever)

-Causes **scarlet fever** (scarletiniform) **rash**

-Immunomodulators (**superantigens**): stimulate T cells to release cytokines

4- **Cardiohepatic toxin**

Enzymes:

1- **Nucleases:** Four antigenic types (A,B,C,D)

Nucleases A, C have **DNase activity**

Nucleases B, D also have **RNase activity**

2- **Streptokinases: Lyse blood clots:** catalyze conversion of plasminogen to plasmin, leading to digestion of fibrin
3- **C5a Peptidase:** destroys C' chemotactic signals (C5a)
4- **Hyaluronidase:** hydrolyzes hyaluronic acid
5- **Others:** Proteinase, NADase, ATPase, phosphatase, etc.

Lab Identification of

***S. pyogenes* (Group A)**

- Primary culture by pour or streak plate
- Domed,grayish/opalescent colonies

  - Encapsulated cells produce **mucoid** colonies

Beta-hemolytic :Zone several times greater than diameter of colon

Treatment and prevention

Penicillin is the drug of choice; erythromycin is suitable for patients hypersensitive to penicillin. No vaccine is available.

Streptococcus agalactiae (group B)

This species is increasingly recognized as a human pathogen, especially as a cause of neonatal meningitis and sepsis.

Genus *Streptococcus*

Streptococcus pneumoniae

- Commonly referred to as **pneumococcus**
- Formerly *Diplococcus pneumoniae*

***Streptococcus pneumoniae* Infections**

- Infections from endogenous spread from naso- or orapharynx
- Pneumonia; sinusitis; otitis media; bacteremia; meningitis
- Colonization highest in children
- Antecedent viral respiratory tract disease increases risk
- Most common in cold months
- Polyvalent vaccine available (newly available for children)

VIRULENCE FACTOR	BIOLOGICAL EFFECT
COLONIZATION AND MIGRATION	
Protein adhesin	Binds to epithelial cells
Secretory IgA protease	Disrupts secretory IgA-mediated clearance
Pneumolysin	Possibly destroys ciliated epithelial cells
PHAGOCYTIC SURVIVAL	
Capsule	Antiphagocytic
Pneumolysin	Suppresses phagocytic oxidative burst

Streptococcus pneumoniae

Viridans Streptococci

Viridans streptococci (characterized by :)

- Large group of commensal streptococci
- Either **alpha –hemolytic** producing a green coloration on blood agar (hence the name viridans in latin it is green) **or non hemolytic**
- They possess **no lancefield** antigens
- In general pathogenicity is low

Viridans streptococci	***Streptococcus pneumoniae***
1- optochin test resistant	1- sensitive

2- Lack polysaccharide-based capsule (Quellung test) : negative 3- solubility in bile : **insoluble** 4- Fermentation of inulin : **NO** 5- Pathogenicity: **Nonpathogenic**	2- present (Quellung test) : positive 3- soluble 4- yes 5- pathogenic

Characteristics and Pathogenicity

- The most abundant organism in mouth
- Oral streptococci, which live principally in the oropharynx
- One member of the group S. mutans is the etiologic agent of dental caries
- Others may be envolved in other mouth or gingival infections
- They are the most common causes of subacute bacterial endocarditis.
- typically show **α-haemolysis** on blood agar, but this is not a constant feature as some strains are **non-haemolytic** and others **β-haemolytic**.
- Oral streptococci can be divided into **four main species groups** as follows:

  1. mutans group

  2. salivarius group

  3. anginosus group 4. mitis group.

  Each of these groups comprises a number of species

Table 11.1 Some recognized species of oral streptococci

Group	Species
mutans group	*S. mutans*, serotypes *c*, *e*, *f*
	S. sobrinus, serotypes *d*, *g*
	S. rattus, serotype *b* and others
salivarius group	*S. salivarius*
anginosus group	*S. intermedius*
	S. anginosus
mitis group	*S. sanguinis*
	S. gordonii
	S. parasanguinis
	S. oralis and others

Habitat and transmission

-Streptococci make up a large proportion of the resident oral flora.

-It is known that roughly **one-quarter** of the total cultivable flora from **supragingival and gingival plaque**

-and **half** of the isolates from the **tongue and saliva** are streptococci.

-They are **vertically** transmitted from mother to child.

Infective endocarditis caused by these organisms is generally a result of their entry into the blood stream during **intraoral surgical procedures** (e.g. **tooth extraction**), and sometimes even during **tooth-brushing.**

Streptococcus mutans

-is a facultatively anaerobic, gram-positive coccus found in mouth

-This bacterium, along with the closely related species ***Streptococcus sobrinus*****,** can cohabit the mouth: Both contribute to oral disease

-for clinical purposes they are often considered together as a group, called the mutans streptococci

-S. mutans is naturally present in the human oral microbiota, along with at least 25 other species of oral streptococci

-S. mutans is most prevalent on the **pits and fissures**, constituting **39%** of the total streptococci in the oral cavity.

-Fewer S. mutans bacteria are found on the **buccal surface (2–9%)**

Cariogenicity

-**Early colonizers** of the tooth surface are mainly streptococci, including **S. mutans, S oralis , S mitis and also Neisseria** spp

-The growth and metabolism of these **pioneer species** changes local environmental conditions (e.g., pH, coaggregation, and substrate availability)

-enabling more fastidious organisms to further colonize after them, forming **dental plaque.**

-Along with **S. sobrinus, S. mutans** plays a major role in **tooth decay**, **metabolizing sucrose** to **lactic acid** using the enzyme **glucansucrase**

-The production of lactic acid leading to **highly mineralized** tooth enamel to be vulnerable to decay

-**S. mutans** is one of a few specialized organisms equipped with **receptors that improve adhesion to the surface of teeth**. Sucrose is used by S.

mutans to produce a **sticky**, extracellular, **dextran-based polysaccharide** that allows them to cohere, forming plaque.

-S. mutans produces **dextran** via the enzyme **dextransucrase** using sucrose as a substrate

-However, many other sugars—glucose, fructose, lactose—can also be -digested by S. mutans, but they produce lactic acid as an end product.

-The **combination of plaque and acid leads to dental decay (cariogenic process**)

-If the adherence of S. mutans to the surface of teeth or the physiological ability (**acidogenity and aciduricity**) of S. mutans in dental biofilms can be reduced or eliminated, the acidification potential of dental biofilms and later cavity formations can be decreased.

-the most common bacteria that are found in the **supragingival** plaque are: gram positive cocci (Streptococcus mitis, Streptococcus oralis, Streptococcus sanguis, Streptococcus mutans, Streptococcus gordonii,)

Infections of Viridans streptococci :Pathogenicity

- 1- *S. mutans* in combination with Lactobacilli the leading cause of dental carries .
- 2- Viridans S. (*S. mutans, S. sanguis, S. salivarius, and S. mitis*) are the most common cause of infective endocarditis.
- 3- Viridans S. (*S. milleri, and S. intermedius*) causing brain abscesses often in combination with mouth anaerobes.
- 4- Viridans S. are involved in mixed aerobic-anaerobic infections in other areas of the body eg, abdominal abscesses

Gram-positive anaerobic cocci

--Gram-positive anaerobic cocci **(GPAC)** all belonged to the genus ***Peptostreptococcus*** until recently. However, they now comprise **three genera**, namely ***Peptostreptococcus***, ***Micromonas*** and ***Finegoldia***.

--The representative species are ***Peptostreptococcus anaerobius***, ***Finegoldia magnus*** (previously Peptostreptococcus magnus) and ***Micromonas micros*** (previously Peptostreptococcus micros).

--These **GPAC** can often be **isolated from dental plaque** and the **female genital tract**. They are also found in **carious dentine**, **subgingival plaque**, **dentoalveolar abscesses** and in **advanced periodontal disease**, usually in mixed culture.

--Their pathogenic role is still unclear.

ا.م.د جتين عزالدين علي
ا.د هديل مزهر يونس

oral Microbiology

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

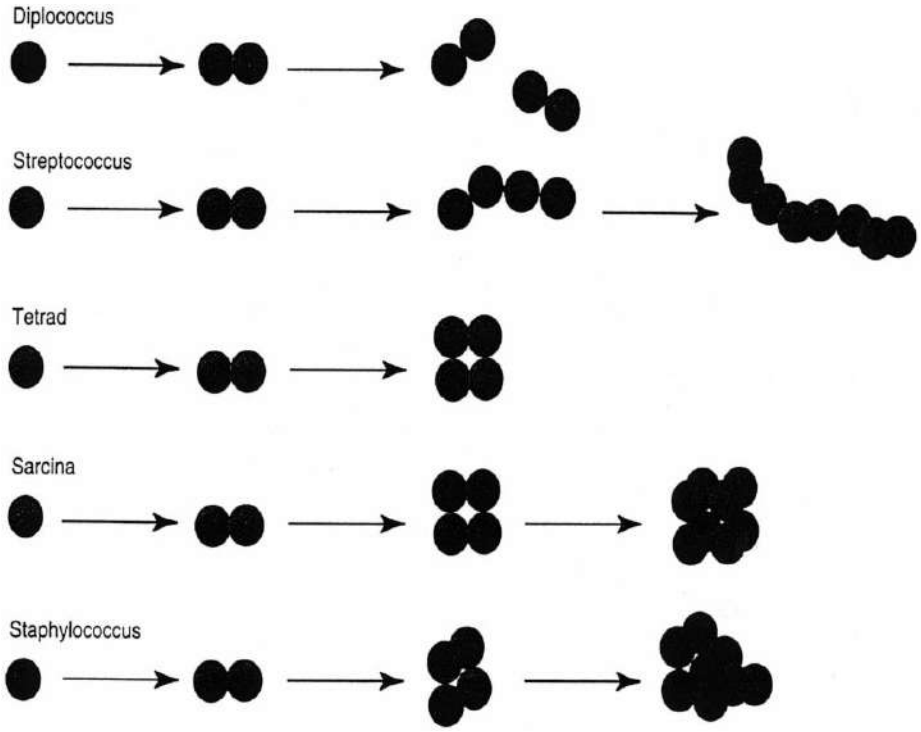
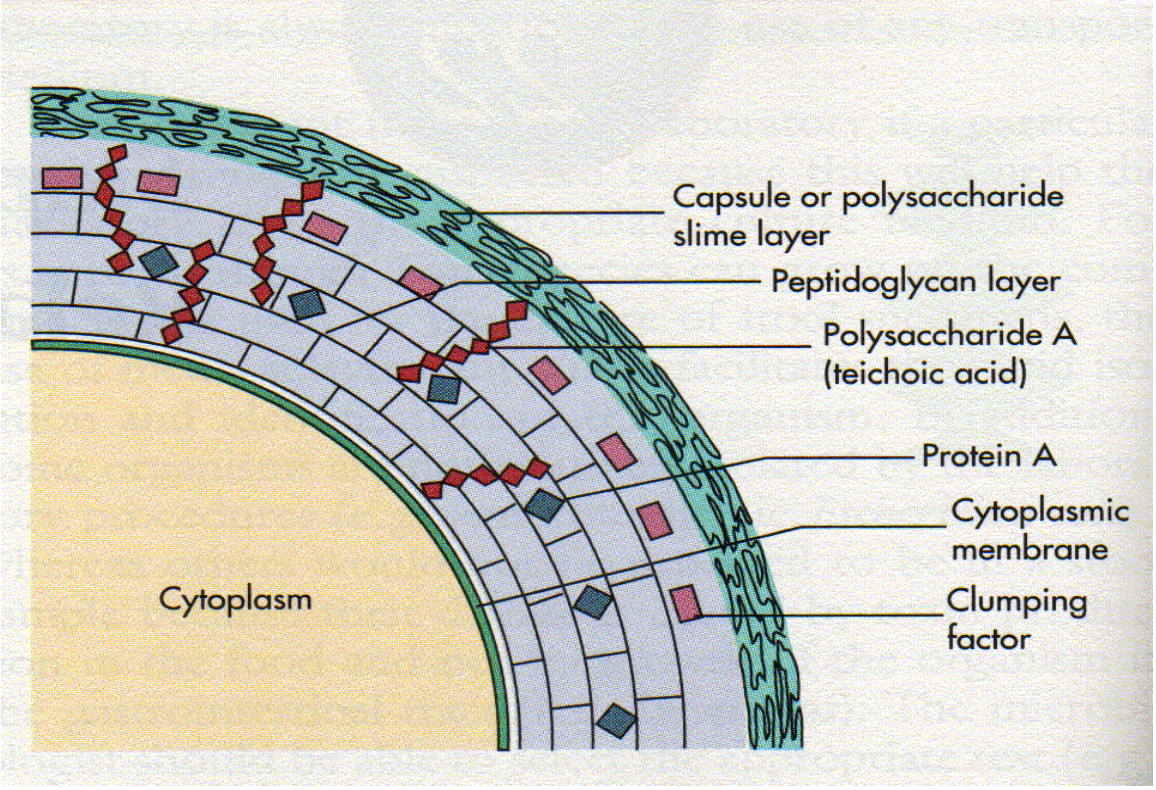


TABLE 22–2. *Staphylococcus*, *Micrococcus*, *Stomatococcus*, and *Alloiococcus* and Their Diseases

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

Staph. **Antigenic Structure:**



Peptidoglycan :

important in pathogenesis of infection

- 1- elicite prouction of IL-1(endogenous pyrogen)
- 2- elicite prouction 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 components 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 (polysaccharide A) — ribitol teichoic acid

S. epidermidis (polysaccharide B) — glycerol teichoic acid

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**

TABLE 22–3. *Staphylococcus aureus* **Virulence Factors**

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

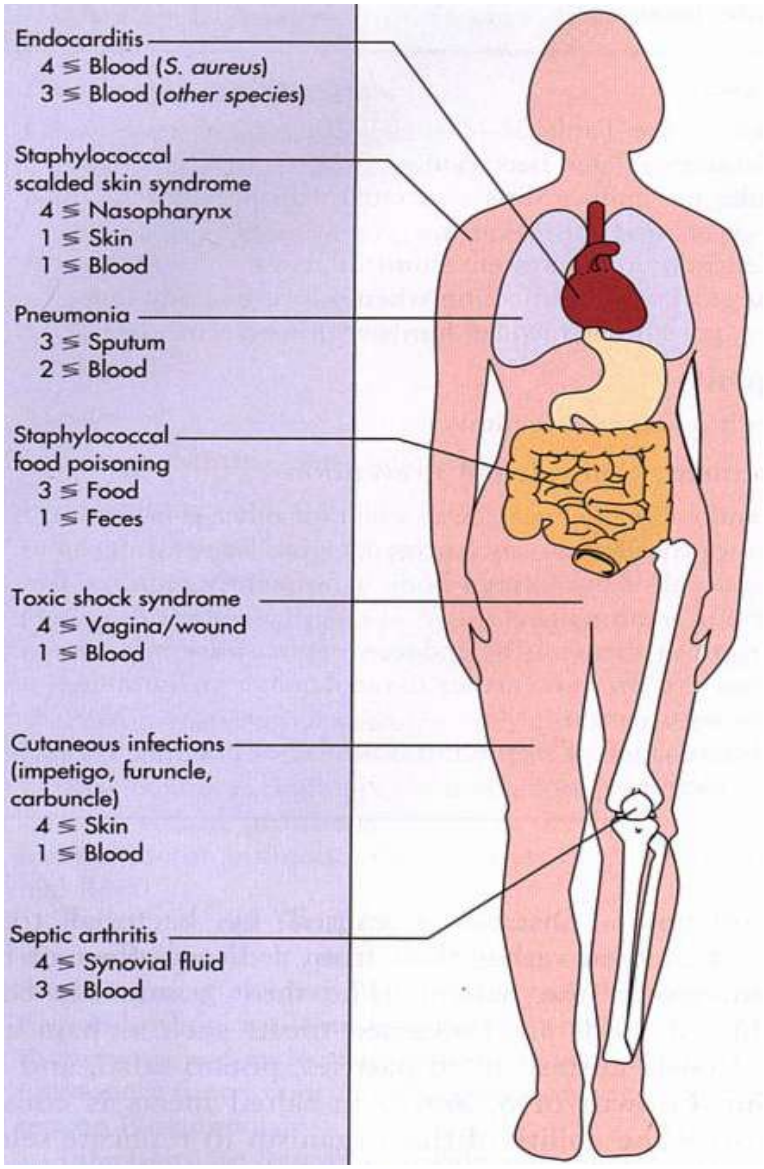
Clinical Manifestations/Disease

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

$2H_2O_2 \rightarrow O_2 + 2H_2O$

Streptococci *negative* vs. Staphylococci *positive*

Treatment

1-Drain infected area

2-Deep/metastatic infections

semi-synthetic peniclins

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

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Gram Negative cocci ***Neisseria***

The **Neisseriaceae Family** : include the genera ***Neisseria*** and ***Moraxella*****.**
Two species of Neisseria are human pathogens:

General Characteristics of Neisseria spp.

- **Aerobic**
- **Gram-negative** cocci often arranged in pairs (**diplococci**) with **adjacent sides flattened** (like coffe beans)
- **Oxidase positive**
- Most **catalase positive**
- Non motile
- Acid from oxidation of carbohydrates, not from fermentation

Important Human Pathogens

1- Neisseria gonorrhoeae

2- Neisseria meningitidis

-Other species normally colonize mucosal surfaces of **oropharynx** and **nasopharynx** and occasionally **anogenital** mucosal membranes

-The majority of human-associated Neisseria species are non-pathogenic and are normal inhabitants of the upper respiratory tract.

-Human associated species include: *N. gonorrhoeae, N. meningitidis, N. lactamica , N. mucosa, N. sicca, N. subflava* including the biovars *subflava, flava,* and *perflava*,

-N. elongata

-Only *N. gonorrhoeae* and *N. meningitidis* are regarded as pathogens

Orally important Species

-*N. mucosa*

-*N. sicca*

-*N. subflava* including the biovars subflava, flava, and perflava

Neisseria Associated Diseases

Organism	Diseases
N. gonorrhoeae	Urethritis, cervicitis, salpingitis, pelvic inflammatory disease, proctitis, bacteremia, arthritis, conjunctivitis, pharyngitis
N. meningitidis	Meningitis, meningoencephalitis, bacteremia, pneumonia, arthritis, urethritis
Other *Neisseria* species	Opportunistic infections

Neisseria gonorrhoeae

(gonococcus)

General Overview of Neisseria gonorrhoeae

- **Readily transmitted by sexual contact** (so urogenital , and anogenital inhabitant)

- **Gram-negative diplococci flattened along the adjoining side**
- **Fastidious**
    - Requires complex media pre-warmed to 35-37C
    - Soluble starch added to neutralize fatty acid toxicity
    - Grow best in moist atmosphere supplemented with CO_2
- Produce **acid from glucose**, but not from other sugars
- Though aerobic, most strains of *N. gonorrhoeae* are **capnophilic** (they require increased carbon dioxide for growth); and need **haemolysed blood**
-

Differences

Neisseria gonorrhoeae	***Neisseria meningitidis***
1-no polysaccharide capsule	1-prominent polysaccharide capsule
2-divided into more than 100 serotypes on the basis of the antigenicity of pilus protein	2- divided into more than 13 serotypeson the basis of the antigenicity of polysaccharide capsule
3-The endotoxin is **LOS**	3- the endotoxin is **LPS**
4-have three outer membrane proteins (protein I, II, and III)	4- do not have these proteins

Epidemiology of Gonorrhea

- Found **only in humans** with strikingly different epidemiological presentations for females and males
- Transmission primarily by sexual contact
- Asymptomatic carriage is major reservoir
- Lack of protective immunity and therefore **reinfection**, partly due to **antigenic diversity** of strains

Differences Between Men & Women with Gonorrhea

IN MEN:

- **Causing Urethritis**; Epididymitis
- Most infections among men are acute and symptomatic with **purulent discharge & dysuria** (painful urination) after 2-5 day incubation period
- The two bacterial agents primarily responsible for **urethritis** among men are *N. gonorrhoeae* and *Chlamydia trachomatis*

IN WOMEN:

- **Cervicitis**; Vaginitis; **Pelvic Inflammatory Disease (PID); Disseminated Gonococcal Infection (DGI)**
- Women often **asymptomatic**, Often untreated until PID complications develop
- **Pelvic Inflammatory Disease (PID)**

- Can cause scarring of **fallopian tubes** leading to **infertility** or ectopic pregnancy

Pathogenesis of Neisseria gonorrhoeae

- **Fimbriated cells** attach to **intact mucus membrane** epithelium so : Adherence to mucosal epithelium and start infection

➢ **Most common sites of inoculation:**

- **Cervix (cervicitis)** or vagina in the female
- **Urethra (urethritis)** or penis in the male

Gonococcal Virulence Factors

➢ **1- Pili is one of the most important virulence factors.Only fimbriated (piliated) cells are virulent**

➢ **2-Outer membrane proteins** (formerly Proteins I, II, & III)

➢ **3-Acquisition of iron** mediated through Tbp 1 and Tbp 2 (**transferrin-binding proteins**), Lbp (**lactoferrin binding protein**) & Hbp (**hemoglobin-binding protein)**

➢ **4-** some isolates of *N. gonorrhoeae* produce **β-lactamase**, which is plasmid-mediated •

➢ 5- a **tracheal cytotoxin** damages the ciliated cells of the **fallopian tube**, leading to scarring and sterility.

Host defense mechanisms

- The host defense against gonococci are
- 1- IgA and IgG

- 2- complement
- 3-neutrophils
- 4-Ab-mediated opsonization and killing within phagocytes is also important
- But repeated gonococcal infections are common as a result of antigenic changes of pili and the outer membrane proteins

Prevention & Treatment

- Penicillin no longer drug of choice due to:
    - Continuing rise in the MIC
    - Plasmid-encoded beta-lactamase production
    - Chromosomally-mediated resistance
- Uncomplicated infxn: ceftriaxone, cefixime or fluoroquinolone
- Combined with doxycycline or azithromycin for dual infections with *Chlamydia*
- **Chemoprophylaxis of newborns against opthalmia neonatorum** with 1% silver nitrate, 1% tetracycline, or 0.5% erythromycin eye ointments
- Treatment of newborns with opthalmia neonatorum with ceftriaxone
- Measures to limit epidemic include **education, aggressive detection, and follow-up screening** of sexual partners, use of **condoms** or **spermicides** with nonoxynol 9

Neisseria meningitidis

(meningococcus)

General Overview of Neisseria meningitidis

- Encapsulated small, gram-negative diplococci
- Second most common cause (behind ***S. pneumoniae)*** of **community-acquired meningitis**
- **<u>Pathogenicity</u>:**
  - 1- **receptor-specific colonization of nonciliated epithelial cells of nasopharynx**
  - **2-Antiphagocytic** polysaccharide **capsule** allows systemic spread in absence of specific immunity
  - 3-Toxic effects mediated by **hyperproduction of lipooligosaccharide**

In susceptible individuals , meningococci **spread** from the **nasopharynx** into the blood stream (**septicaemia**), and then to the meninges. Septicaemia is accompanied by a rash. The **antiphagocytic properties** of the **capsule** help **dissemination**, while the toxic effects are mainly due to the meningococcal endotoxin. Treatment and

Diseases Associated with Neisseria meningitidis

- **Following dissemination of virulent organisms from the nasopharynx:**
  - ✓ **Meningitis**
  - ✓ Septicemia (**meningococcemia**) with or without meningitis
  - ✓ **Meningoencephalitis**
  - ✓ Pneumonia
  - ✓ Arthritis

    Urethritis

Virulence Factors

- 1- A **polysaccharide capsule** that enables the organism to resist phagocytosis
- 2- **Endotoxin LPS** causing fever, shock and pathophysiological change
- 3-**IgA protease** helping the bacteria attach to the membranes of the upper respiratory tract by cleaving secretory IgA

Epidemiology of Meningococcal Disease

- **Humans only natural hosts**
- **Person-to-person transmission** by aerosolization of **respiratory tract secretions** in crowded conditions
- **Close contact** with infectious person (e.g., family members, day care centers, military barracks, prisons, and other institutional settings)
- Highest incidence in **children younger than 5 years** and particularly those **younger than 1 year** of age as passive maternal antibody declines and as infants immune system matures
- Commonly **colonize nasopharynx** of healthy individuals; highest oral and nasopharyngeal carriage rates in school-age children, young adults and lower socioeconomic groups

Prevention and Treatment of Meningococcal Disease

- **Penicillin is drug of choice** for treatment in adjunct with supportive therapy for meningeal symptoms
    - **Chloramphenicol** or **cephalosporins** as alternatives

- **Chemoprophylaxis of close contacts** with rifampin or sulfadiazine (if susceptible)
- **Polyvalent vaccine** containing serogroups A, C, Y, and W135 is effective in people older than 2 years of age for **immunoprophylaxis** as an **adjunct to chemoprophylaxis**

Commensal Neisseria species

Commensal Neisseria species are common in the **oral cavity**, **nose** and **pharynx**, and sometimes in the **female genital tract**. The taxonomy of the group is confused. The **three main species** are ***Neisseria subflava*, *N. mucosa* and *N. sicca*.** The main difference between these and the pathogenic Neisseria species is the **ability** of the **commensal species to grow on ordinary agar at room temperature in the absence of carbon dioxide supplements.**

These organisms are essentially **non-pathogenic** and are almost always found in oral specimens from **saliva or mucosa**. **Neisseria species** are among the **earliest colonizers of a clean tooth surface**. They **consume oxygen** during the early plaque formation and facilitate subsequent growth of **facultative and obligate anaerobic late colonizers**.

Moraxella

Moraxella are :

-Obligate aerobic bacteria

-Gram-negative cocci closely related to the **non-pathogenic Neisseria species**.

-They are **commensals** of the **human respiratory tract**

-are recognized **opportunistic pathogens** causing **meningitis, endocarditis, otitis media, maxillary sinusitis and chronic obstructive pulmonary disease**.

-As the **majority of strains produce β-lactamase**, they may **indirectly 'protect'** other **pathogens** and thus complicate antibiotic therapy.

- **Asaccharolytic** : differentiate it fro *Neisseria*

Veillonella

Veillonella species are :

- **obligate anaerobic**, Gram-negative cocci

-frequently isolated from **oral samples**.

-**Three oral species** are recognized: ***Veillonella parvula*** (the type species), ***Veillonella dispar*** and ***Veillonella atypica***.

-***Veillonella parvula*** Gram-negative, small anaerobic cocci. Found in the human oral cavity, mostly in **dental plaque**, they are considered as '**benevolent organisms'** in relation to **dental caries** as they **metabolize the lactic acid** produced by **cariogenic bacteria** into **weaker acids** (acetic and propionic) with a **reduced ability to solubilize enamel**.

-No known pathogenic potential.

Diphtheria

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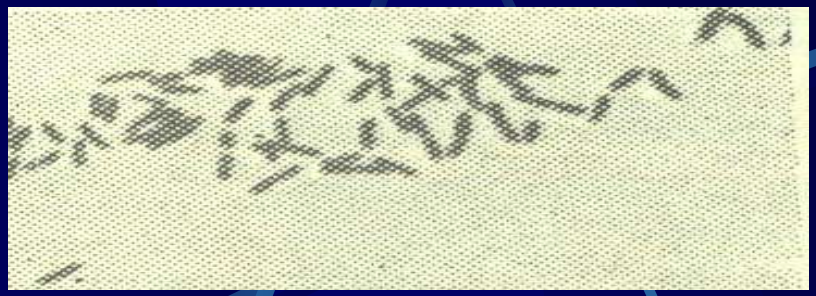
- Acute disease from the group of respiratory infections which characterized by fibrinous inflammation of mucous membranes of oral cavity, nasopharynx, larynx with toxic lesion of cardiovascular and nervous systems



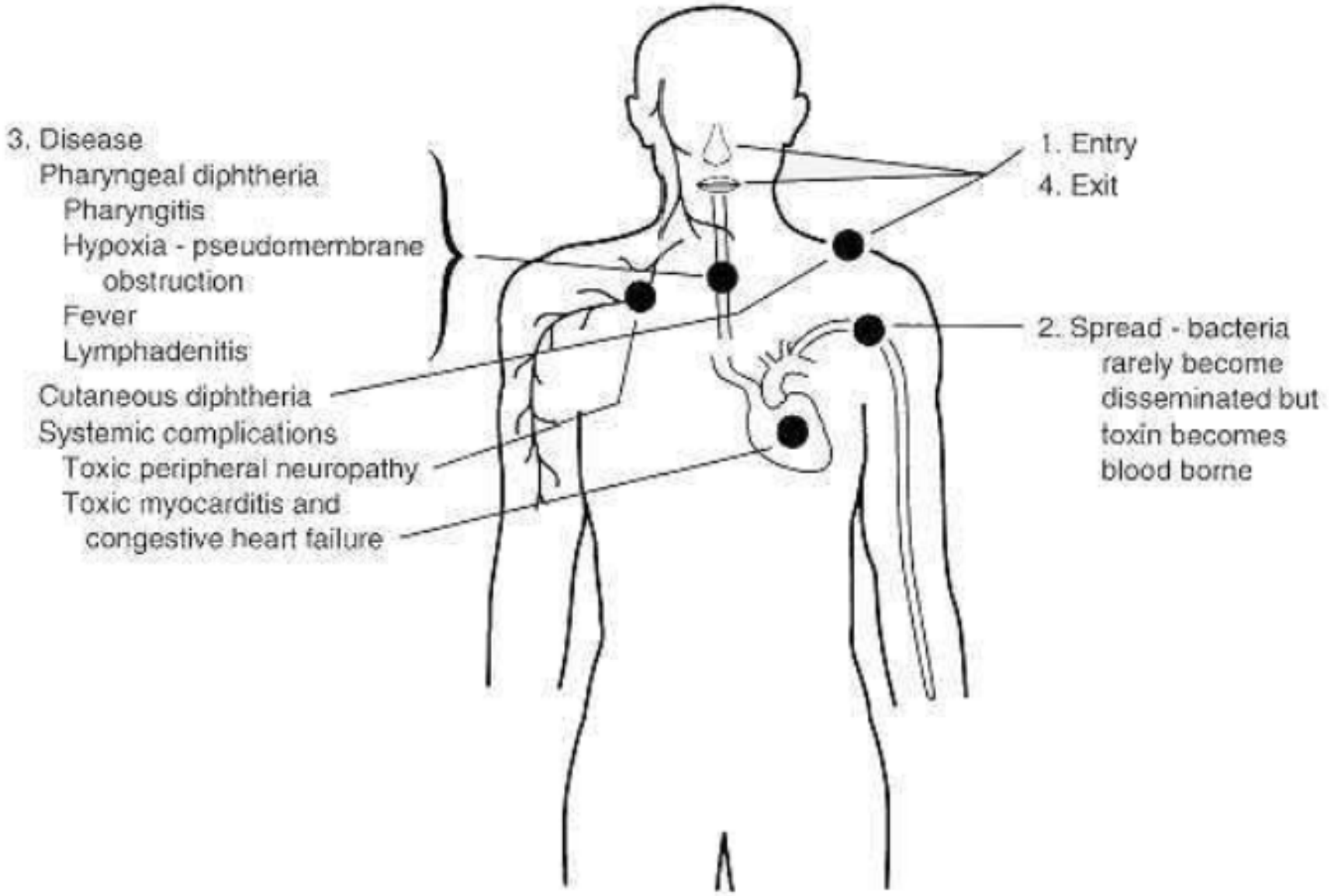
Etiology

Corynebacterium diphtheriae

- Grampositive, nonmotile
- Don’t forms spores and capsules
- brown-yellow color
- Sub species *C. diphtheriae* - mitis, gravis, intermedius
- Production of very strong exotoxin Structure of exotocin - dermanecrotoxin, hemolysin, neurotoxic

Epidemiology

- Source – sick person or carrier
- **Ways of transmission** - airborne, contact - Sensibility is high, adults more often become sick (80 %)
- Seasonal character - autumn - winter


3. Disease
Pharyngeal diphtheria
Pharyngitis
Hypoxia - pseudomembrane obstruction
Fever
Lymphadenitis
Cutaneous diphtheria
Systemic complications
Toxic peripheral neuropathy
Toxic myocarditis and congestive heart failure
1. Entry
4. Exit
2. Spread - bacteria rarely become disseminated but toxin becomes blood borne

Corynebacterium

- Gram + Non Acid fast, Non motile,
- Irregularly stained with granules,
- Club shaped swelling at one or both ends so the name
- Important Pathogen

Corynebacterium diphtheria,

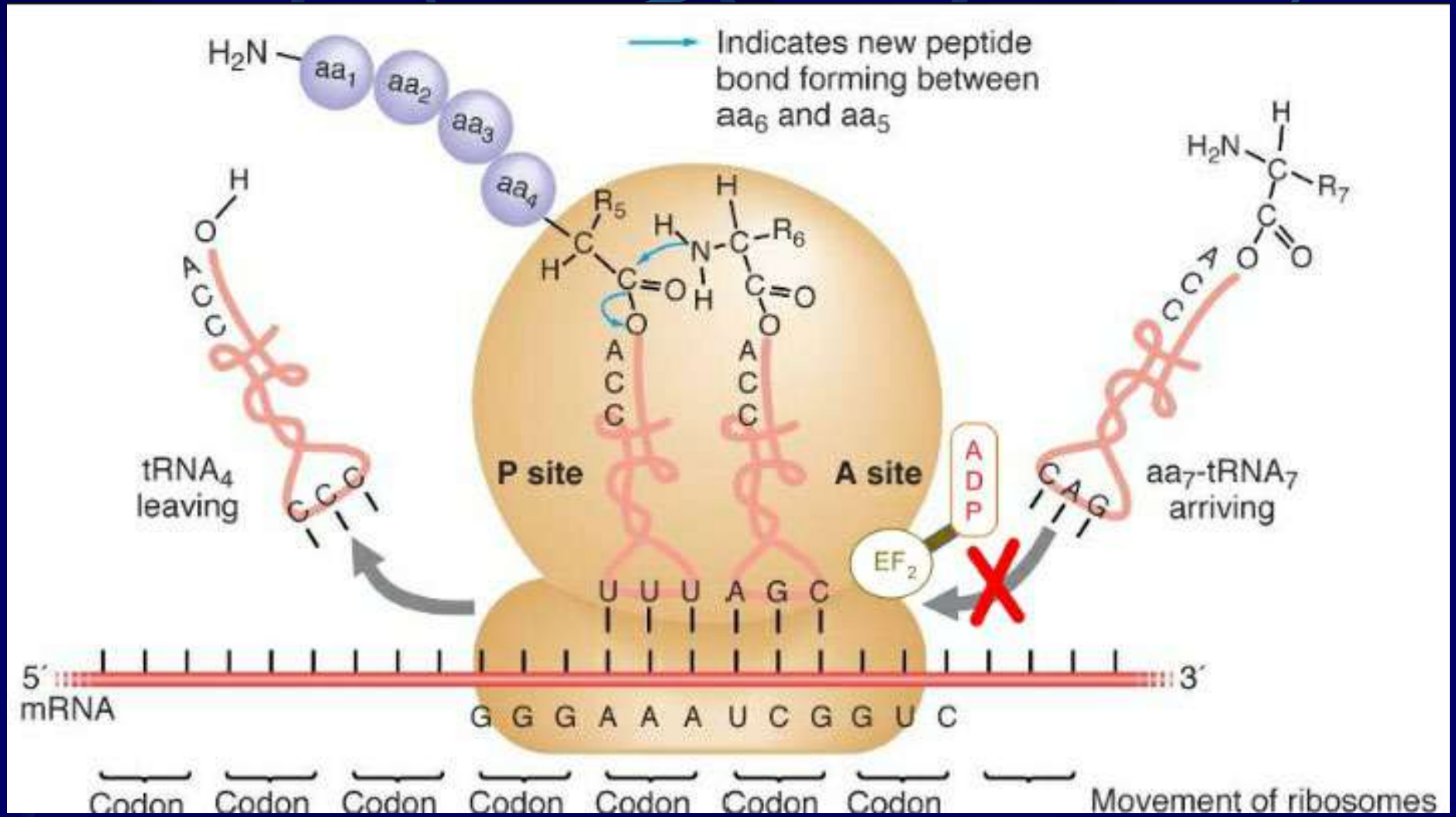
Diptheros meaning leather,

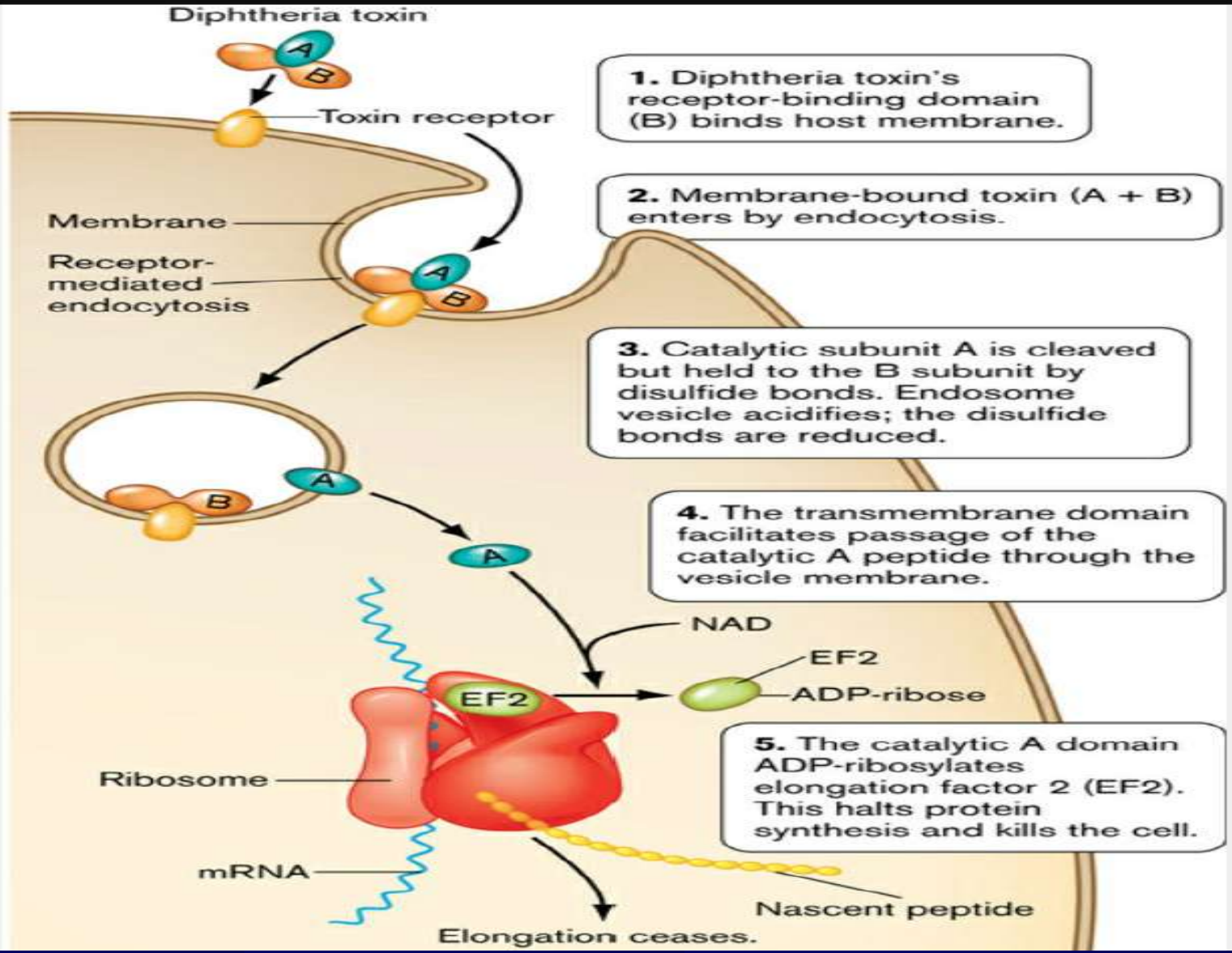
Diphtheria Epidemiology

- Reservoir — Human carriers
Usually asymptomatic
- Transmission — Respiratory
Skin and fomites rarely
- Temporal pattern — Winter and spring
- Communicability — Up to several weeks without antibiotics

Pathogenesis

- Penetration of the agent through entrance gate (mucous of upper respiratory tract, sometimes conjunctivas, skin)
- Production of exotoxin
- Local and systemic effects of the toxin:
- Dermonecrotoxin - necrosis of a surface epithelium, retardation of blood stream, rising of a permeability of vessels, their fragility, transuding of plasma in ambient tissues, formation of a fibrinous film, edema of tissues; downstroke of pain sensitivity


Indicates new peptide bond forming between aa6 and aa5
H2N
aa1
aa2
aa3
aa4
tRNA4 leaving
P site
A site
ADP
EF2
aa7-tRNA7 arriving
5′
mRNA
3′
U U U A G C
G G G A A A U C G G U C
Codon
Codon
Codon
Codon
Codon
Codon
Codon
Movement of ribosomes


Diphtheria toxin
A
B
Toxin receptor
1. Diphtheria toxin's receptor-binding domain (B) binds host membrane.
Membrane
Receptor-mediated endocytosis
2. Membrane-bound toxin (A + B) enters by endocytosis.
3. Catalytic subunit A is cleaved but held to the B subunit by disulfide bonds. Endosome vesicle acidifies; the disulfide bonds are reduced.
4. The transmembrane domain facilitates passage of the catalytic A peptide through the vesicle membrane.
NAD
EF2
ADP-ribose
Ribosome
5. The catalytic A domain ADP-ribosylates elongation factor 2 (EF2). This halts protein synthesis and kills the cell.
mRNA
Nascent peptide
Elongation ceases.

Clinical manifestation

- **Incubation period – 2-10 days**
- (high fever, general weakness, headache)
- Changes of a throat mucous - soft hyperemia, edema of tonsills, covers on their surface (grey colour, dense, hard to remove with bleeding, slime), spread out of tonsills limits

- Edema at neck

Clinical features: (depend on site of membrane formation)

Complications:

Tonsillopharyngeal (50%)

Slow commencement of sore throat and low grade fever.

Markes cervical adenitis and oedema (classical 'bullneck')

Laryngeal

Hoarseness, croupy cough and stridor.

Inspiratory recession of tissues and cyanosis.

Anterior nasal

Blood stained unilateral nasal discharge

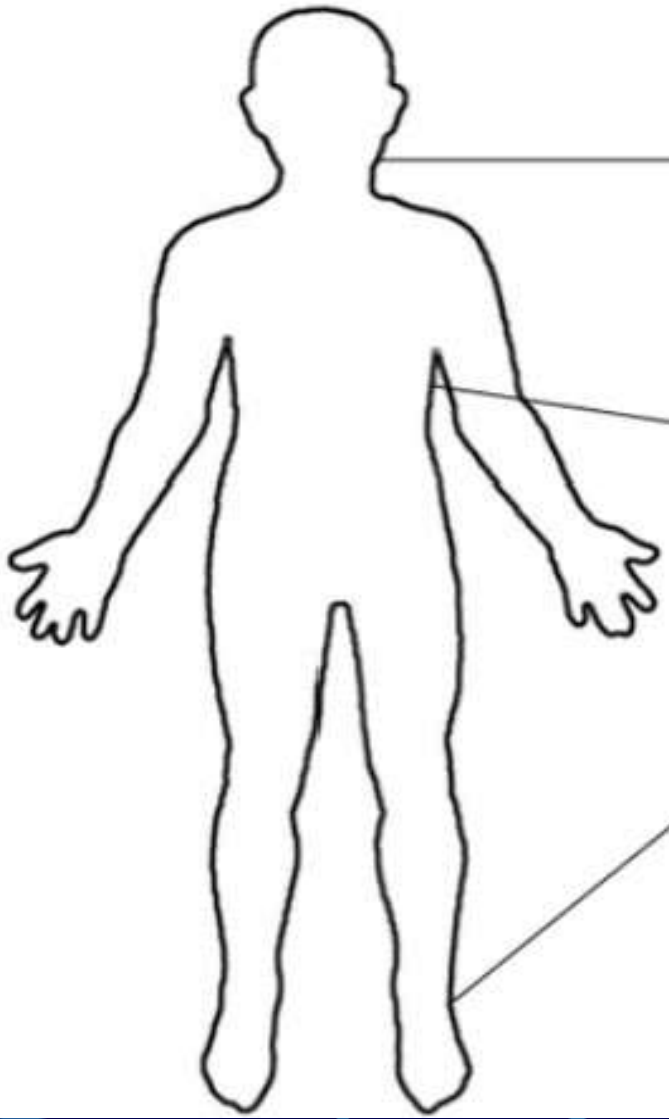
Mild symptoms of toxicity.

Cutaneous

Blood stained unilateral nasal discharge

Chronic ulcers (reservoirs of infection) with grey membrane.

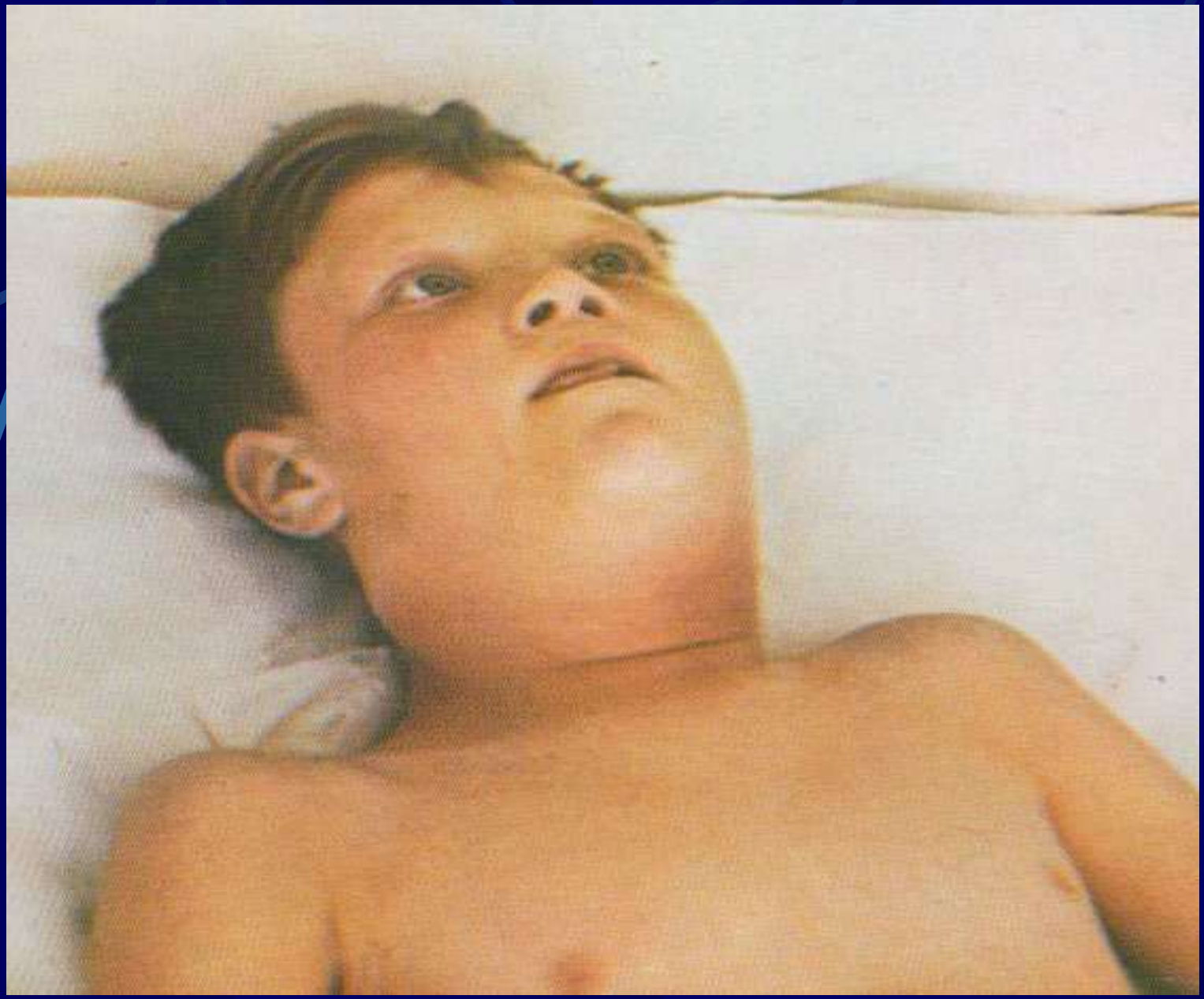
Mild symptoms of toxicity.



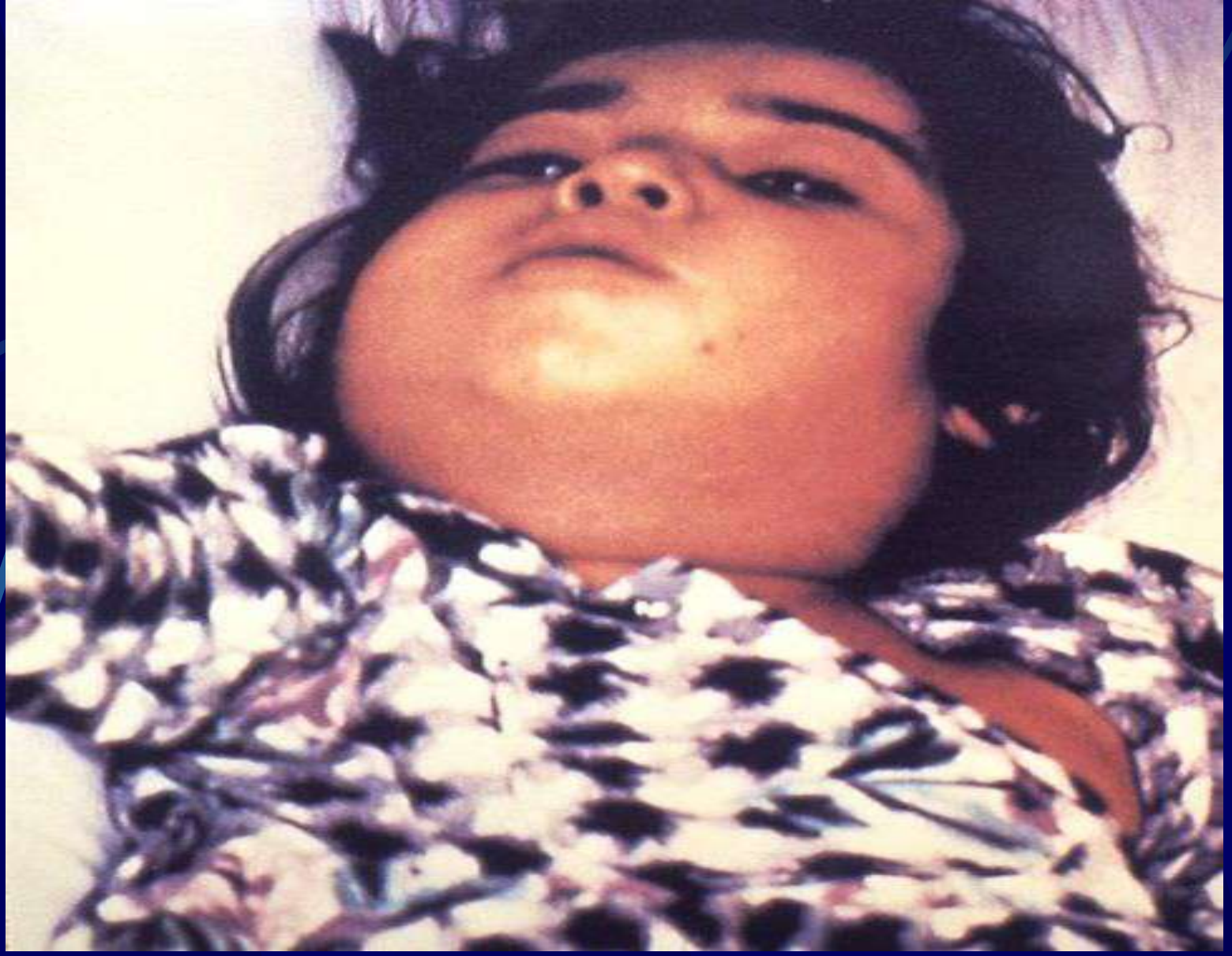
Laryngeal obstruction or paralysis

Myocarditis

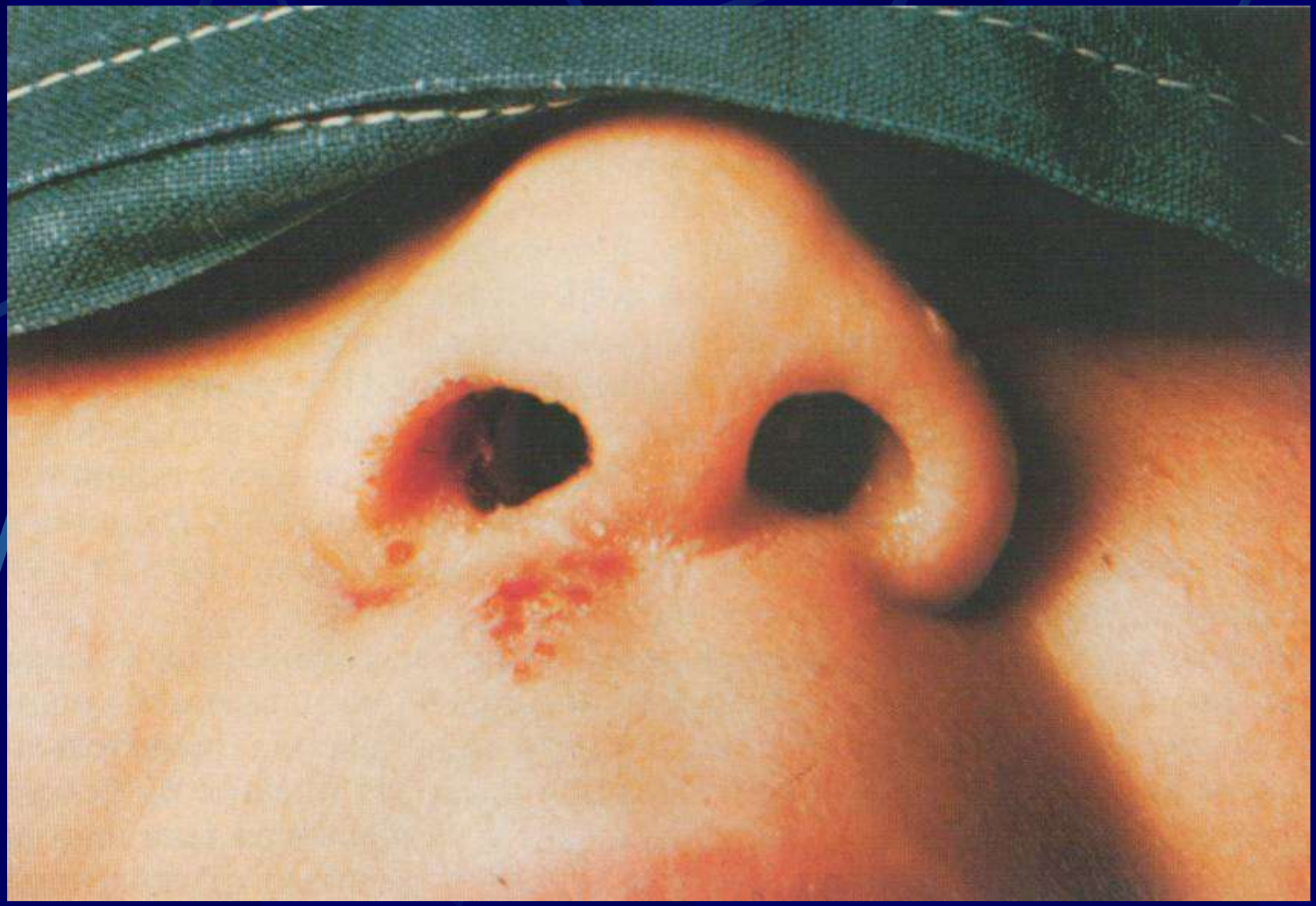
Peripheral neuropathy



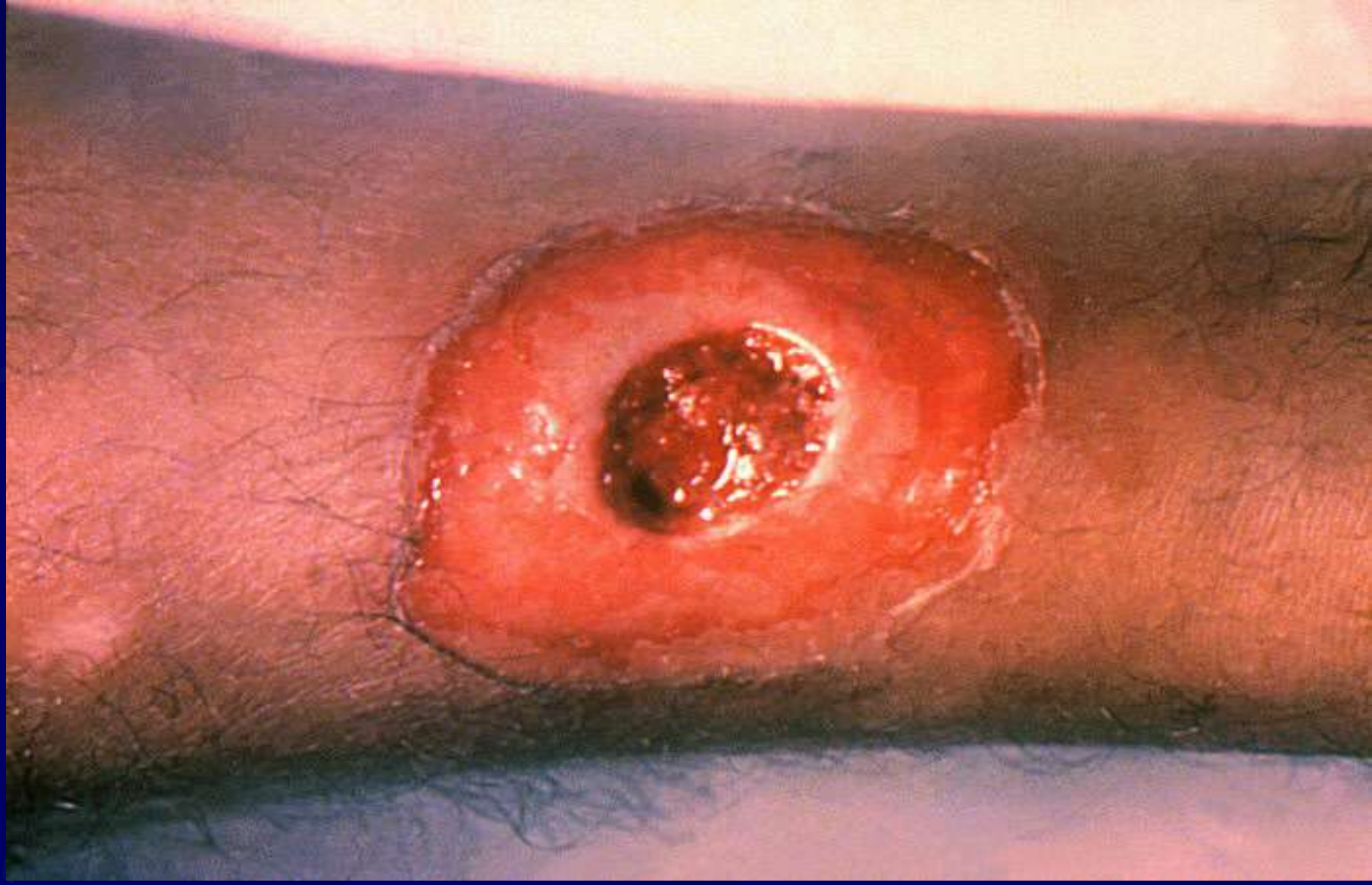
Edema of a hypodermic tissues of a neck



Swollen neck in diphtheria



Diphtheria of the nose



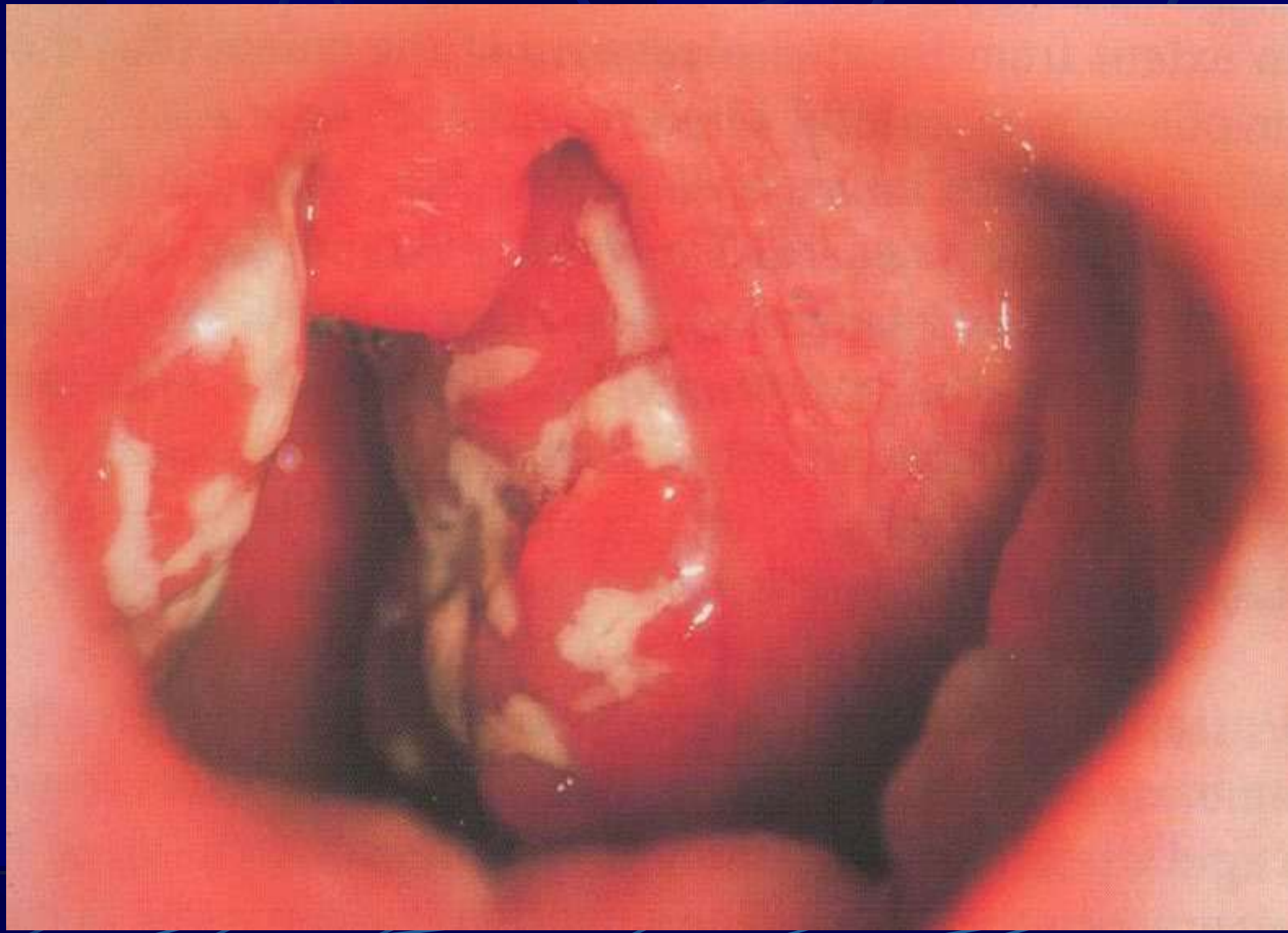
A diphtheria skin lesion on the leg

Features of diphtheria toxicosis

- Edema of the neck tissue
- Paleness of skin
- Cyanosis of lips
- Decreasing of arterial pressure
- Tachycardia
- Decreasing of a body temperature

Treatment

- Immediate hospitalization
- Glucocorticoids
- Antibiotics (penicilini, tetracyclini, erythromycini)



LABORATORY DIAGNOSIS : CULTURE

- If the swabs can not be inoculated promptly, they should be kept moistened with serum;
- Inoculate on :
  - **Loeffler's serum slope**
  - **Tellurite blood agar or Tinsdale medium**
  - **Blood agar (for differentiating Staphylococcal or Streptococcal pharyngitis that simulate diphtheria);**
- *Tellurite medium is particulary useful for isolating the organism from – convalescents, contacts or carriers;*

Corynebacterium diphtheriae

- **LABORATORY DIAGNOSIS**
- **3. Catalase test (+)**
- **4. Urease test (-)**
- **5. Toxigenicity test**
  - **Elek test (in vitro)**
  - **Animal inoculation test (in vivo)**

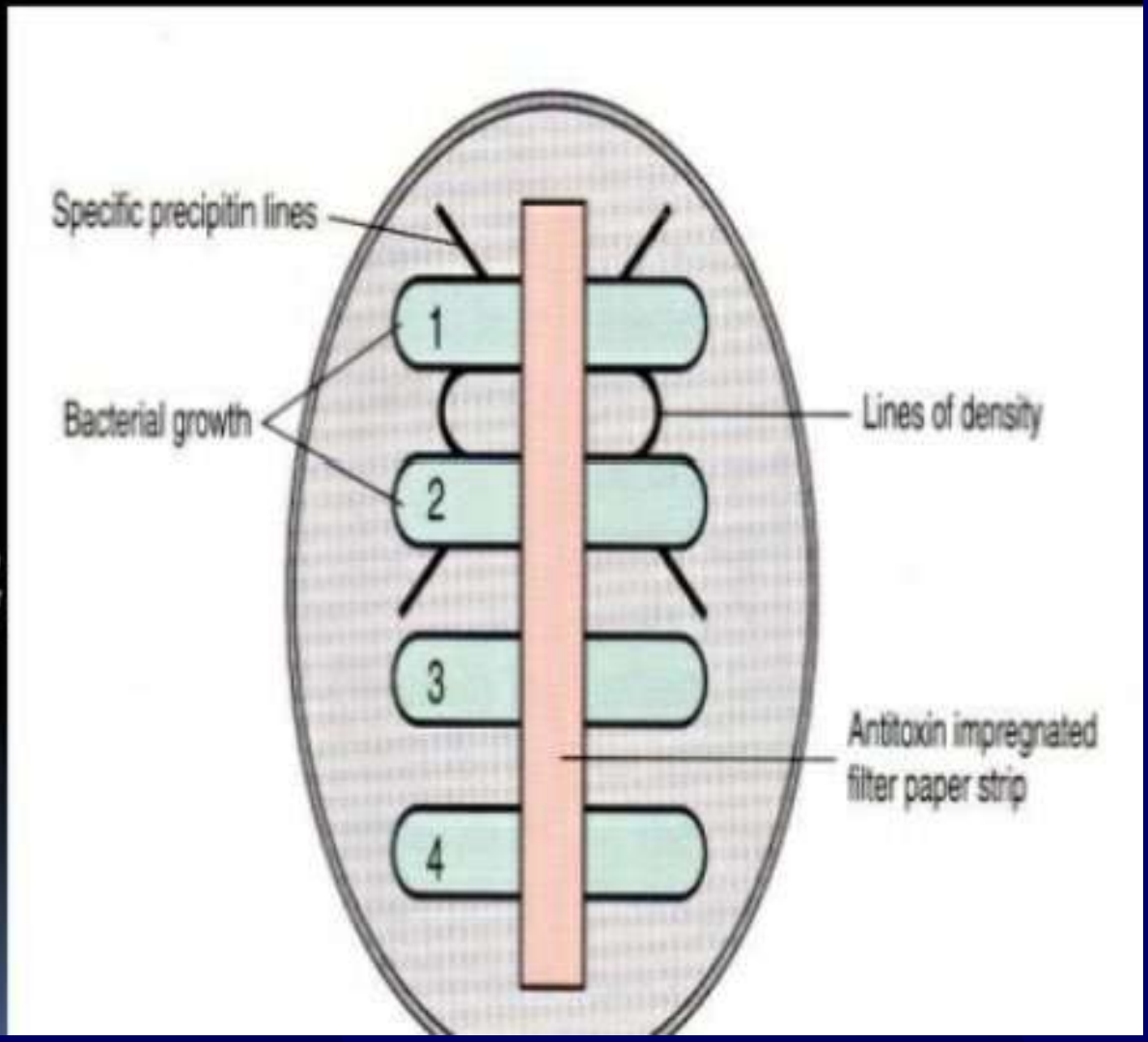
Virulence tests In Vitro: Elek's Test

The organism is streaked on a plate containing low iron.

A filter strip containing anti-toxin antibody is placed perpendicular to the streak of the organism.

Diffusion of the antibody into the medium and secretion of the toxin into the medium occur.

At the zone of equivalence, a precipitate will form.



Aerobic Actinomycetes

Aerobic Actinomycetes:Nocardia species

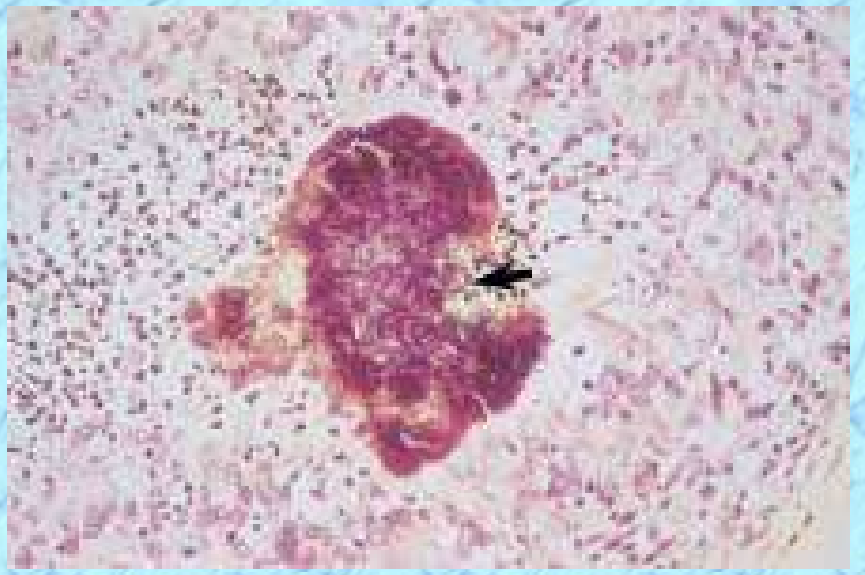
- **General Characteristics**
  - ✓ **Aerobic, gram-positive, filamentous rods, sometimes resembling branched hyphae**
  - ✓ **Weakly acid-fast and may stain gram-variable**
  - ✓ **Morphologically resemble fungi, both in culture and in types of infections produced**
  - ✓ **Generally found in the environment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

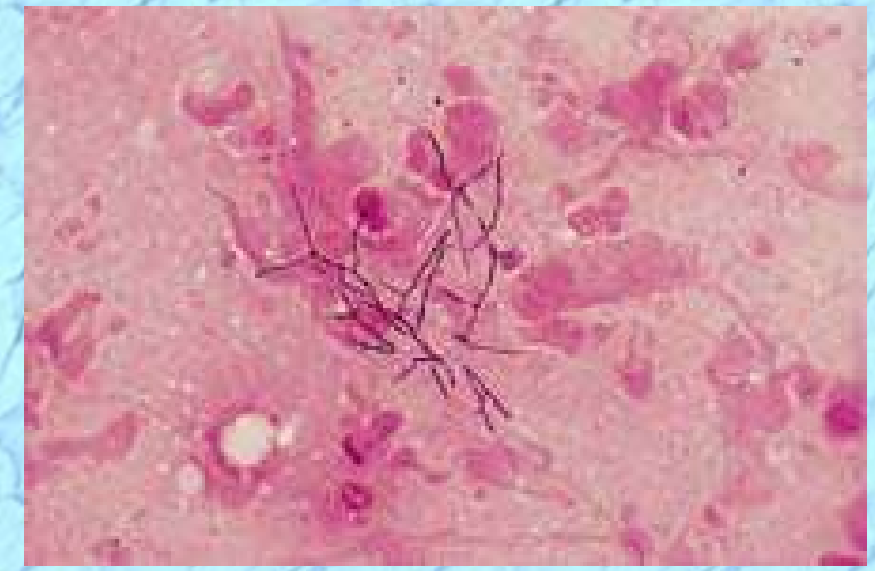
- **Clinical infections**
  - ✓ **Pulmonary form**
  - ✓ **Mycetomas**



Sulfur granules collected from draining sinus

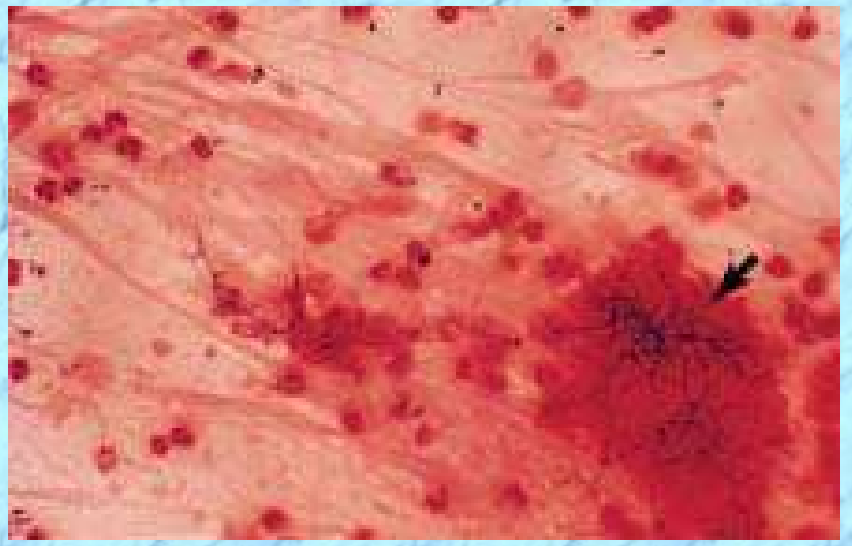
Laboratory Diagnosis: *Nocardia, Actinomadura, and Streptomyces species*

- **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**

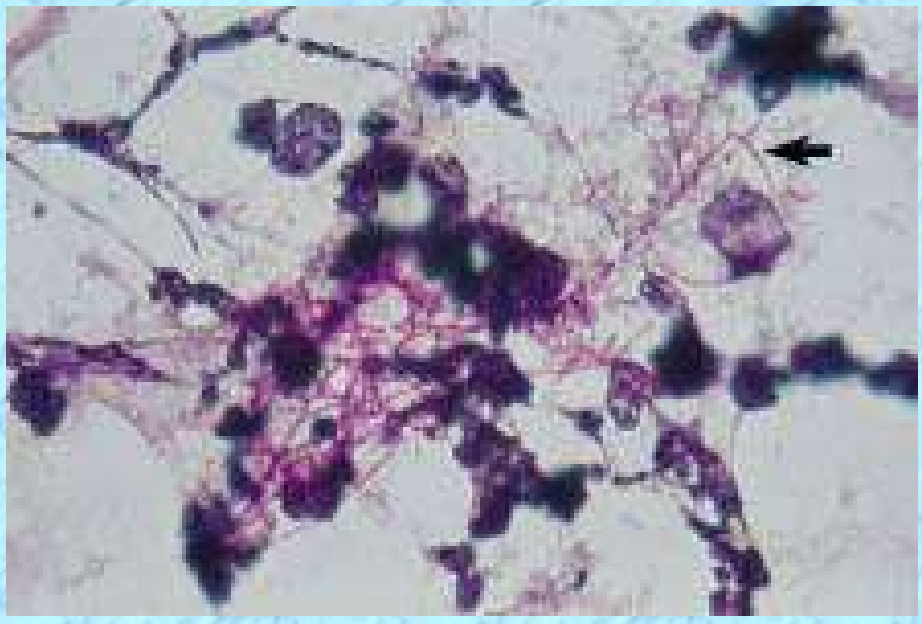
Laboratory Diagnosis:

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**

Listeria monocytogenes: General Characteristics

- Gram-positive, non–spore-forming rods
- Only human pathogen in genus
- Widespread in nature
- Known to infect a wide variety of animals
- Human exposure is limited; direct or indirect
- Transient colonization occurs without disease

Listeria monocytogenes: Clinical Infections

- Adults
  - Septicemia/meningitis in the compromised/elderly
  - Mild flu-like syndrome in pregnant women could be fatal to fetus
  - Ingestion of contaminated food (cottage cheese, coleslaw, chicken, hot dogs, lunch meat)
- Neonatal
  - Early onset from intrauterine transmission results in sepsis; high mortality rate

Listeria monocytogenes: Virulence Factors

- Hemolysin (Listeriolysin O)
  - damages host cell membrane
- Superoxide dismutase
  - Resists toxic effects of the host
- P60 surface protein
  - Induces phagocytosis thru adhesion and penetration

Differentiating Characteristics between *L. monocytogenes* and Other Gram Positive Bacteria

Species	Catalase	Hemolysis	Motility At R. T.	Esculin Hydrolysis	Growth 6.5% NaCl
L. monocytogenes	+	Beta	+	+	+
Corynebacterium sp.	+	None, alpha	=/+	=	+/=
S. agalactiae	=	Beta	=	=	=/+
Enterococcus sp.	=	None, alpha beta	=	+	+

Thanks for your Attention!