Vivekanand College, Kolhapur (Autonomous)
Department of Microbiology
PPT Bank (2018-2023)
Index

Sr. No.	Name of Topic	Class	Course
1	Cell membrane	B. Sc. I	DSC 1010 A Introduction to Microbiology
2	Capsule and slime layer		
3	Plasmids		
4	Contributions of scientists		
5	HMP Pathway	B. Sc. II	D.SC 1010 C1 Microbial physiology and metabolism
6	Oxidative phosphorylation		
7	Median		DSC 1010C2 Industrial and applied Microbiology
8	Mode		
9	Appications of Biostatistics		
10	Antigens		DSC1010D2 Basics in Medical Microbiology and Immunology
11	Antibodies		
12	Immune response		
13	Transduction		
14	Bacitracin and Tetracycline	B. Sc. III	DSE1010 E2 Medical Microbiology
15	Drug resistance		
16	Helicobacter pylori		
17	Nystatin and Griseofulvin		
18	Egg inoculation technique		DSE1010 F1 Virology
19	Structure of viruses		
20	Multiplication of Adenovirus		

Cell membrane

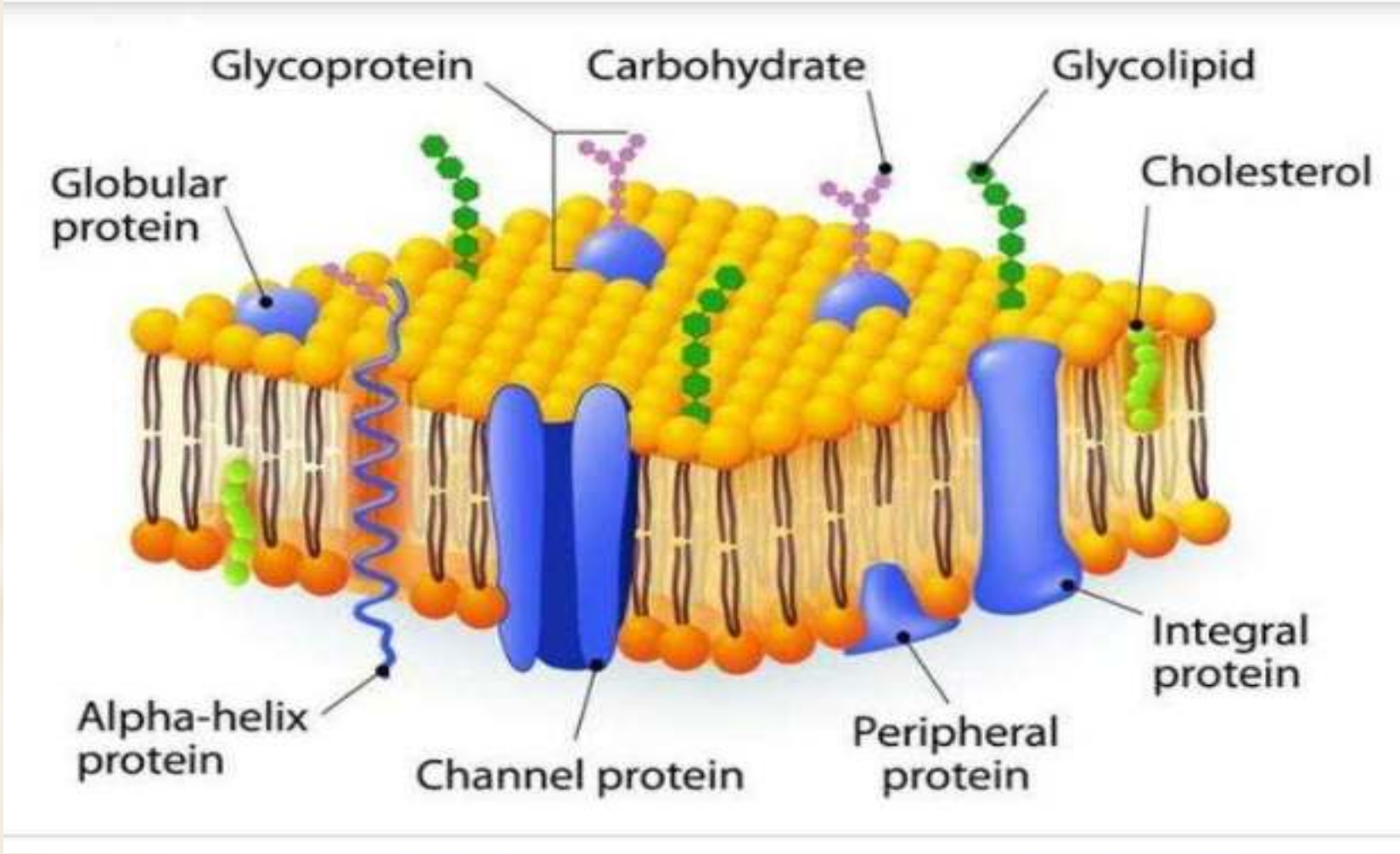
Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

Cell membrane

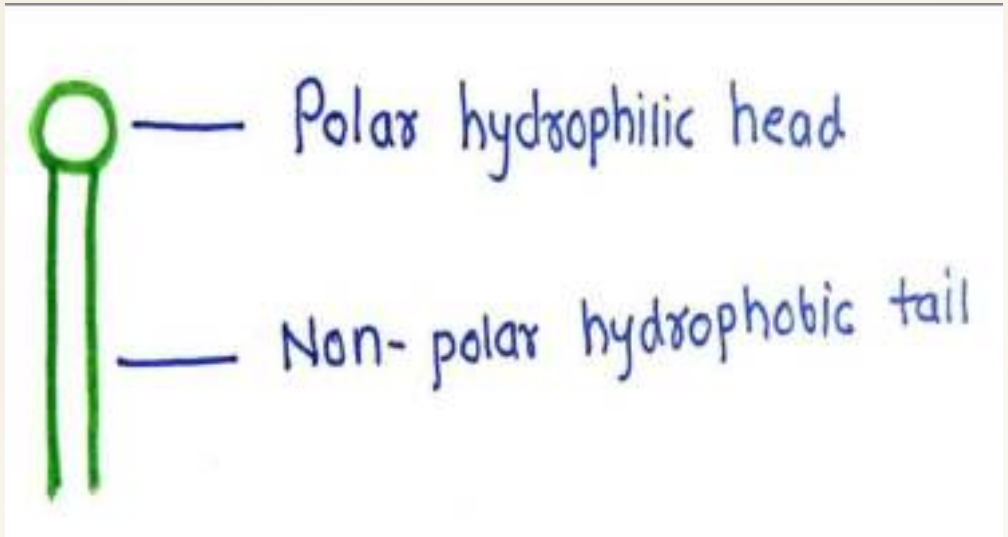
- Also known as **cytoplasmic membrane** or **plasma membrane**.

- **Structure of cell membrane:**
  - To explain structure various models were proposed.
  - Most accepted model is "**fluid mosaic model**".
  - It was proposed by **S. J. Singer** and **G. L. Nicolson** in 1972.
  - According to this model, cell membrane is phospholipid bilayer in which proteins floats.

❖ The model is based on studies using transmission **electron microscopy (TEM)** and **atomic force microscopy.**



- Phospholipids are amphipathic in nature.



- Lipid composition varies with environmental temperature.

a. Bacteria growing at lower temp. have more **unsaturated fatty acids**.

b. While bacteria at higher temp. Have more **saturated fatty acids**.

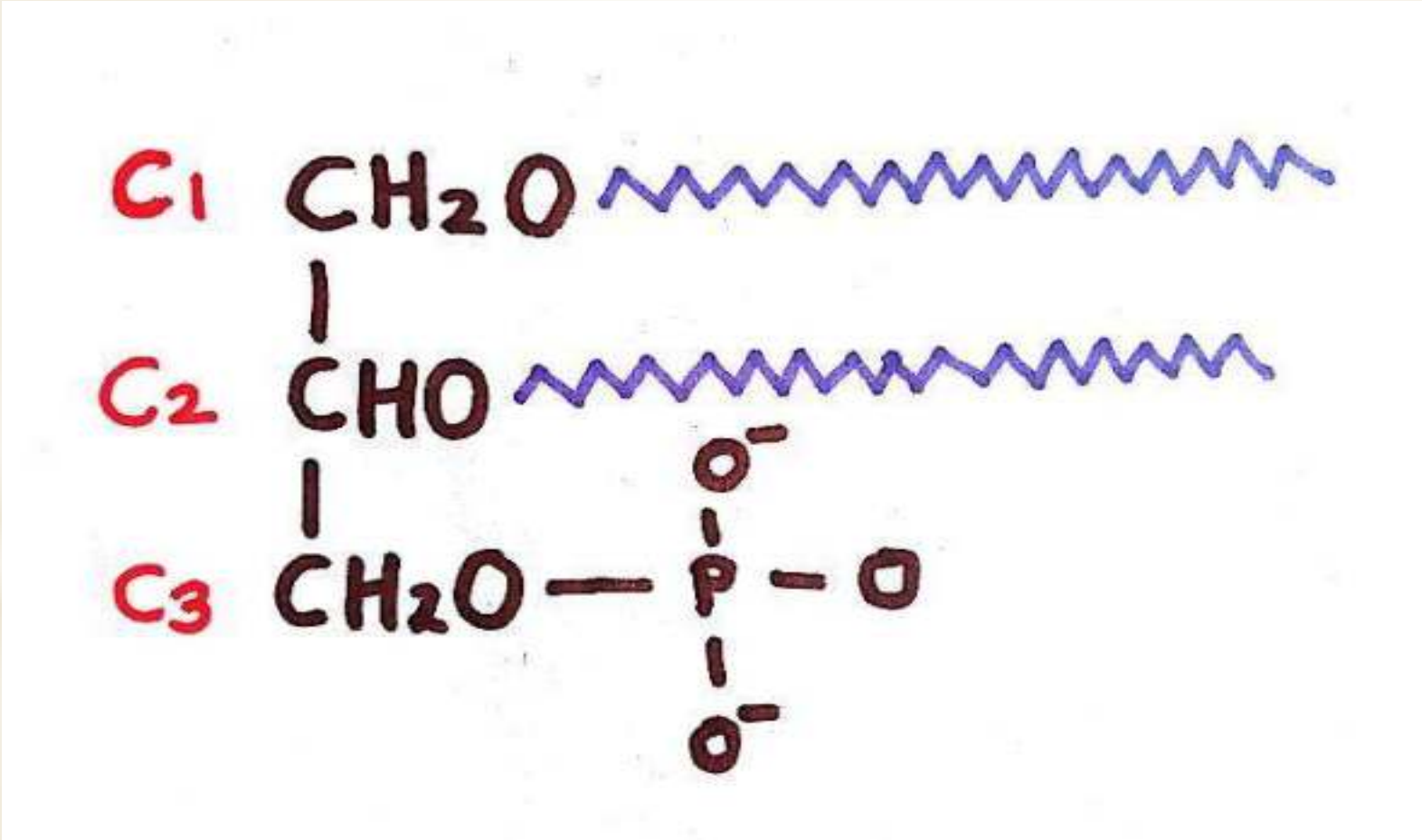
- In both cases lipid remains fluid in nature.

CAPSULE and SLIME LAYER

Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur

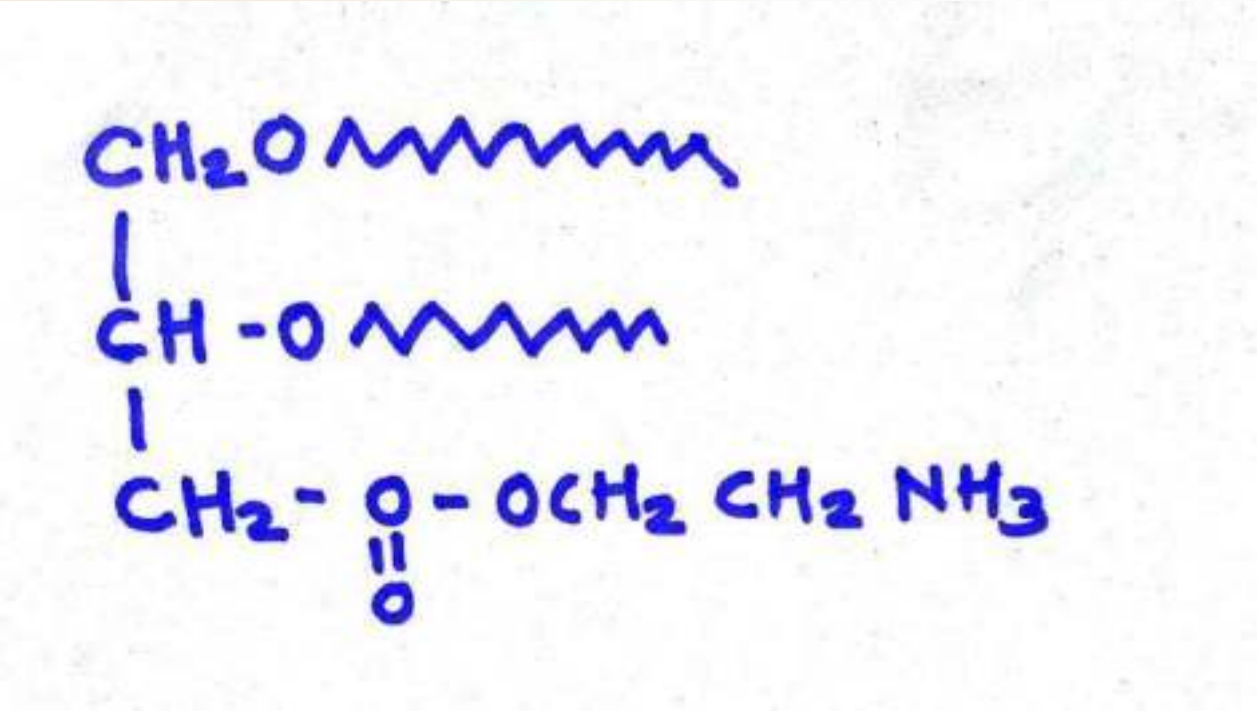
1. PHOSPHOLIPIDS

- Phospholipids of membrane are glycero-phospholipids.



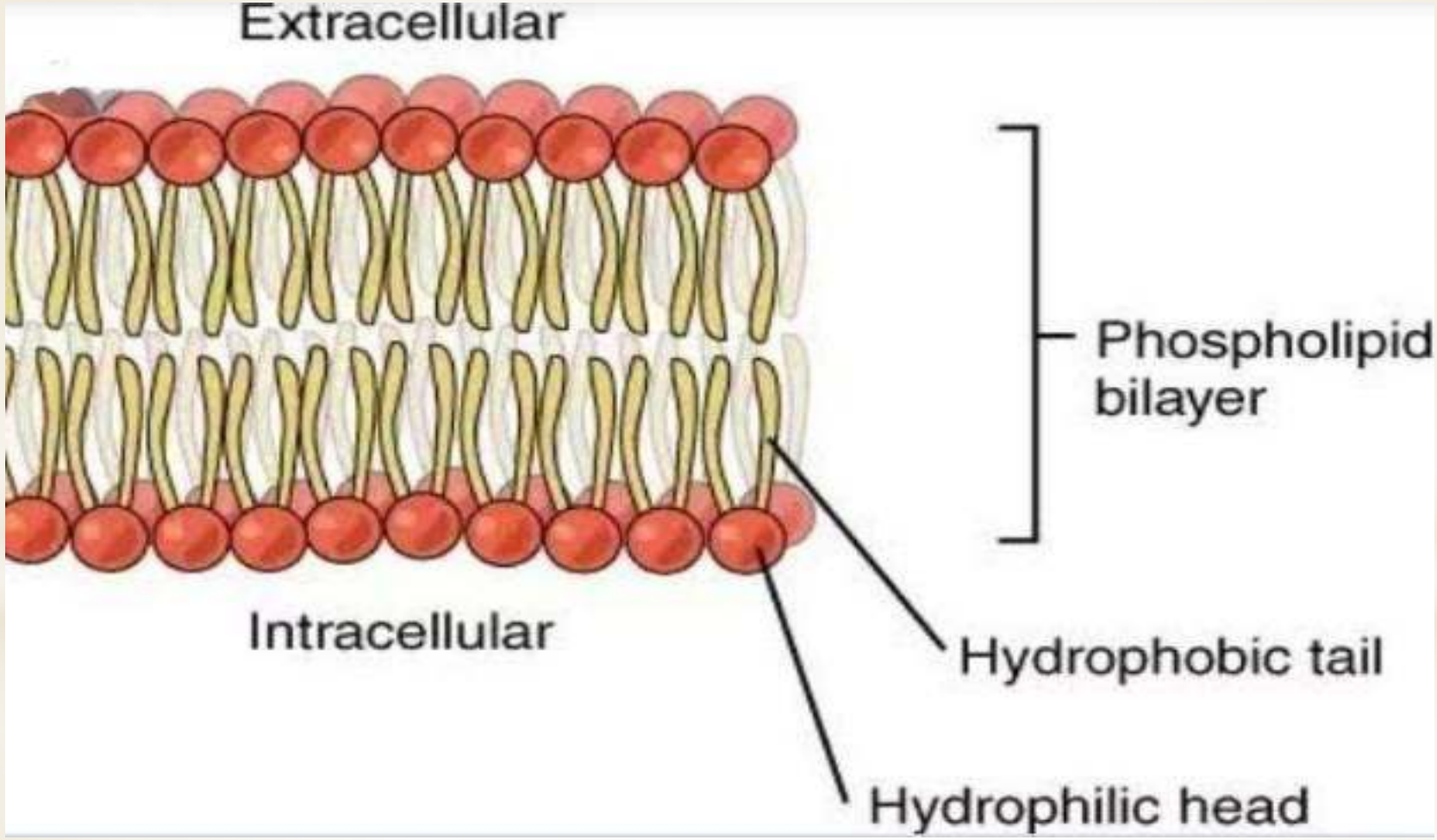
➢Proton of phosphate at carbon 3 is replaced by any organic moiety.

e.g. Ethanolamine



Phosphatidyl ethanolamine

❖ **Phospholipid is always present in bilayer.**

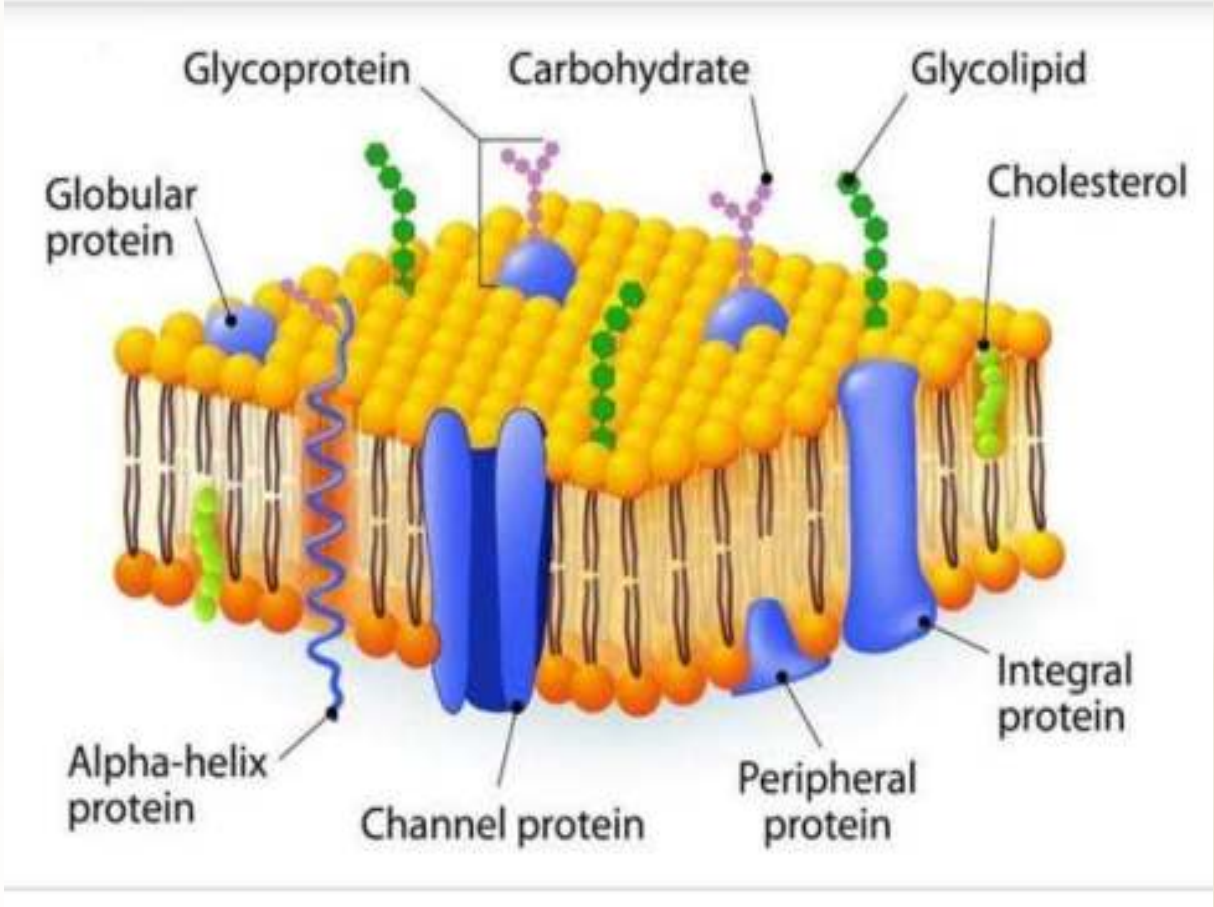


2. PROTEIN

- The membrane proteins are globular proteins.
- It is of 2 types:

i. Peripheral membrane proteins

ii. Integral membrane proteins



1. Peripheral proteins:

- ❑ Loosely connected to membrane.
- ❑ Soluble in aqueous solution.
- ❑ Accounts for 20-30% of total membrane proteins.
- ❑ These are also known as ‘extrinsic proteins’.

2. Integral membrane proteins:

- ❑ Can not ne easily extracted.
- ❑ Insoluble
- ❑ Known a ‘intrinsic proteins’.

Functions of cell membrane

1. Cell membrane is an ultimate organelle of the cell.
2. It is selective permeable membrane.
3. It is an osmotic barrier.
4. It gives attachment site for chromosome and plasmid during replication.
5. It is a site for several crucial metabolic processes as:
   a. Respiration and photosynthesis.
   b. Synthesis of lipids and cell wall constituents.
6. Plays imp. Role in cell division and sporulation during septum formation.
7. Provides anchorage site for flagella.

CAPSULE and SLIME LAYER

Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

Capsule and slime layer

- Bacteria are surrounded by extracellular polymeric substance, which forms an extra coating around cell
- This covering is called exopolymer or glycocalyx.
- Glycocalyx is of 2 types:

  a. Capsule

  b. Slime layer

1. CAPSULE

- **Definition**: **The outermost, slimy, gummy, mucilagenous coating present around cell wall.**
- It is well organized and not easily washed off.
- It is not required for growth and survival of bacteria.
- It increases virulence of an organism.
- The organism which have capsule is called '**capsulated organism**'.
- Thickness of capsule is 0.1 µm to 10 µm.

Depending upon the thickness, capsules are of two types:

i. Macrocapsules
ii. Microcapsules

1. Macrocapsules:

Capsules are with atleast 0.2 µm in thickness.

Can be seen easily under compound microscope.

2. Microcapsules:

Capsules are with less than 0.2 µm in thickness.

Can not ne seen under compound microscope.

It’s presence can be detected immunologically.

2. SLIME LAYER

- It is loose, unorganized, soluble covering around cell.
- It has viscous substance around cell, with less definite shape.
- Gliding bacteria posses slime layer more often.

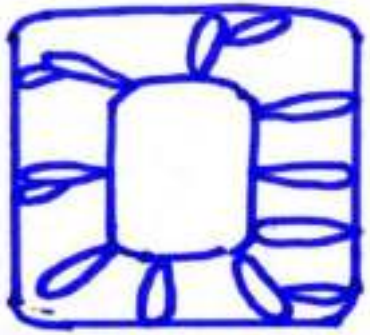
Difference between capsule an slime layer

SR. NO.	CAPSULE	SLIME LAYER
1	It is the part of cell	It is only secretion of cell
2	Has definite shape	Amorphous and thus have less definite shape
3	Have more r less same density throughout	Density decreases with increase in distance from cell surface.
4	Capsule production is under genetic control	Slime production is not under genetic control
5	Capsular material doe not diffuse in surrounding medium	Slime diffuses in medium and becomes sticky
6	Has great immunological significance	No immunological significance
7	Examples of capsulated organisms: ***Streptococcus, Leuconostoc, Klebsiella, Azotobacter, Rhizobium***	Examples of slime forming organisms: ***E.coli, Salmonella, Aerobacter, Cloaceae***

Ultrastructure of capsule

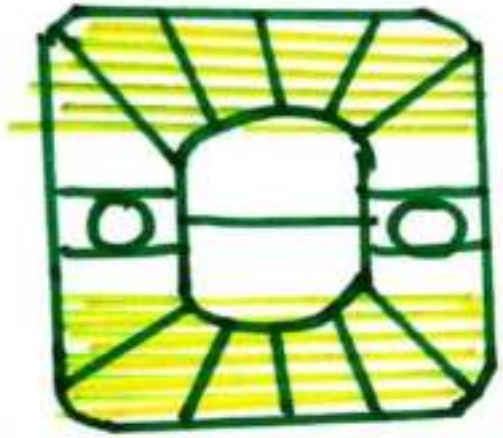
1. Continous layer

Cell surrounded by continuous layer of capsule



2. Banded fibrils

Cell surrounded by capsular layer as banded fibrils



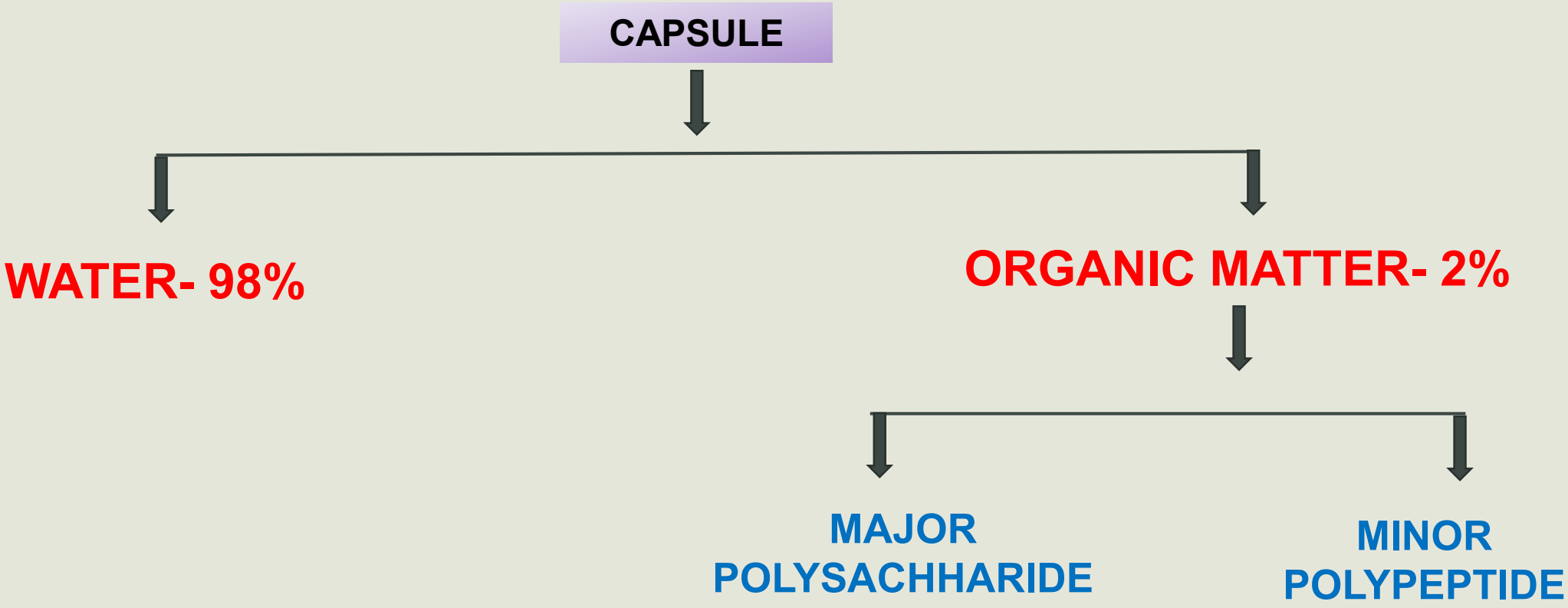
3. Localized patches

Cell surrounded by capsule with localized patches of polysaccharide

4. Discontinous layer

Cell surrounded by capsule as discontinous layer

Chemical composition of capsule



POLYSACCHARIDE

HOMOPOLYSACCHARIDE

EX. Glucans: *Leuconostoc*, *Pediococcus*

Levans: *Xanthomonas*, *Pseudomonas*

Cellulose: *Acetobacter xylinum*

HETEROPOLYSACCHARIDE

Pseudomonas aeruginosa

D-glucose, D-galactose,

D-mannose, D-rhamnose,

D- glucoronic acid

Functions of capsule

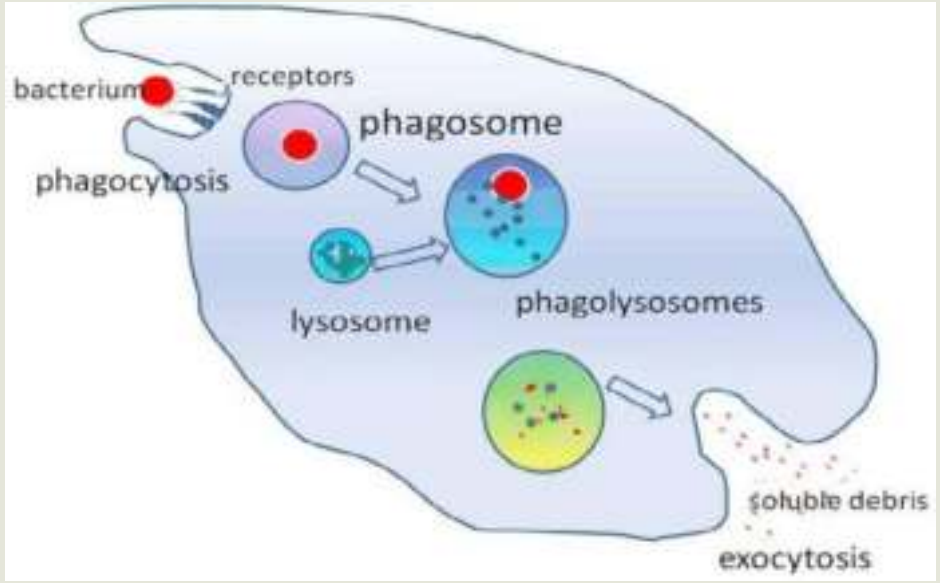
1. Protection: Capsule protects bacterial cell from:-

a. Desiccation or drying:

b. Starvation

c. Phagocytosis

d. Phage infection



2. Adherence:

Capsule is slimy and gummy, the cell acquires sticky property.

Because of sticky appearance, organisms can easily adhere to substratum like teeth, rock, roots.

❖ **Use of capsular material for man:**

1. Dextrans: Produced by ***Leuconostoc mesenteroids***.

    a. Used as plasma in the hospitals.

    b. Used as gel in gel filtration.

2. Used for **immunological differentiation** of related species.

Bacterial nucleoid

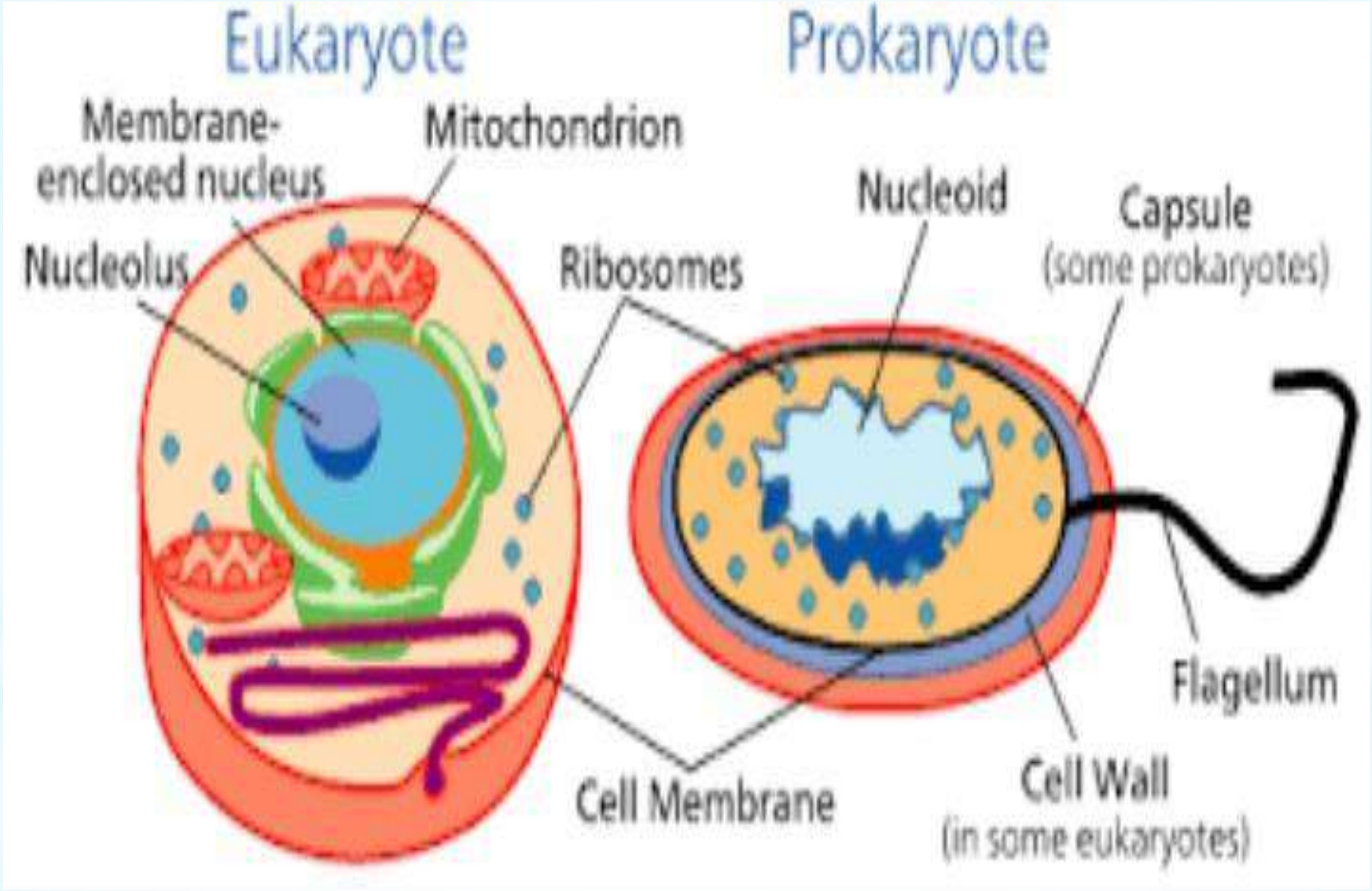
Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

Nucleoid or bacterial chromosome

- Bacteria being prokaryotic have primitive nucleus.
- Nucleus is not surrounded by by nuclear membrane unlike eukaryotes.
- Hence bacterial chromosome is called **‘chromatin or nucleoid’.**

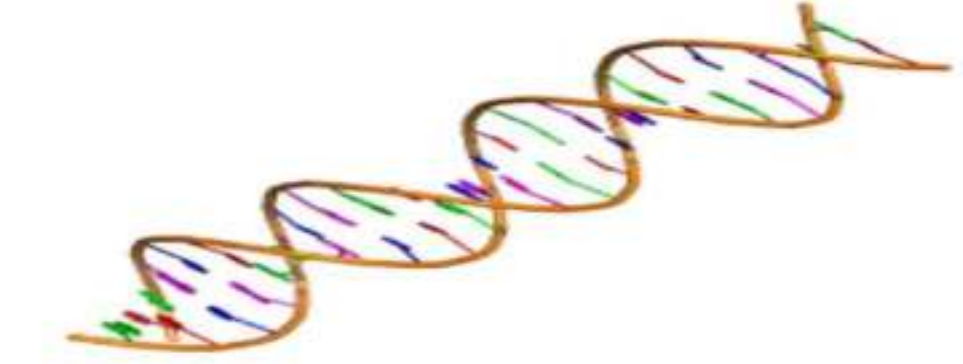
❖Nucleoid:

It is irregular shaped region that contains chromosome (DNA &many proteins.


Eukaryote
Prokaryote
Membrane-
enclosed nucleus
Mitochondrion
Nucleolus
Ribosomes
Nucleoid
Capsule
(some prokaryotes)
Flagellum
Cell Membrane
Cell Wall
(in some eukaryotes)

❖**The chromosome of most of the bacteria is a single circular double stranded DNA.**

❖**But some bacteria may have linear chromosome.**

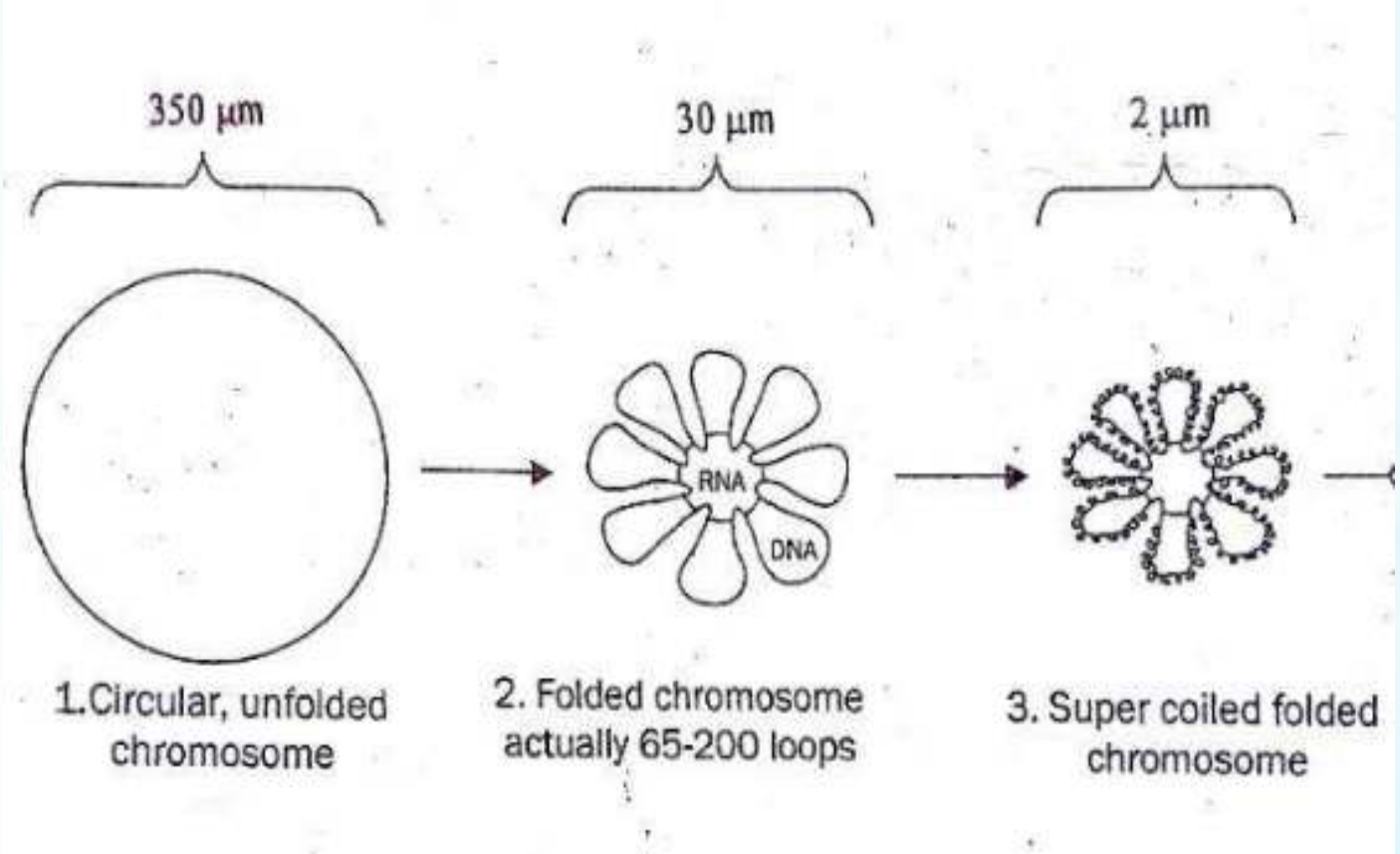


❖**Some bacteria may have more than one chromosome.**
e.g. *Vibrio cholerae* and *Borrelia burgdorferri*

✓Bacterial chromosomes are longer than the length of bacterial cell.

✓e.g. ***E. coli's*** circular chromosome:

It is approx 1400 µm which is 230-700 times longer than length of bacterial cell.



- For most of bacteria nucleus is without covering.
- But in some bacteria nuclear covering is observed.
- e.g. ***Planctomycetes pirrulia***: It has single membrane that surrounds nucleus.

  This is known as **pirellulosome**.
- While in ***Gemmata obscuriglobus*** two membranes are observed.
- Bacterial chromosome contains as many as 3500 genes.
- It contains 4×10^9 base pairs with molecular weight 4×10^9 d.

Functions of chromosome

1. It is a genetic material of the cell.
2. DNA determines what kind of proteins and enzymes can be synthesized by the cell.
3. It controls all metabolic processes of life, including cell division.
4. Organism can not live without its. Hence it is called thread of life.

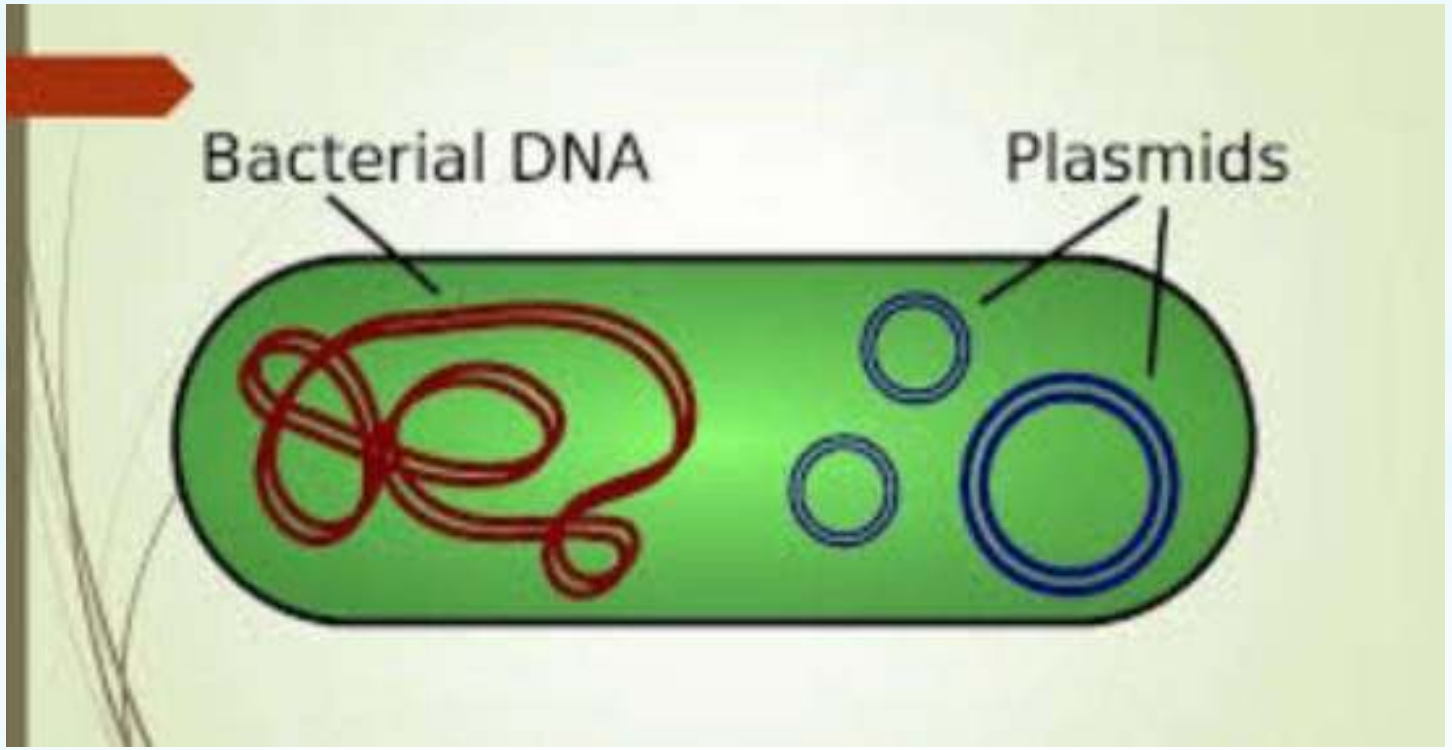
Plasmids

Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

Plasmids

❖ In addition to genetic material, some bacteria contains extrachromosomal DNA molecule known as '**Plasmid**'.

❖ Plasmid is **extrachromosomal circular double stranded DNA** molecule



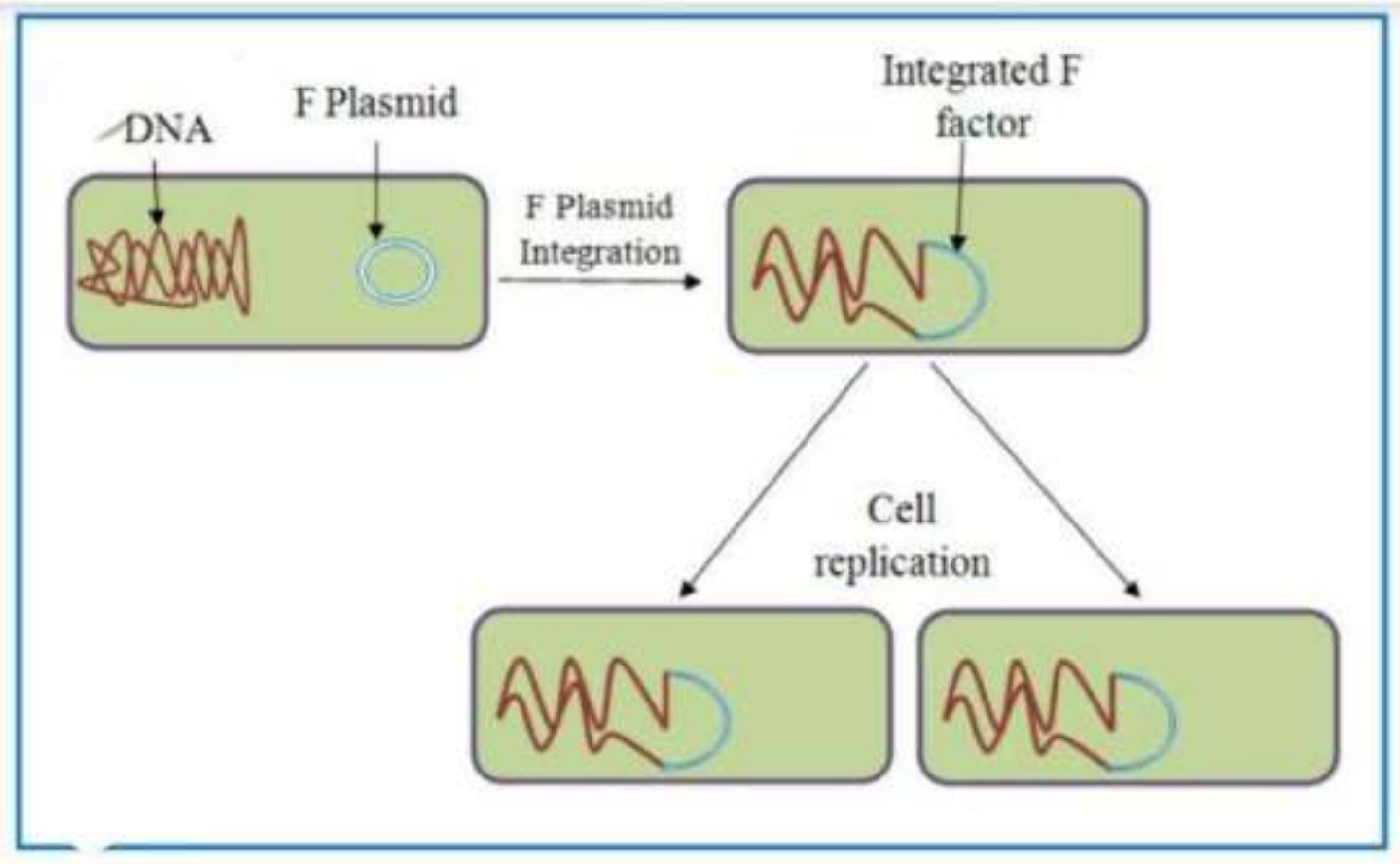
- In some bacteria numerous different plasmids within single species are observed.
- e.g. ***Bacillus burgdorferri***: Contains 12 linear and 9 circular plasmids.
- Plasmids are commonly used in gene transfer and cloning of genes.
- **Structure of plasmids:**

• It is circular or linear double stranded DNA.

• It has few genes, less than 30.

- It replicates autonomously.

•But some plasmids integrate with chromosome. Such plasmids are called **‘episome’.**



- Generally during cell division, plasmids are inherited to daughter cells.
- But some time they may be lost.
- Loss of plasmid is called **curing**.

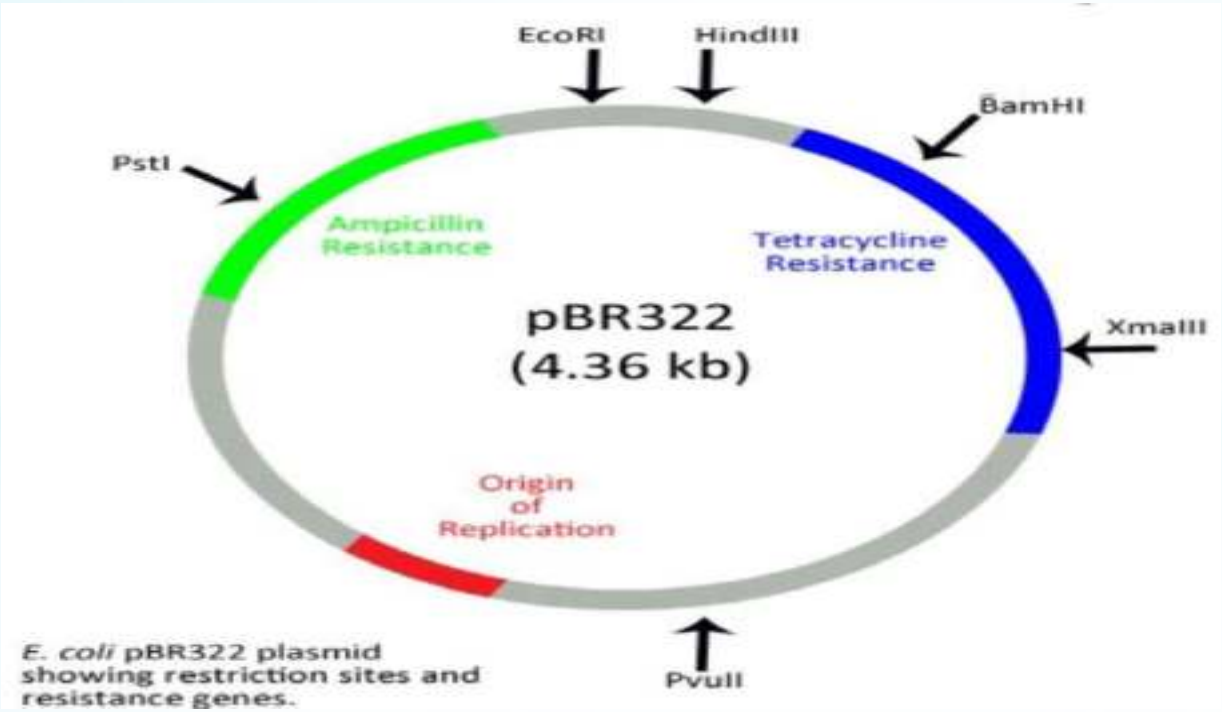
Types of plasmids

Based on their mode of existence, spread and function:

1. R plasmid
2. Toxin coding plasmids and other virulence characteristics
3. Col plasmid
4. Sex factor or F factor
5. Heavy metal resistant plasmids
6. Degradative plasmids

1. R plasmid (resistance plasmid)

- ✓It is most well studied and wide spread plasmid.
- ✓It confers resistance to antibiotics and other growth inhibitors.
- ✓Several antibiotic resistant genes may be present on R plasmid.



E. coli pBR322 plasmid showing restriction sites and resistance genes.

2. Plasmid coding toxins & other virulence characteristics

Plasmid codes for virulence factor in several pathogenic bacteria.

e.g. **Enteropathogenic E. coli**:

It produces hemolysin which lyses RBC's is encoded by plasmid

3. Heavy metal resistant plasmid

Some bacteria can resist action of heavy metals with the help of plasmids.

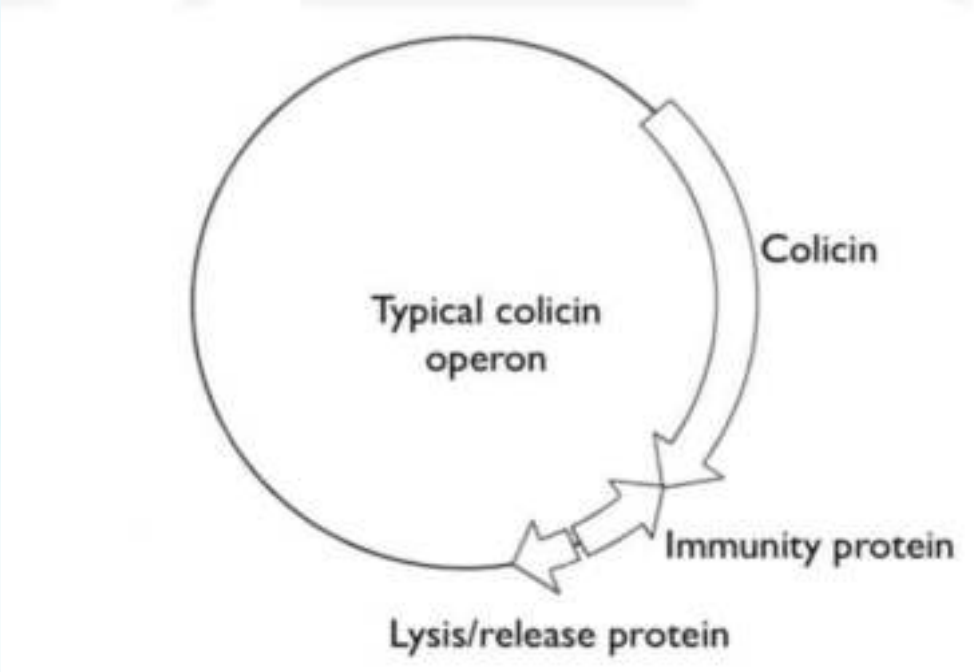
e.g. Resistance to heavy metals like Hg++, Ag++, CO++, Zn++ etc.

4. Bacteriocin or Col plasmid

1. E. coli produces **colicin** (toxin).

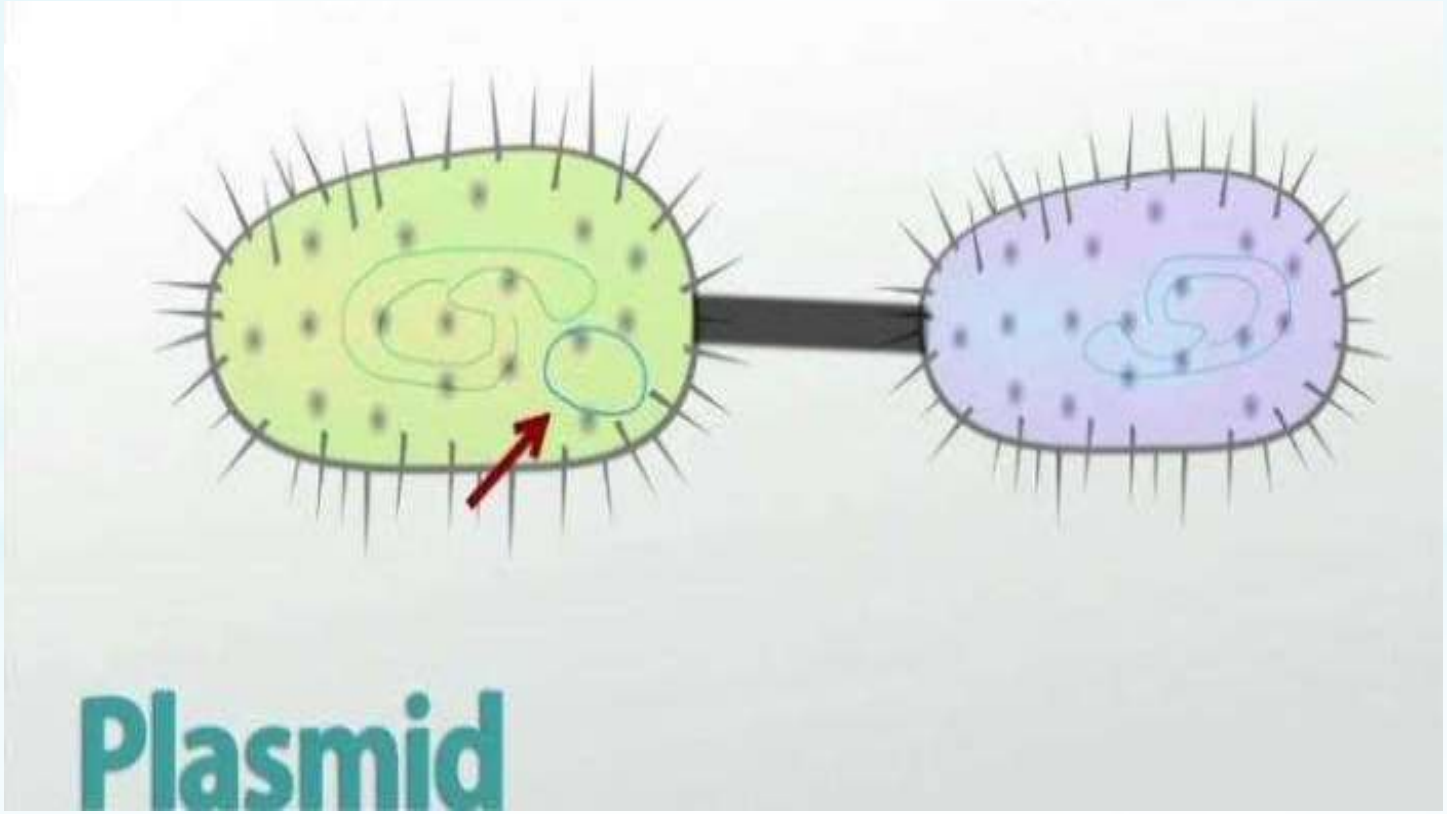
It is produced by col plasmid and it disrupts some cell functions.

2. Yersinia pestis produces **pesticin**



5. F plasmid or F factor

- Also known as Sex factor.



6. Degaradative plasmid

❖***Pseudomonas*** degrades some unusual complex organic compounds like octane, salicylate, naphthalene, xylene, toluene, camphor etc.

Contributions of different scientists in Microbiology

By-Mr. Suraj D. Gabale

Antony Van Leeuwenhoek (1632-1723)

- Delft, Holland
- Cloth merchant
- Not first to see bacteria, but first to report his observations.
- Had hobby of lens grinding and microscope making.
- Made 419 lenses and 247 compound microscopes with magnifying power of 40-270 times.

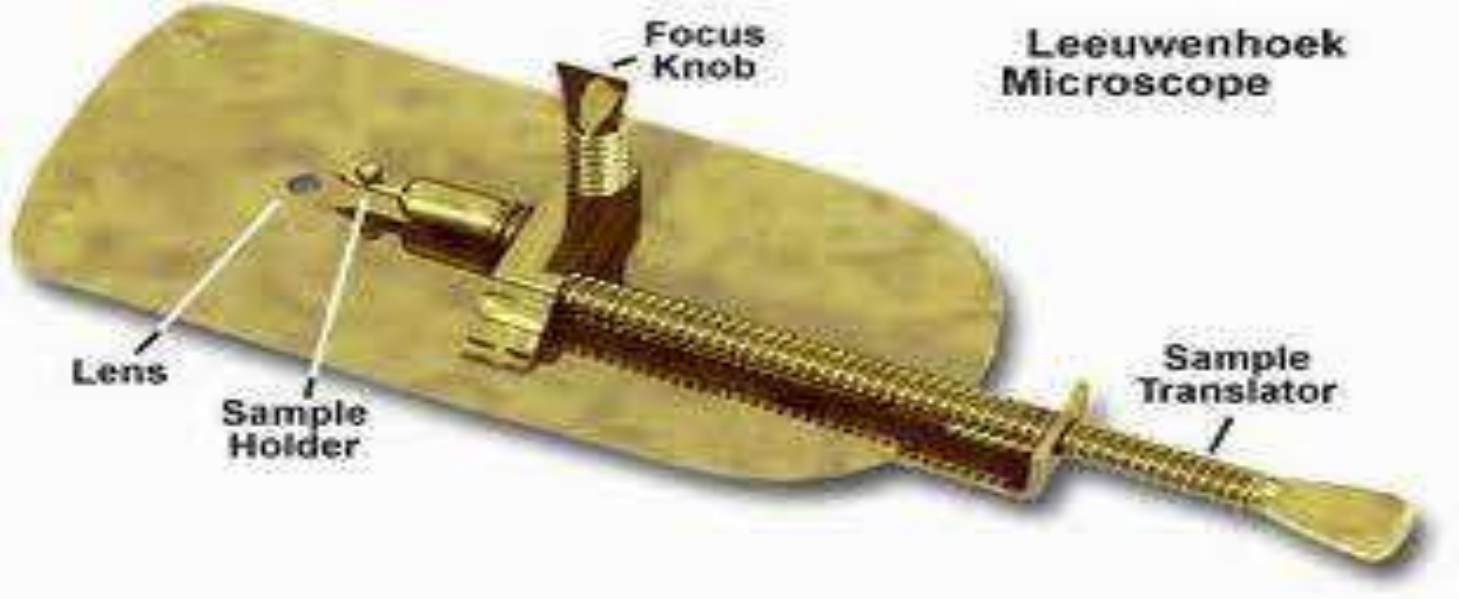
- Microscope used by him was not a compound microscope but simple magnifying glasses.
- These were spheres or bulbs of glasses. (Sometimes filled with water)
- Smaller the bulb, greater the magnification.
- Spheres/bulbs- Magnification **2-300 times**.
- This magnification was not enough to see bacteria.
- **How did he saw bacteria/ protozoa??**
- Reported his observations in series of letters to **British Royal Society**.

  * Firsst letter: Sept. 7, 1674

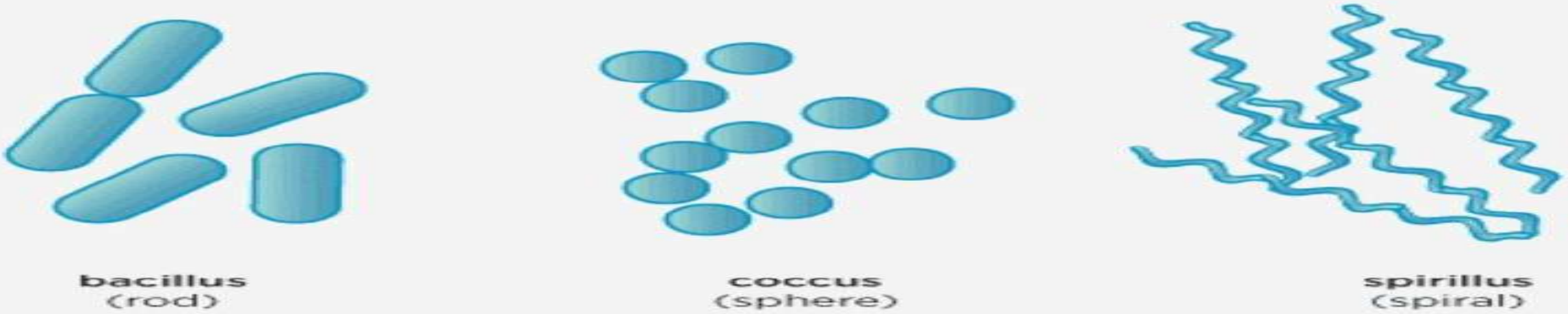
    : **“Very little Animalcules” (Living protozoa)**

  * June 16, 1675: “Many little animalcules of different sorts and sizes and motility.

- October 9, 1676**: Described Bacteria** to Henry Oldenberg, Sectretary, Royal Society.
- 1683: Sketched 3 principle shapes of bacteria: The rods, The spheres (Cocci) and the spirals.
- Death: 1793



Bacterial Shapes



bacillus
(rod)

coccus
(sphere)

spirillus
(spiral)

Edward Jenner (1749-1823)

- British physician
- 1796- Invented small pox vaccine (in Latin: Vacca means Cow)
- He observed that individuals (milkman) develops **lesion** and later on gets immunized.
- Jenner vaccinated 8 year old boy named **James phipps** with cowpox lesions on the hands of a milkmaid.
- Six weeks later, the boy was injected with pus from small pox victim.
- Jenner's smallpox vaccine reached India in 1802.

Louis Pasteur (1822-1895)

- Professor of Chemistry
- French biologist, chemist and microbiologist

1. **Solved problem of beer and wine industry in France**.
   - Sour wine problem
   - Studied methods & process involved in wine manufacturing.
   - Found that fermentation brought by microbes
   - Good product giving batches contains only one type of microbe
   - Good product giving batches contains only another type of microbe
   - Microscopic observation

Suggestion: "By selecting proper microbe, can get good product".

Undesirable microbes can be removed by heating juice-- **Pasteurization**

- During fermentation he discovered some facts:

  a) Each type of fermentation is a product of microbial activity

  b) Fermentative microbe ay be a specific bacterium or yeast.

  c) Each fermentation requires specific condition

  d) Alcohol producing agent grows well in acidic condition

  e) Lactic acid producing agent grows well in neutral medium.

2. Discovered existence of “anaerobic bacteria” during butyric acid fermentation.

3. 1864- Disproved theory of spontaneous generation by ‘ Swan necked’ flask.1865- Proved germ theory of disease

- Role of protozoa in pebrine disease of silkworm.

4. 1865-1869: Studied pebrine , a silkworm disease (France).

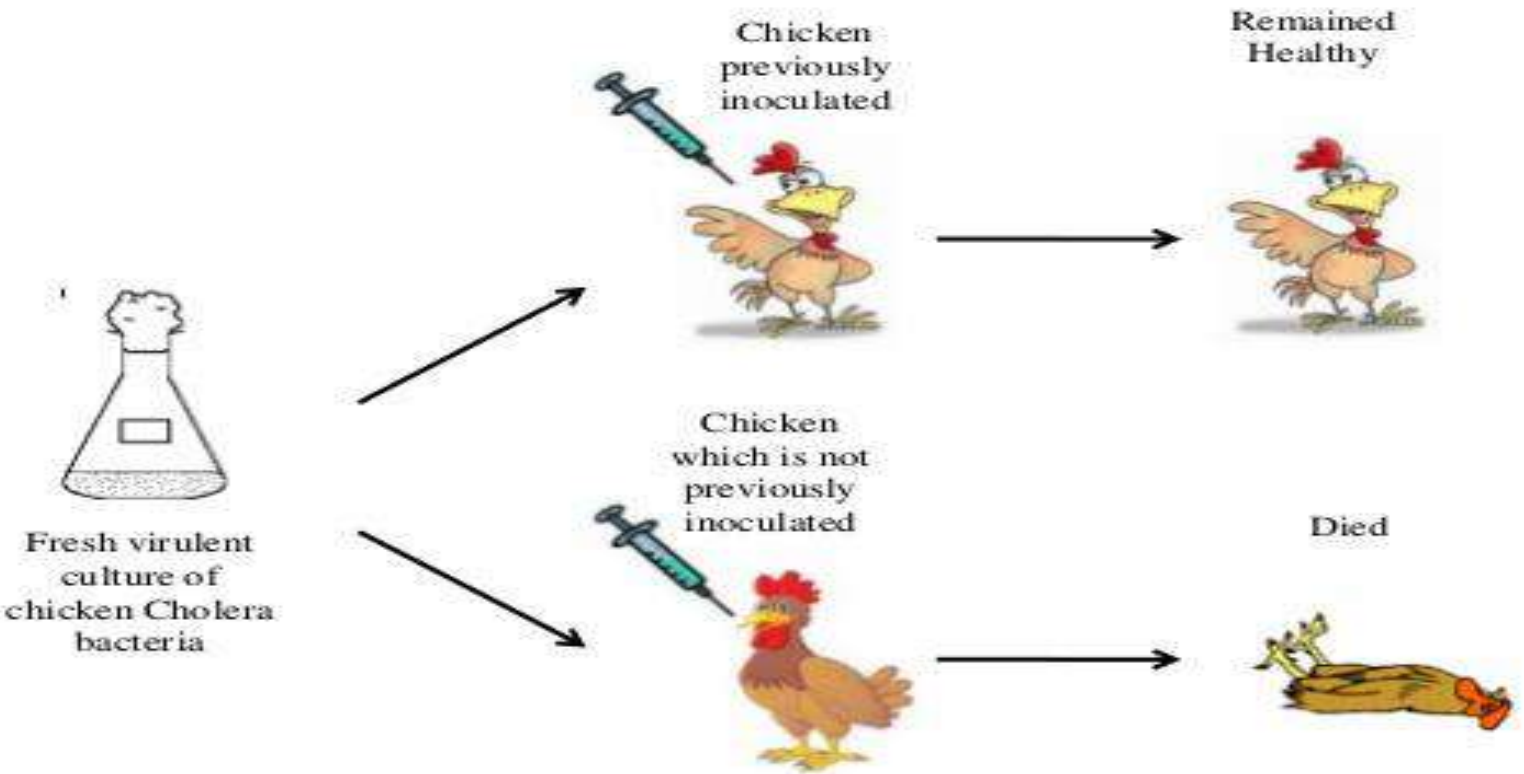
- Suggested that farmers can eliminate disease by using healthy caterpillars for breeding.

6. Discoverd principle of **immunization**.

- 1880: Isolated Pasteurella –Cholera disease

- Grew it in pure culture into chicken- Death

After several weeks…



7. 1881: Prepared Anthrax vaccine

- Anthrax bacillus attenuated by growth at 42-43 degree Celsius

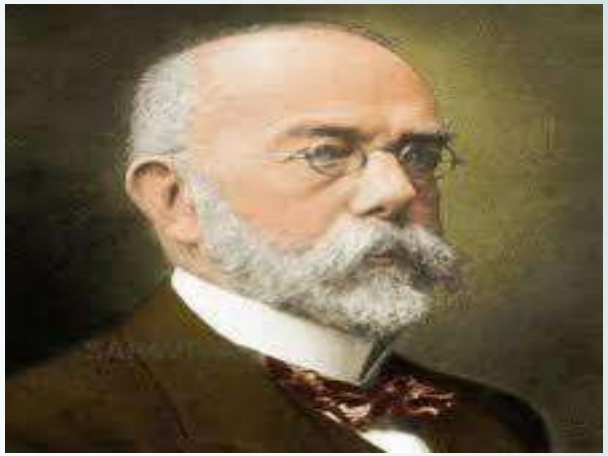
8. Prepared Rabies vaccine

- Attenuated causative agent by growing in abnormal host, rabbit.

- Brain and spinal cords removed and dried preparation usd as vaccine.

-Tried in boy named Joseph Mister bitten by wolf

Robert Koch (1843-1910)



German bacteriologist

✓ Won Nobel prize in 1905

Contributions:

1. Discovered causative agents of anthrax, tuberculosis and cholera.

- Anthrax : *Bacillus anthracis* (1876)
- Tuberculosis : *Mycobacterium tuberculosis* (1882)
- Cholera : *Vibrio cholerae* (1883)

2. Invented **Streak plate technique** ad used gelatin as solidifying agent.

3. **Sporulation** and **germination of endospore,** their thermo resistance and appearance were independently described by **Cohn** anh **Koch**, 1877.

Koch's Postulates

Evidence required to establish etiologic relationship between microorganism and disease:

1. **Microorganism must be observed in every case of the disease**
2. **It must be isolated and grown in pure culture**
3. **The pure culture, when inoculated in animals, must reproduce the disease**
4. **Microorganism must be recovered from the diseased animal**

4. Used **meat infusions** and **meat extract** as the basic ingredient in culture medium.

5. **Nutrient agar** and **Nutrient broth** were are most widely used media in general work, were outcome of Koch's experiment.

6. Developed methods of studying bacteria with microscope.

-Smear on glass slide

- Heat fixed

- Stained

- Observed

Dmitrii Ivanowski (1864-1920)

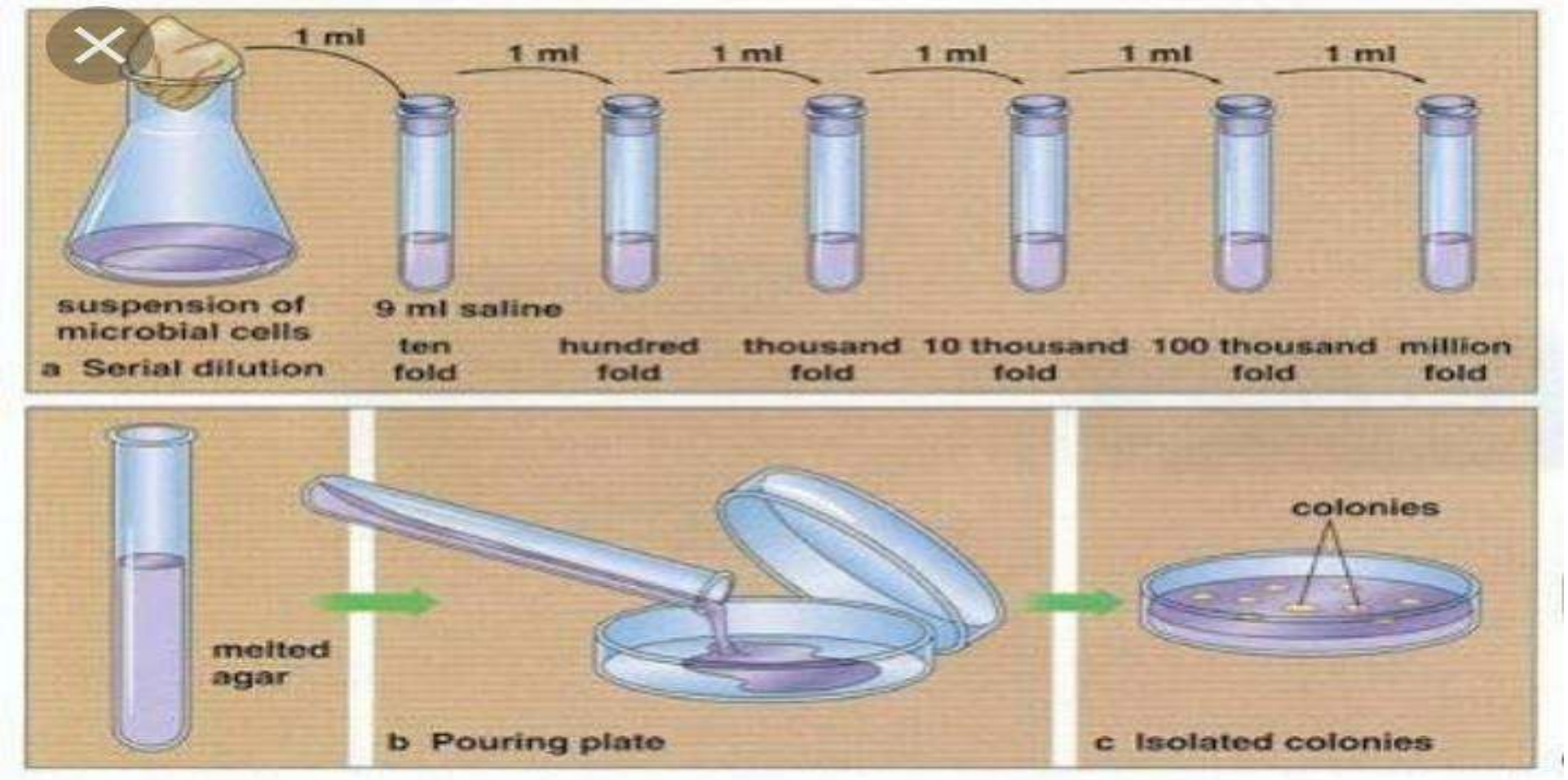
- 1892- Discovered that causative agent of tobacco mosaic disease was filterable.
- Obtained extracts from diseased tobacco leaves and filtered through bacteria proof filter.
- By using filtrate he produced in healthy tobacco plant.
- **This filterable agent was later named as Virus**

Joseph Lister (1827-1912)

- English surgeon
- Developed **antiseptic surgery** and **pure culture technique**.

1. 1867- Used carbolic acid (phenol) to reduce infection of surgical incisions and surgical wounds.
   - Carbolic acid- Disinfectant
   - Wound treated with carbolic acid healed rapidly
   - Later developed practice of spraying phenol in operating room to control infection.
   - Used heat to sterilize surgical instruments
   - Coined the term 'Antiseptic surgery'.

2. 1878: 1st to obtain pure culture of bacteria using serial dilutions in liquid media.



Alexander Fleming (1881-1955)

- Scottish physician
- **Discovered Penicillin antibiotic.**
- 1945 – Won Nobel prize in medicine and physiology along with Chain and Florey

- **Discovery of penicillin:**

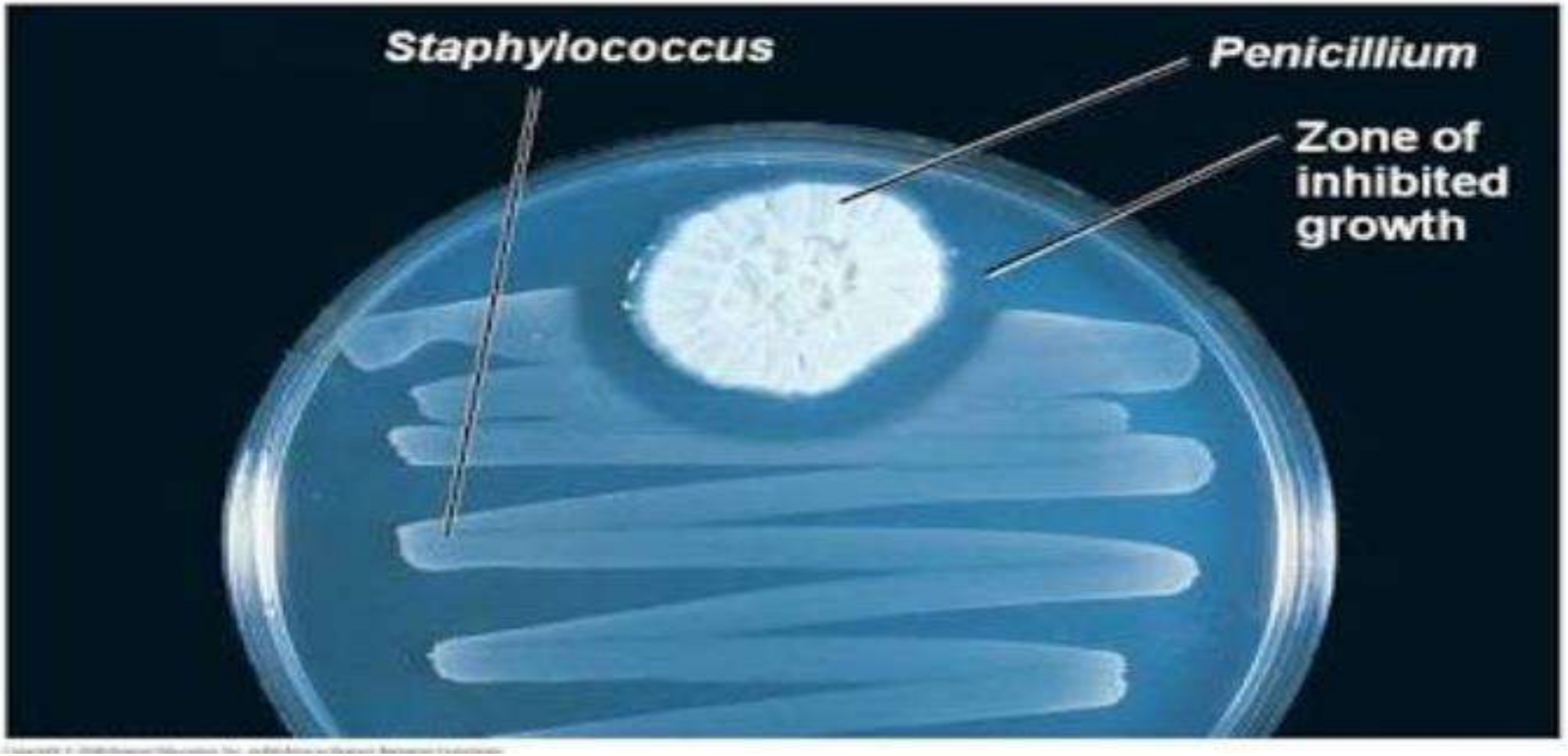
  - Contaminated colony of mold in *Staphylococcus aureus* plate lysed adjacent colony of stahylococci.

  - Named this fungi as '*Penicillium* species'

  - Demonstrated that penicillin has low toxicity to man and animal.

- **Discovered Lysozyme enzyme.**
- **Discovered antibiotic resistance-**

  Very little antibiotic or exposure for short duration.


Staphylococcus
Penicillium
Zone of inhibited growth

Martinus Beijerinck (1851-1931)

- Dutch microbiologist
- 1888- Isolated bacteria now known as *Rhizobium leguminosarum* from root nodules of peas.
- 1890- Introduced **enrichment culture technique** for isolation of bacteria.
- 1898- Confirmed filterable nature of tobacco mosaic virus.
- 1901- Isolated **non-symbiotic nitrogen fixer *Azotobacter chrococcum*** and describe its usefulness in promoting soil fertility.
- 1903- Isolated **anaerobic, spore forming nitrogen fixer**, which he called *Granulobacter butylicum* (Now *Clostridium butylicum*)

Sergius Winogradsky (1856-1953)

➢Russian microbiologist

1. Discovered **Chemoutotrophs** in soil

- **Chemoautotrophs:**

  - Obtain energy from oxidation of reduced inorganic N, S or Fe and use CO_2 as source of carbon.

  - Sulfur bacteria- ***Thiobacillus*** species

  - Nitrifying bacteria- **Nitrobacter** species

2.1890- Applied enrichment culture technique for isolation of chemoautotrophs from soil

3. 1895- Isolated anaerobic nitrogen fixing bacteria ***Clostridium pasteurianum.***

4. Studied decomposition of cellulose in soil.

5. Showed importance of nitrogen fixation for plants.

Har Gobind Khorana (1922-2011)

- Indian born biochemist
- Early work on biochemistry of enzymes
- 1960's- Study of nucleic acid and genetic code

1) Determined sequence of nucleic acids for each of amino acids

Ex.- UCU- Serine, AUG- Methionine ACU- Threonine

- **Described relationship between sequence of codon in mRNA and sequence of amino acids proteins**
- Recieved Nobel prize in medicine and physiology (1968)

2) 1970: First to synthesize **artificial gene** by using polymerase and ligase enzymes.

This was the basis of polymerase chain reaction.

First artificial gene was **yeast tRNA**

3) 1980's: Described biochemistry of **Bacteriorhodopsin,**

Bacteriorhodopsin- Membrane protein that convert light energy into chemical energy

4) 1955: Synthesized **Co-enzyme A**

5) Describd direction of mRNA read during protein synthesis

6) **Characteritics of genetic codon:**

Non-overlaping, commaless, polarity

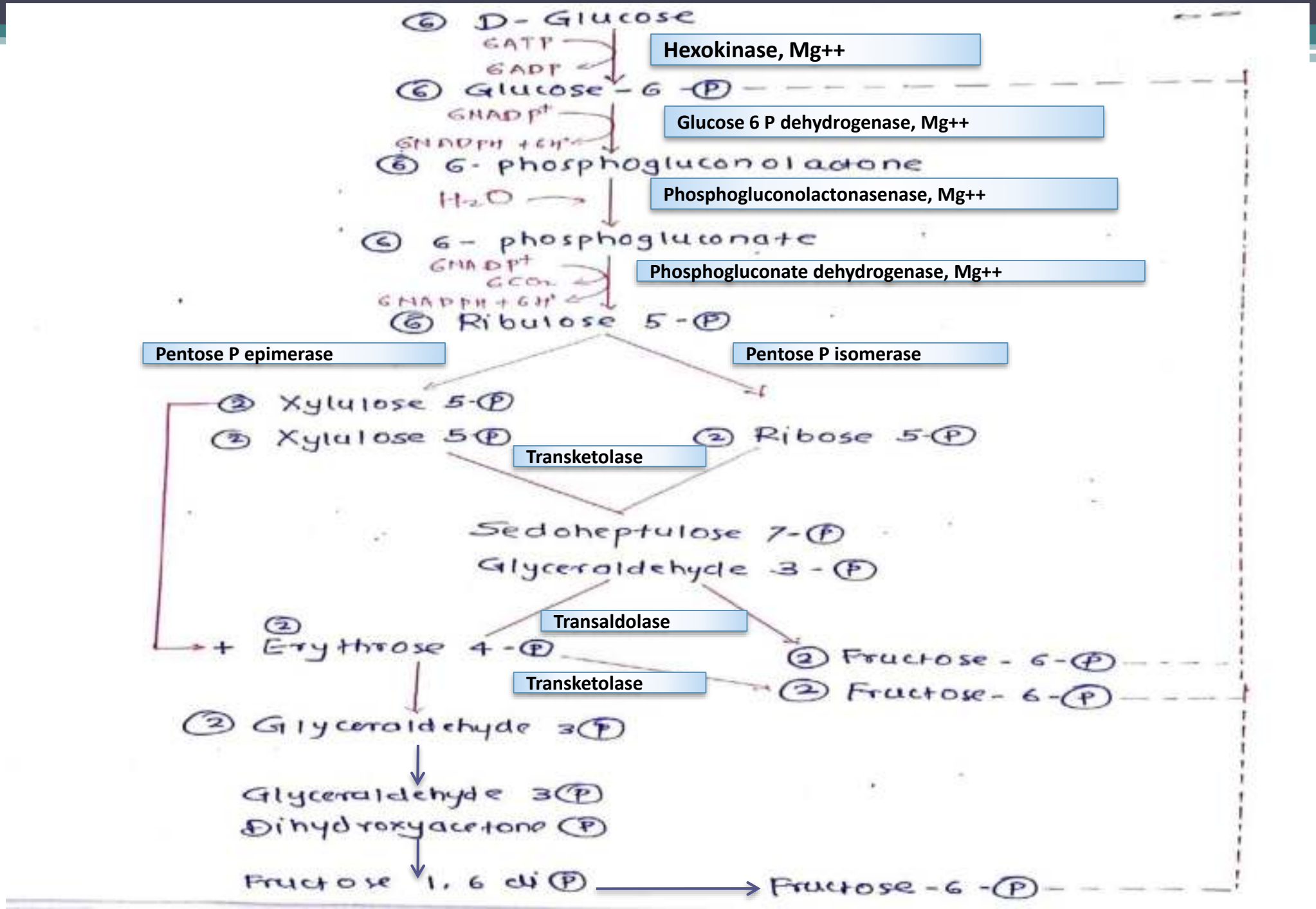
HMP Pathway

Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

HMP pathway

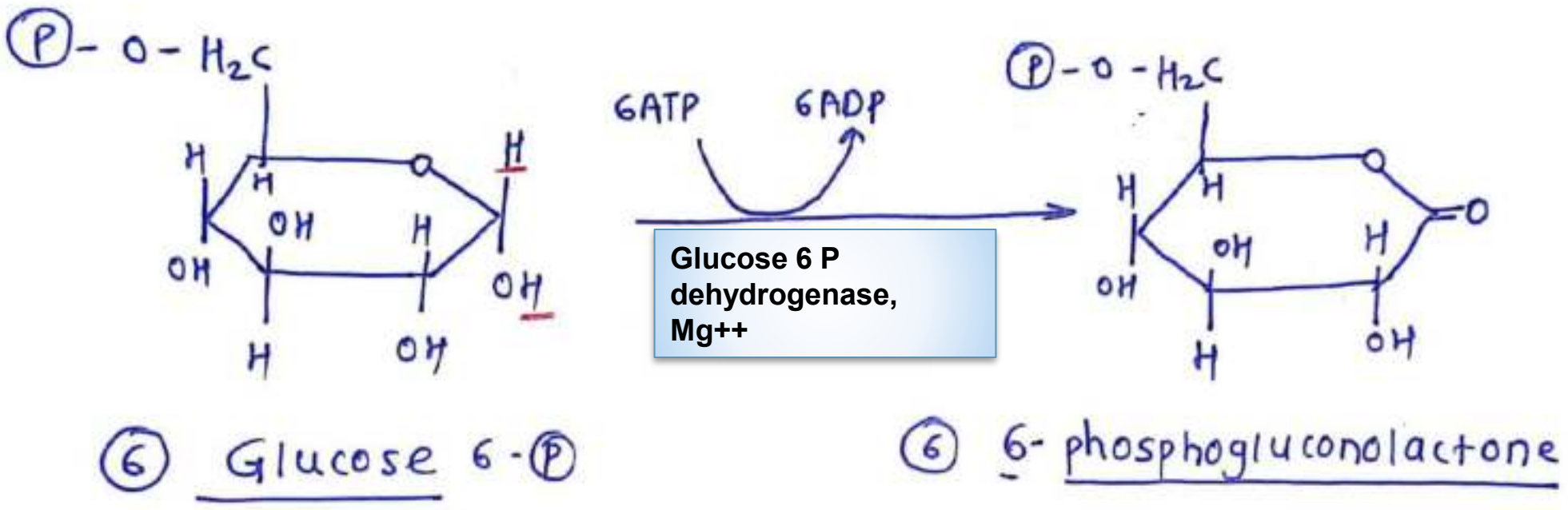
- **HMP- Hexose monophosphate pathway**
- **PPP- Pentose phosphate pathway**
- Also known as **Warberg-Dickens pathway** or **Phosphogluconate pathway.**
- It is an alternative route for metabolism of glucose.
- Does not forms ATP but has functions like:
    a. Formation of NADPH
    b. Synthesis of ribose sugar for nucleic acid and nucleotides

- It meets need of all organisms for a source of NADPH.
- Also called '**shunt pathway**'.
- Reactions occurs in cytoplasm.


D- Glucose
ATP
ADP
Hexokinase, Mg++
Glucose - 6 - P
NADP+
NADPH + H+
Glucose 6 P dehydrogenase, Mg++
6- phosphogluconolactone
H2O
Phosphogluconolactonasenase, Mg++
6 - phosphogluconate
NADP+
CO2
NADPH + H+
Phosphogluconate dehydrogenase, Mg++
Ribulose 5 - P
Pentose P epimerase
Pentose P isomerase
Xylulose 5-P
Xylulose 5-P
Ribose 5-P
Transketolase
Sedoheptulose 7-P
Glyceraldehyde 3 - P
Transaldolase
Erythrose 4 - P
Fructose - 6 - P
Transketolase
Fructose - 6 - P
Glyceraldehyde 3 P
Glyceraldehyde 3 P
Dihydroxyacetone P
Fructose 1, 6 di P
Fructose - 6 - P

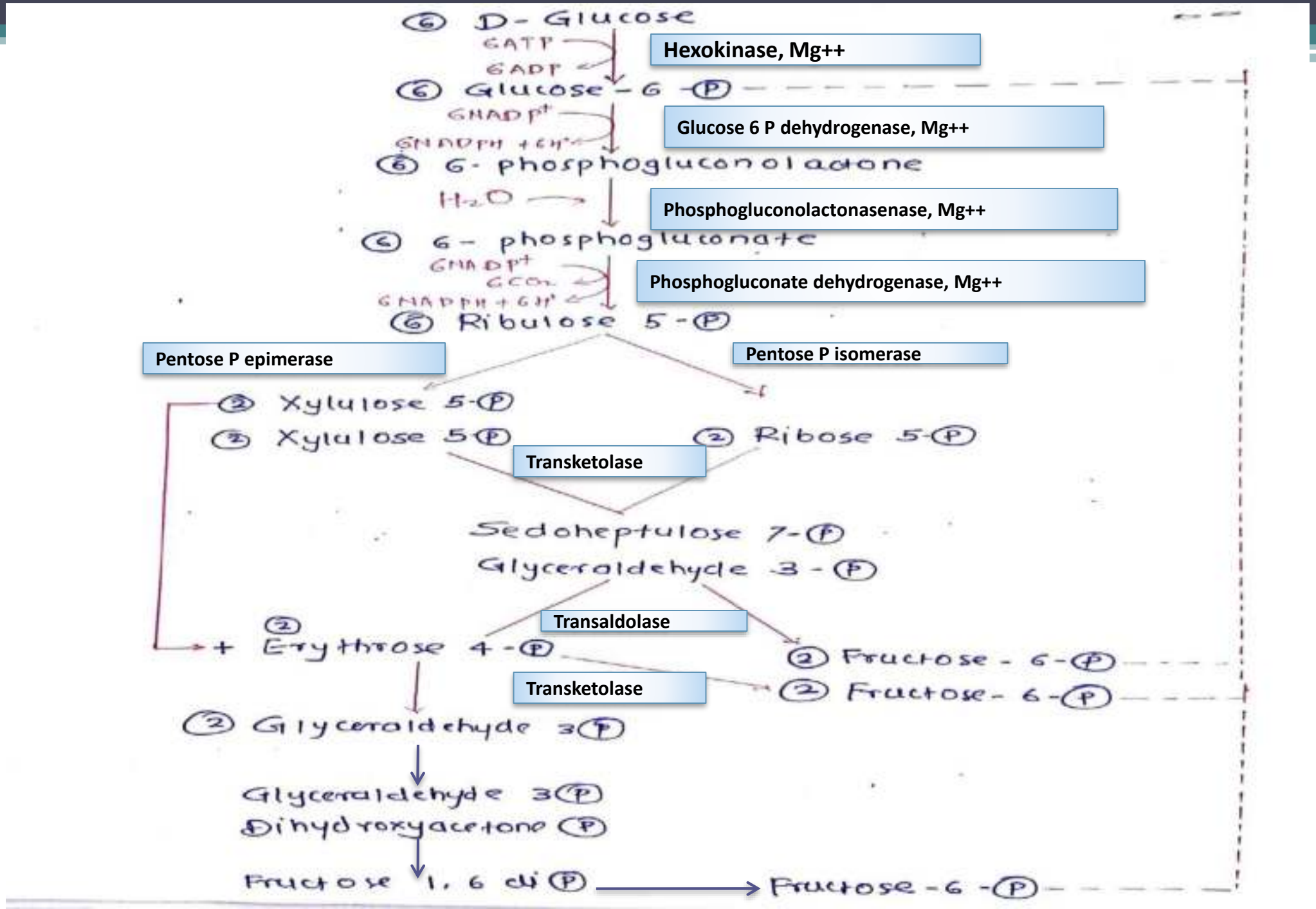
Steps in HMP pathway

1. Dehydrogenation of Glucose 6-phosphare

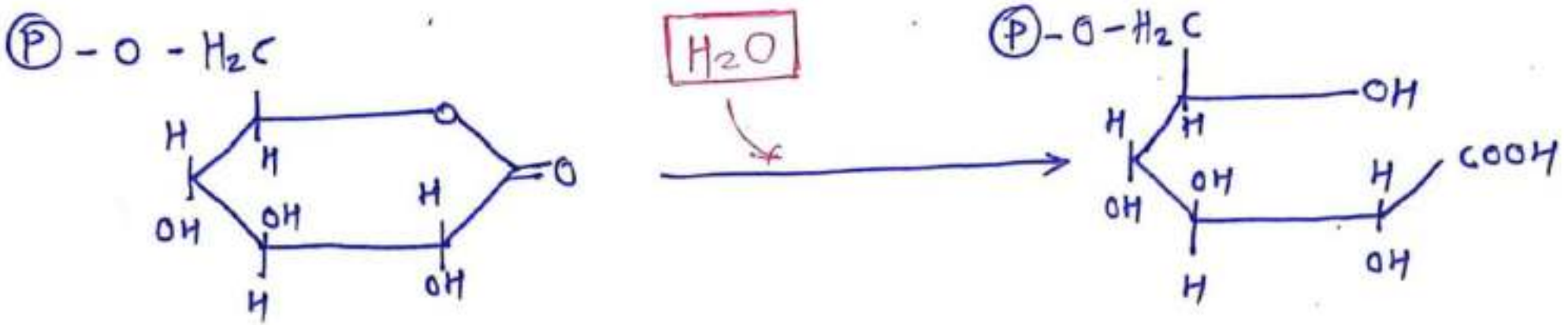


⑥ Glucose 6-Ⓟ

⑥ 6-phosphogluconolactone

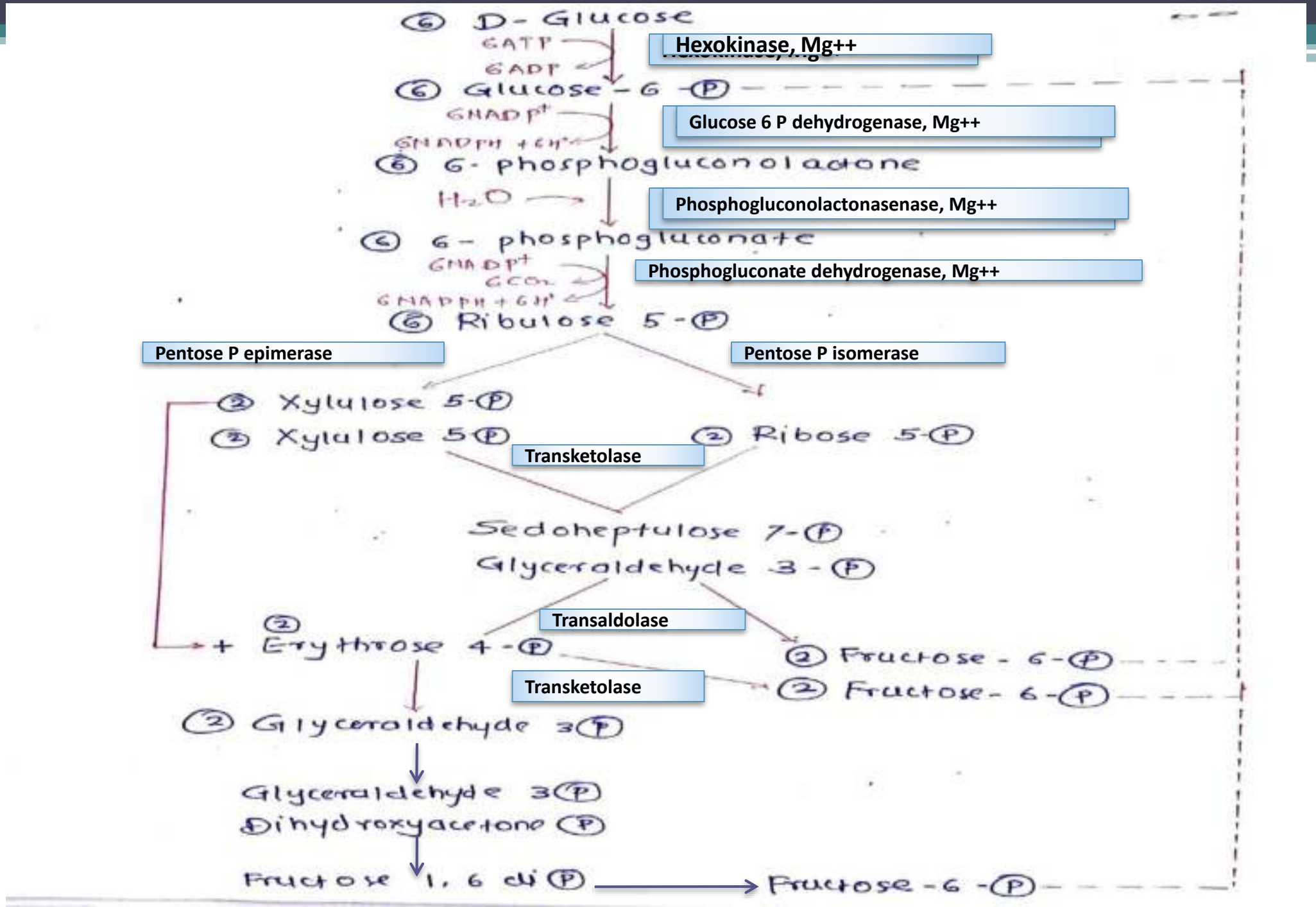

D- Glucose
ATP
ADP
Hexokinase, Mg++
Glucose - 6 - P
NADP+
NADPH + H+
Glucose 6 P dehydrogenase, Mg++
6- phosphogluconolactone
H2O
Phosphogluconolactonasenase, Mg++
6 - phosphogluconate
NADP+
CO2
NADPH + H+
Phosphogluconate dehydrogenase, Mg++
Ribulose 5 - P
Pentose P epimerase
Pentose P isomerase
Xylulose 5-P
Xylulose 5 P
Ribose 5-P
Transketolase
Sedoheptulose 7-P
Glyceraldehyde 3 - P
Transaldolase
Erythrose 4 - P
Fructose - 6 - P
Transketolase
Fructose - 6 - P
Glyceraldehyde 3 P
Glyceraldehyde 3 P
Dihydroxyacetone P
Fructose 1, 6 di P
Fructose - 6 - P

2. Hydrolysis of 6 phospho-gluconoloactone:

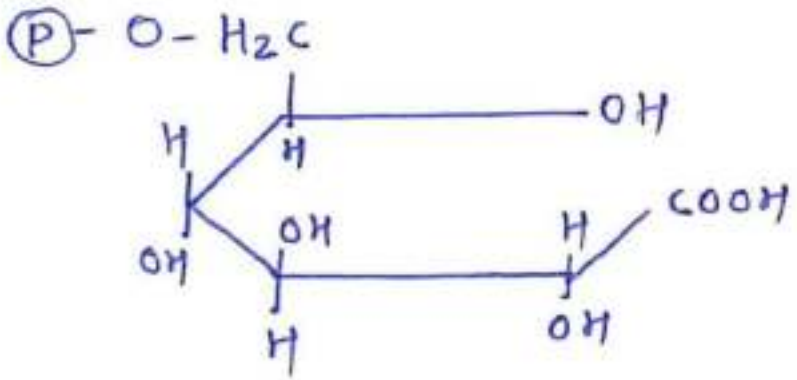


⑥ 6 phosphogluconolactone

⑥ 6-phosphogluconate


D- Glucose
ATP
ADP
Hexokinase, Mg++
Glucose - 6 - P
NADP+
NADPH + H+
Glucose 6 P dehydrogenase, Mg++
6- phosphogluconolactone
H2O
Phosphogluconolactonasenase, Mg++
6 - phosphogluconate
NADP+
CO2
NADPH + H+
Phosphogluconate dehydrogenase, Mg++
Ribulose 5 - P
Pentose P epimerase
Pentose P isomerase
Xylulose 5-P
Xylulose 5 P
Ribose 5-P
Transketolase
Sedoheptulose 7-P
Glyceraldehyde 3 - P
Transaldolase
Erythrose 4 - P
Fructose - 6 - P
Transketolase
Fructose - 6 - P
Glyceraldehyde 3 P
Glyceraldehyde 3 P
Dihydroxyacetone P
Fructose 1, 6 di P
Fructose - 6 - P

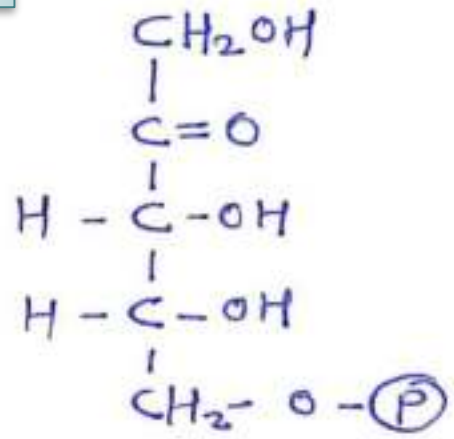
2. Oxidative decarboxylation of phosphogluconate:



6 NADP+

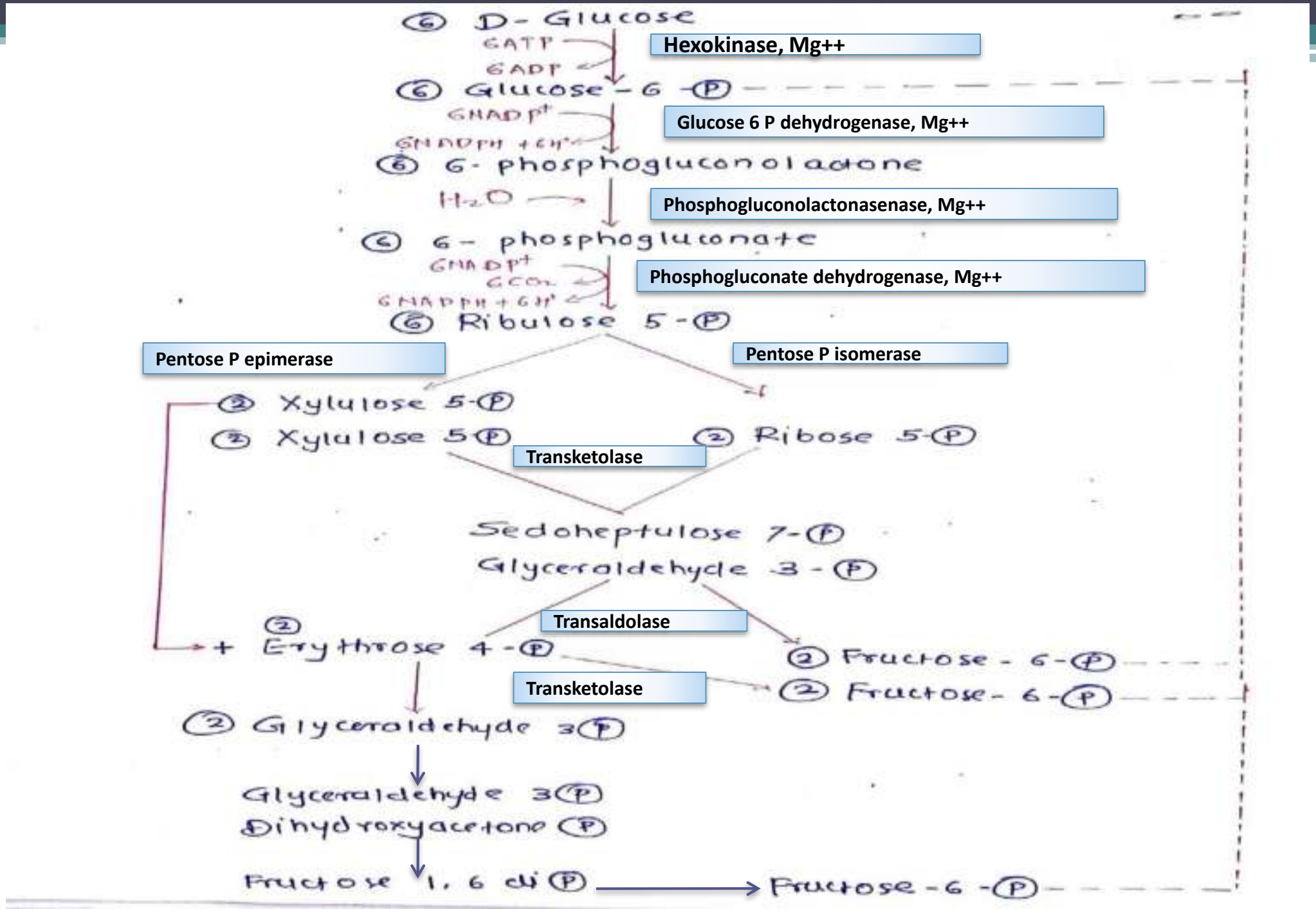
6 NADPH + H+

CO2

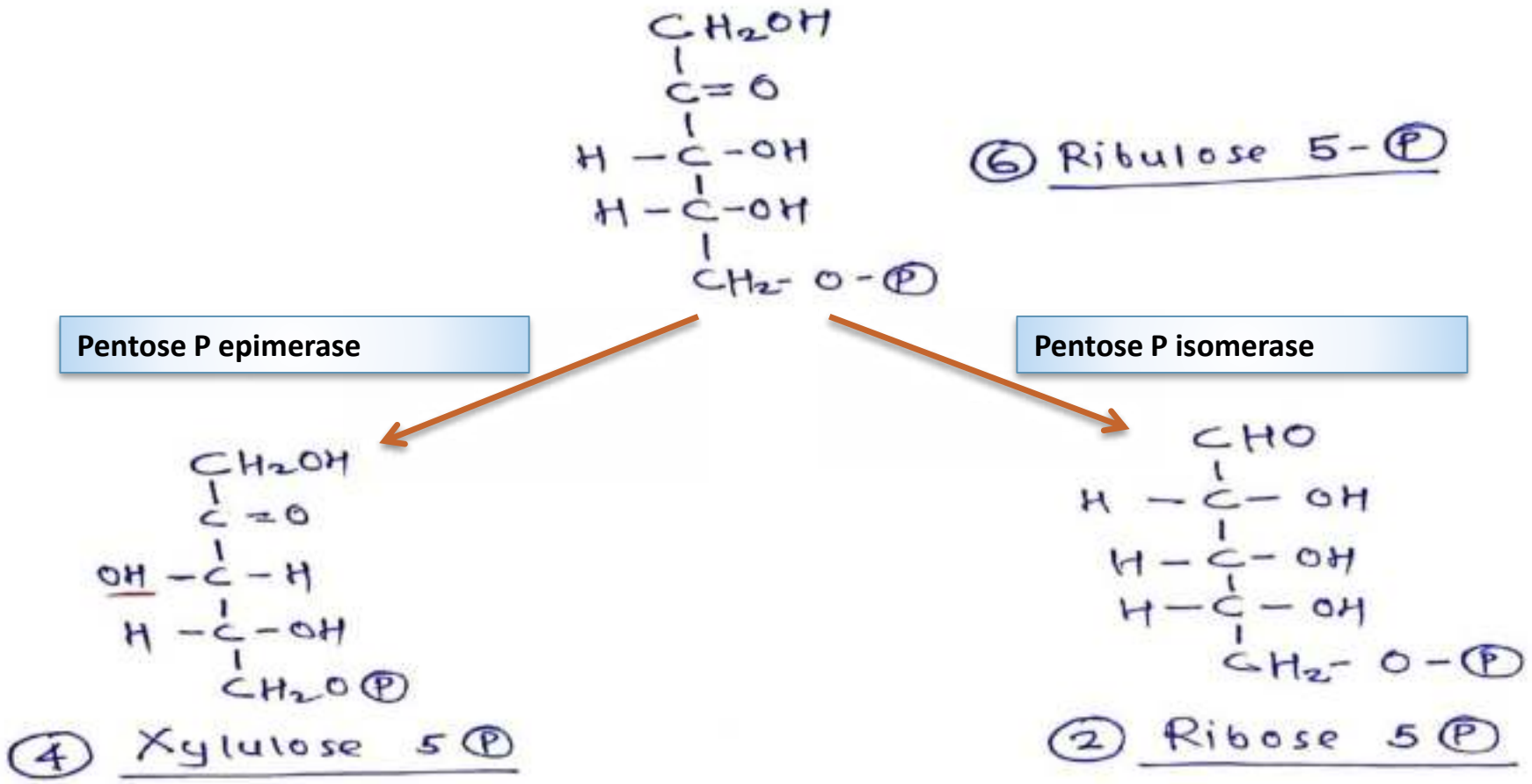


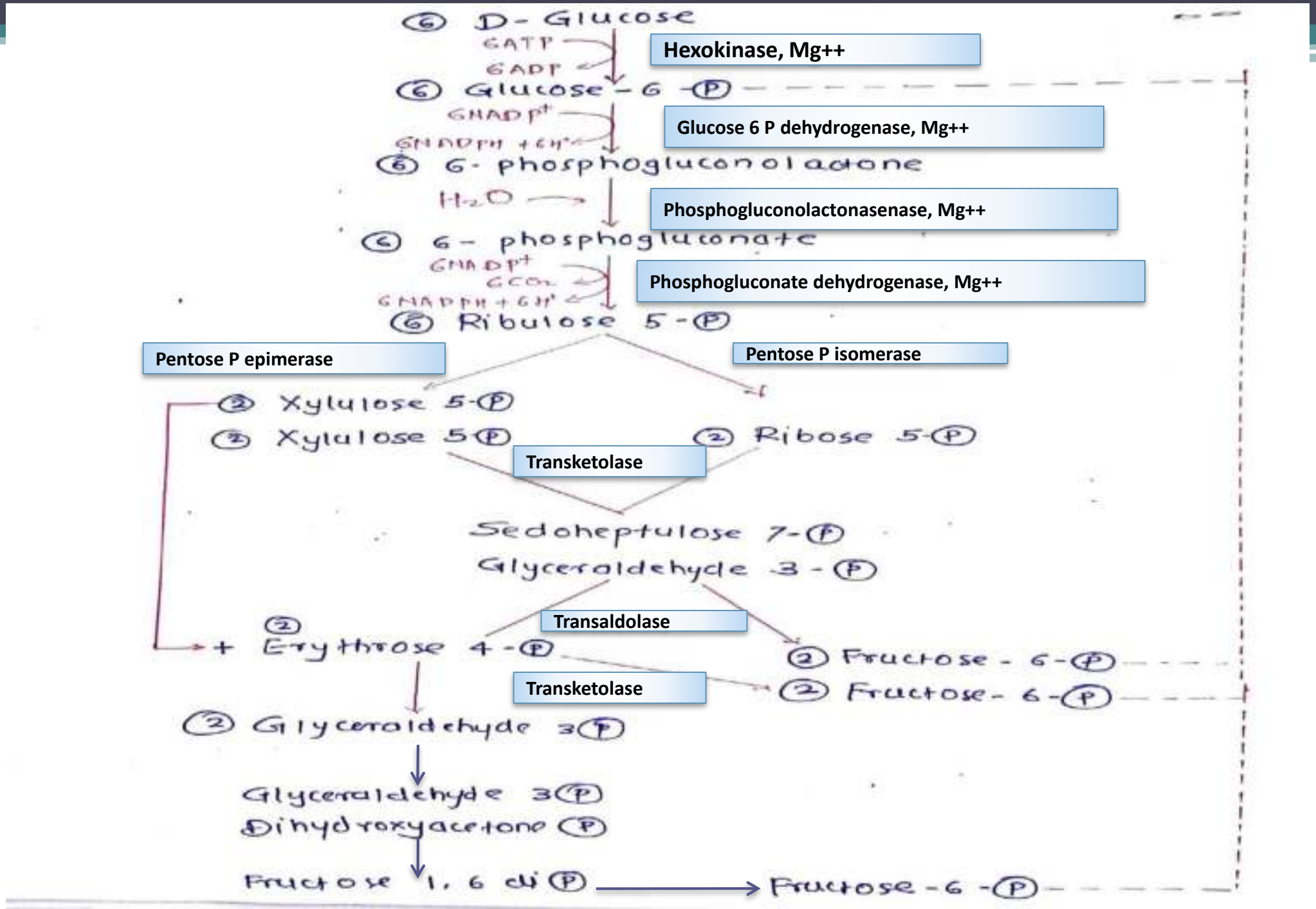
(6) 6-phosphogluconate

(6) Ribulose - 5 - P

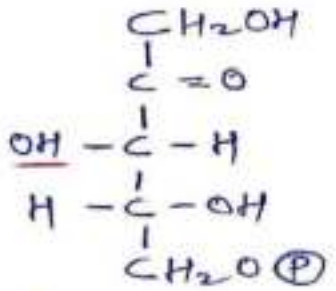

D- Glucose
ATP
ADP
Hexokinase, Mg++
Glucose - 6 - P
NADP+
NADPH + H+
Glucose 6 P dehydrogenase, Mg++
6- phosphogluconolactone
H2O
Phosphogluconolactonasenase, Mg++
6 - phosphogluconate
NADP+
CO2
NADPH + H+
Phosphogluconate dehydrogenase, Mg++
Ribulose 5 - P
Pentose P epimerase
Pentose P isomerase
Xylulose 5-P
Xylulose 5 P
Ribose 5-P
Transketolase
Sedoheptulose 7-P
Glyceraldehyde 3 - P
Transaldolase
Erythrose 4 - P
Fructose - 6 - P
Transketolase
Fructose - 6 - P
Glyceraldehyde 3 P
Glyceraldehyde 3 P
Dihydroxyacetone P
Fructose 1, 6 di P
Fructose - 6 - P

4. Isomerization and epimerization of Ribulose 5 phosphate:

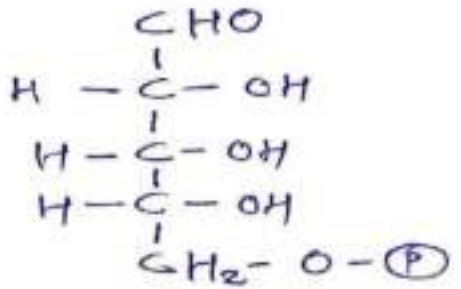



D- Glucose
ATP
ADP
Hexokinase, Mg++
Glucose - 6 - P
NADP+
NADPH + H+
Glucose 6 P dehydrogenase, Mg++
6- phosphogluconolactone
H2O
Phosphogluconolactonasenase, Mg++
6 - phosphogluconate
NADP+
CO2
NADPH + H+
Phosphogluconate dehydrogenase, Mg++
Ribulose 5 - P
Pentose P epimerase
Pentose P isomerase
Xylulose 5-P
Xylulose 5 P
Ribose 5-P
Transketolase
Sedoheptulose 7-P
Glyceraldehyde 3 - P
Transaldolase
Erythrose 4 - P
Fructose - 6 - P
Transketolase
Fructose - 6 - P
Glyceraldehyde 3 P
Glyceraldehyde 3 P
Dihydroxyacetone P
Fructose 1, 6 di P
Fructose - 6 - P

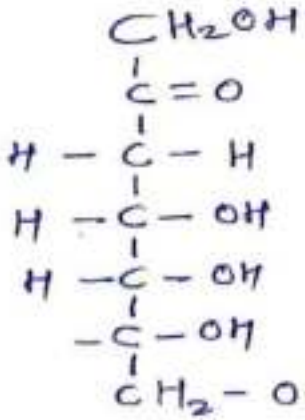
5. Transfer of ketose group from xylulose to ribose



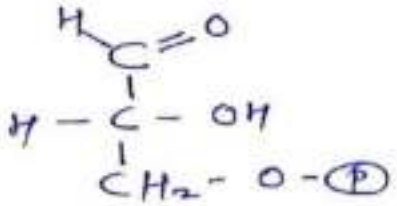
④ Xylulose 5 Ⓟ



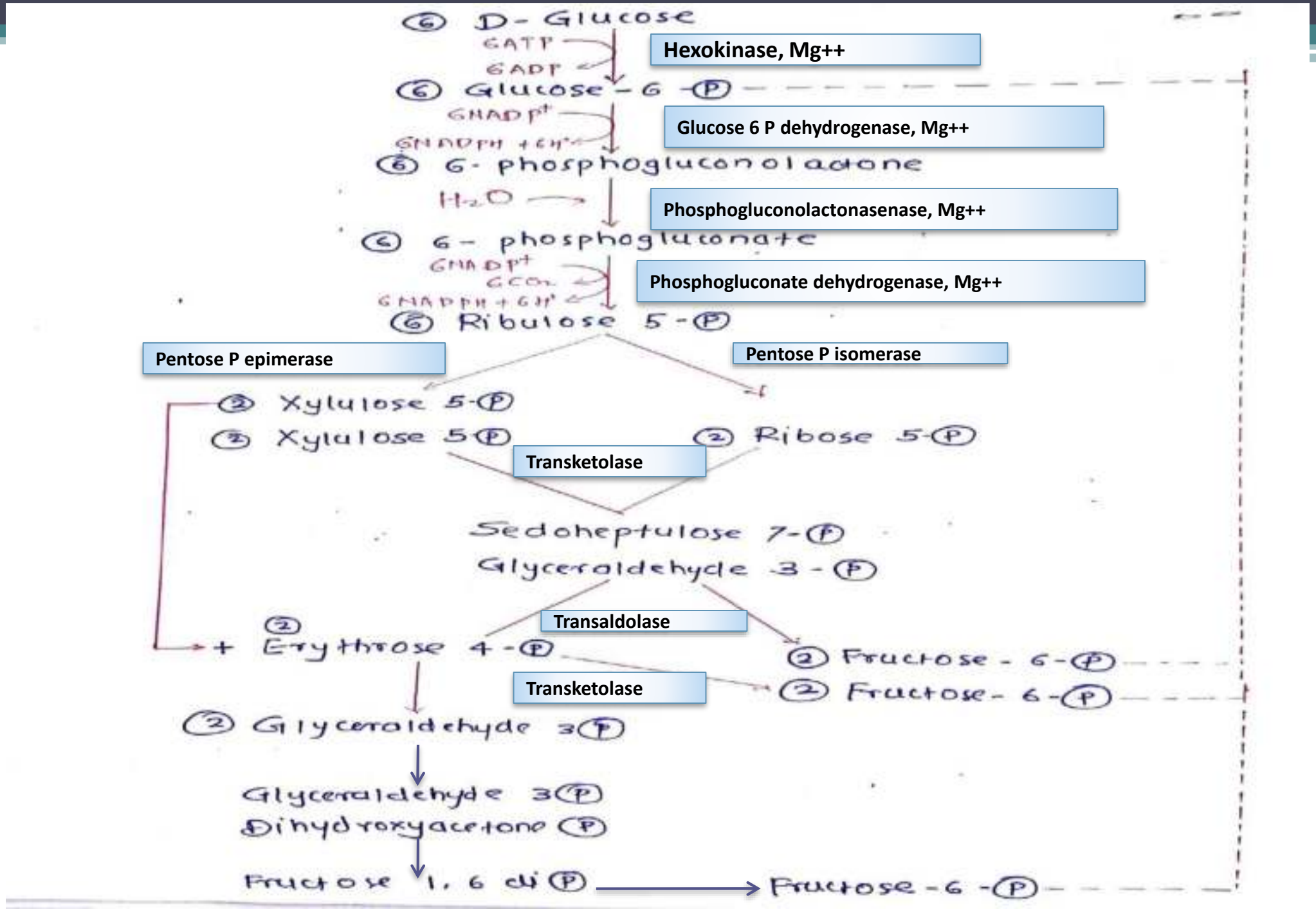
② Ribose 5 Ⓟ



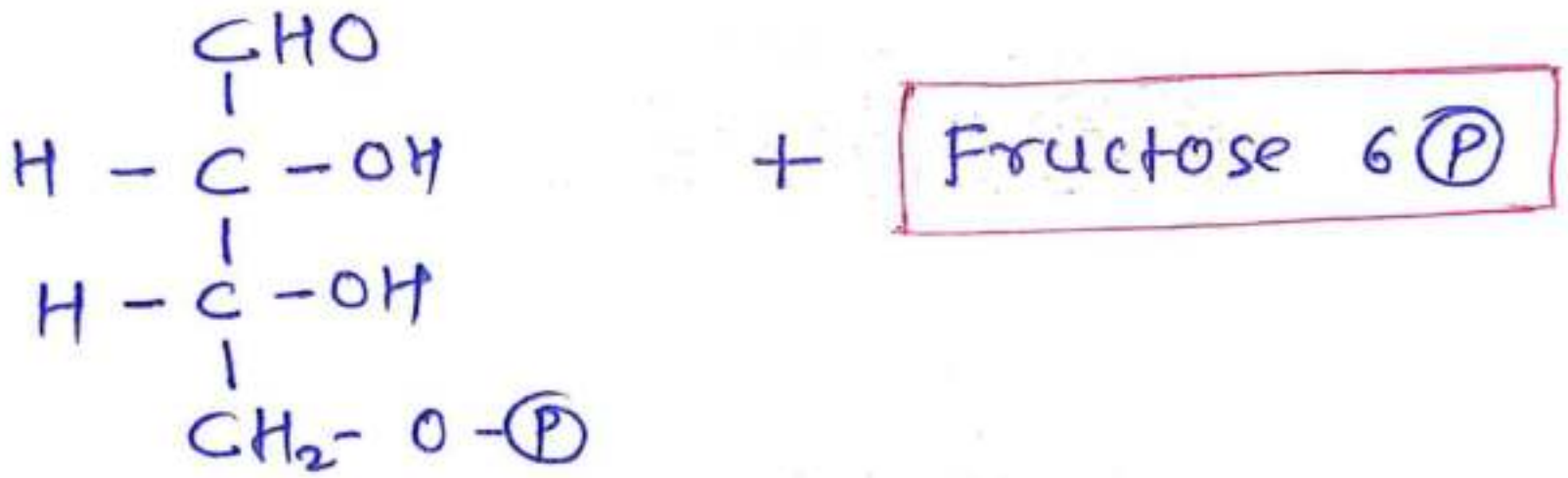
② Sedoheptulose - 7 Ⓟ

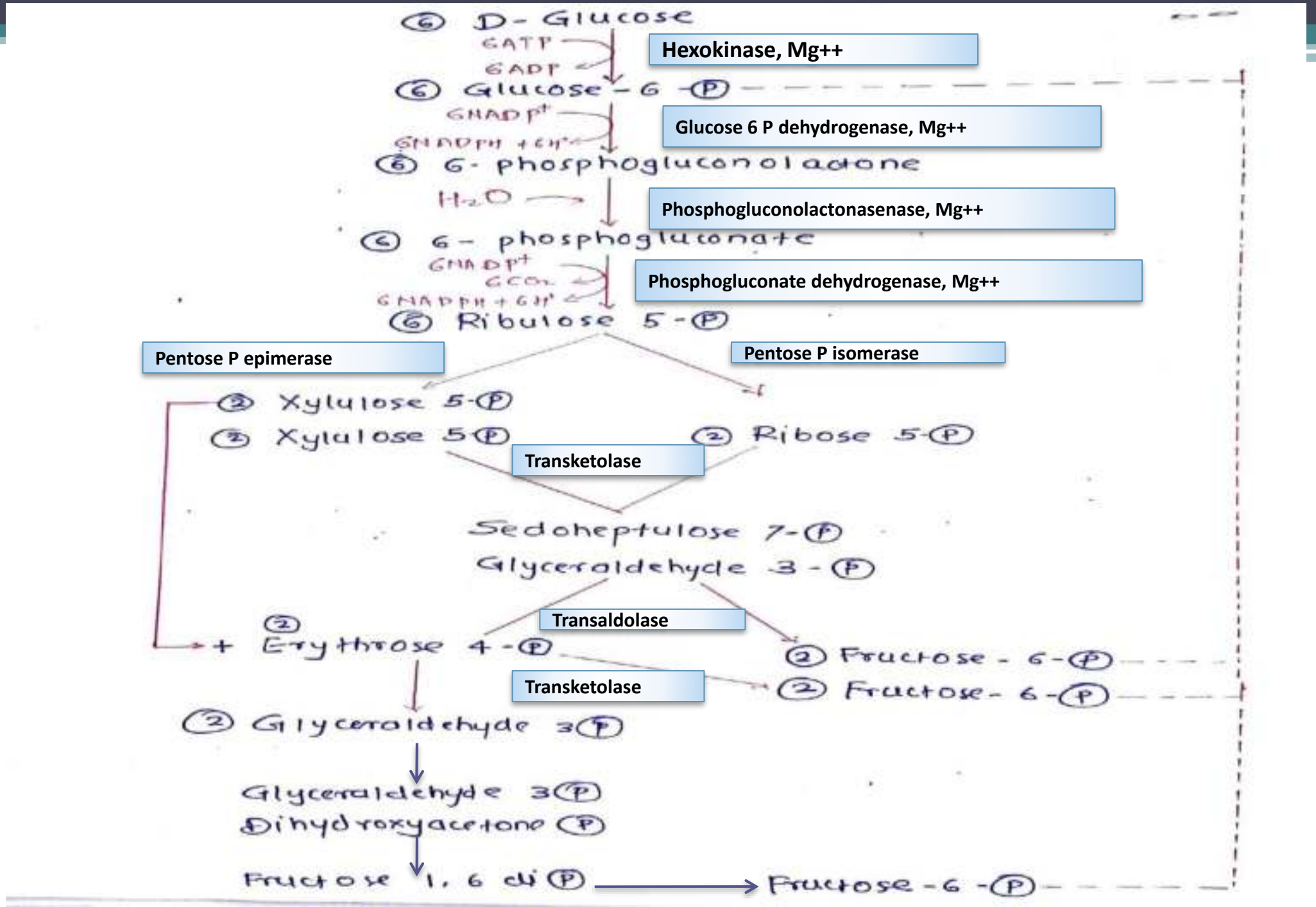


② Glyceraldehyde 3 Ⓟ


D- Glucose
ATP
ADP
Hexokinase, Mg++
Glucose - 6 - P
NADP+
NADPH + H+
Glucose 6 P dehydrogenase, Mg++
6- phosphogluconolactone
H2O
Phosphogluconolactonasenase, Mg++
6 - phosphogluconate
NADP+
CO2
NADPH + H+
Phosphogluconate dehydrogenase, Mg++
Ribulose 5 - P
Pentose P epimerase
Pentose P isomerase
Xylulose 5-P
Xylulose 5 P
Ribose 5-P
Transketolase
Sedoheptulose 7-P
Glyceraldehyde 3 - P
Transaldolase
Erythrose 4 - P
Fructose - 6 - P
Transketolase
Fructose - 6 - P
Glyceraldehyde 3 P
Glyceraldehyde 3 P
Dihydroxyacetone P
Fructose 1, 6 di P
Fructose - 6 - P

6. Formation of fructose 6 phosphate from sedoheptulose 7 phophate



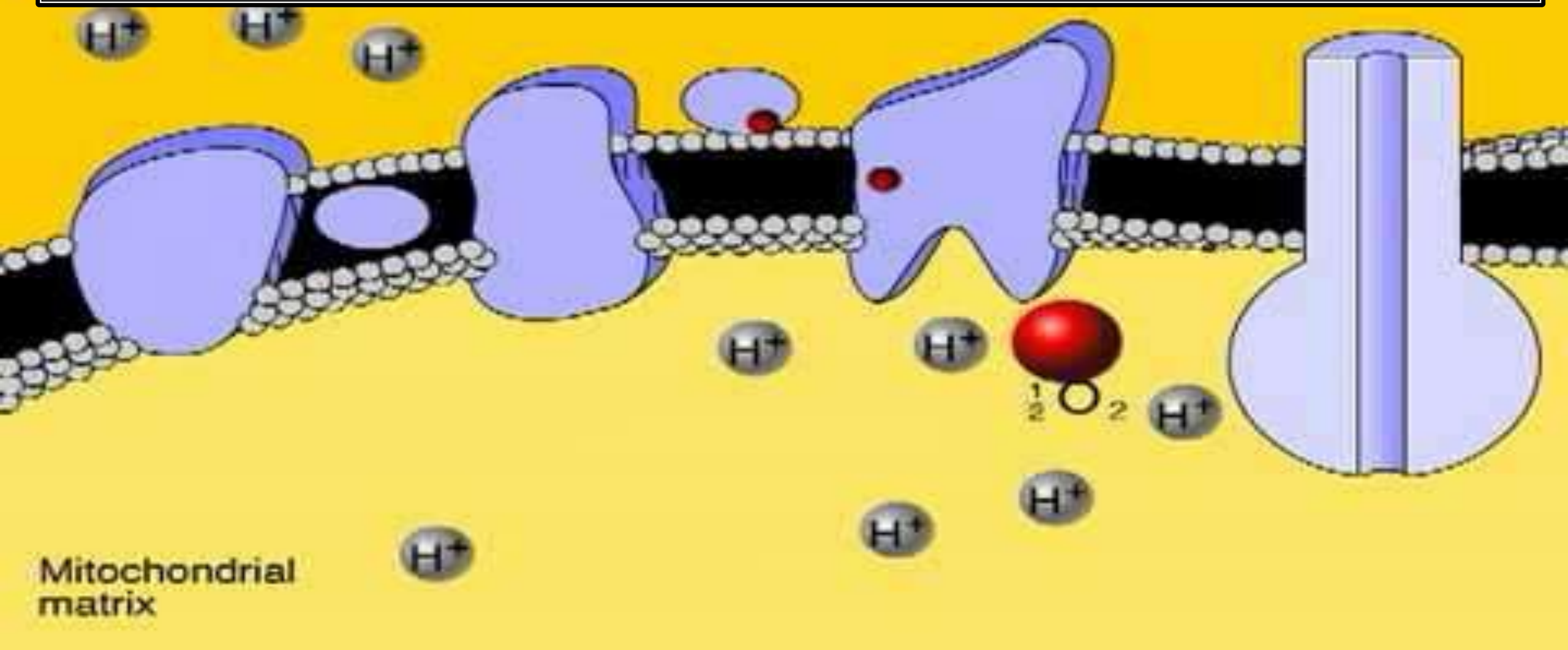

D- Glucose
ATP
ADP
Hexokinase, Mg++
Glucose - 6 - P
NADP+
NADPH + H+
Glucose 6 P dehydrogenase, Mg++
6- phosphogluconolactone
H2O
Phosphogluconolactonasenase, Mg++
6 - phosphogluconate
NADP+
CO2
NADPH + H+
Phosphogluconate dehydrogenase, Mg++
Ribulose 5 - P
Pentose P epimerase
Pentose P isomerase
Xylulose 5-P
Xylulose 5 P
Ribose 5-P
Transketolase
Sedoheptulose 7-P
Glyceraldehyde 3 - P
Transaldolase
Erythrose 4 - P
Fructose - 6 - P
Transketolase
Fructose - 6 - P
Glyceraldehyde 3 P
Glyceraldehyde 3 P
Dihydroxyacetone P
Fructose 1, 6 di P
Fructose - 6 - P

Significance of HMP pathway

1. Provides pentose rapidly dividing cells for synthesis of DNA, RNA and coenzymes as ATP, NADH, FADH2 etc.
2. Provides reducing power as NADH which id needed for synthesis of fatty acids, steroid hormones and cholesterol

Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur

Oxidative Phosphorylation



OXIDATIVE PHOSPHORYLATION

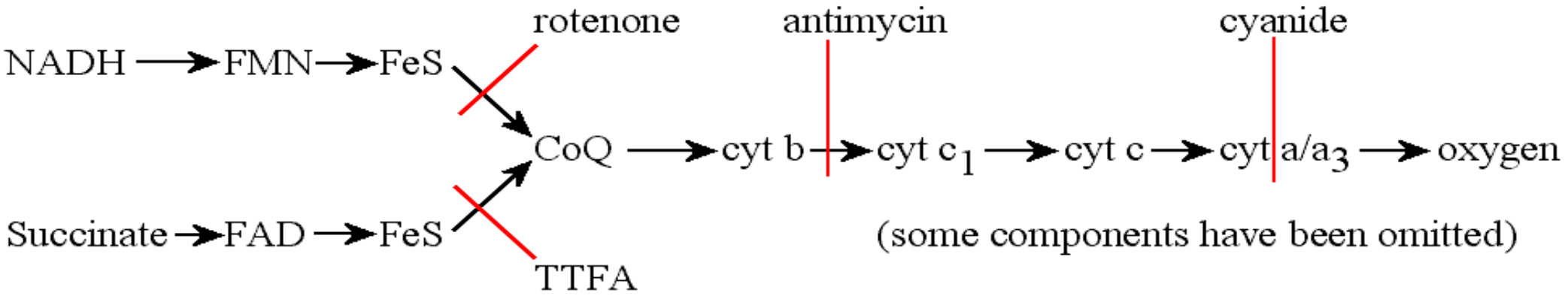
- **Definitions:**
- 1) It is a mode of ATP generation in which synthesis of ATP from ADP and inorganic phosphate occurs by using energy released during flow of electrons from substrate to O2, NO3 or SO4.
- 2) It is a mode of ATP generation in aerobic organisms, in which energy released during oxidation of substrate is used to synthesize ATP.

- In Oxidative phosphorylation substrate is oxidized which causes release of electrons. These electrons flows through membrane bound carriers like NADH2, FADH2 etc.

Respiratory Electron Transport Chain:

- The sequence of carriers' that mediates oxidation of substrate and reduction of terminal electron acceptor is known as respiratory ETC.
- It involves large no. of enzymes and electron carriers.
- In glycolysis, Krebs cycle electrons released after oxidation of substrate enters ETC. The first electron acceptor is NAD+ while in some cases it is FAD+.
- The terminal electron acceptor in aerobes is Oxygen while in anaerobes it may nitrate, sulfate or carbonate.

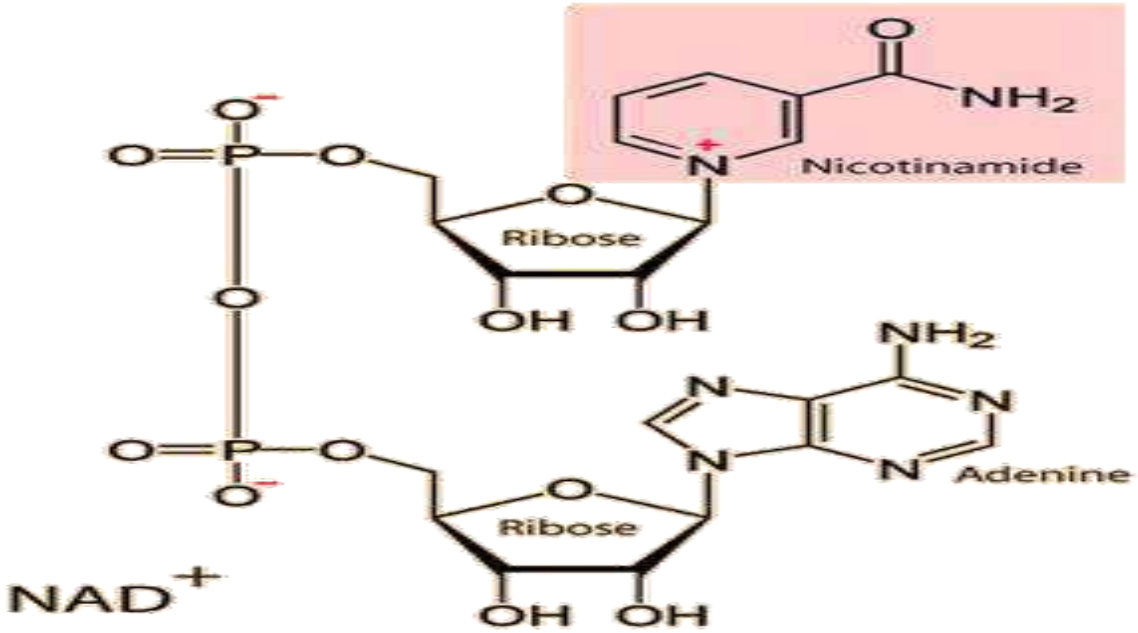
- Sequence of ETC was first elucidated by **D. Keilin** and **P. Mitchell** in 1927.



Components of ETC

- NAD and NADP
- Flavproteins
- Coenzyme Q
- Iron-sulfur proteims
- Cytochromes

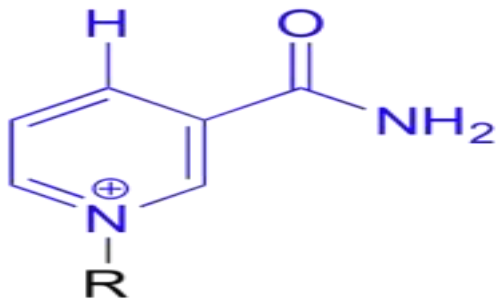
1) Nicotinamide adenine dinucleotide



- Contains 2 nucleotides- Adenylic acid (AMP) and Nicotinamide ribotide.
- Also known as Pyridine nucleotides.
- It is a type of Co-enzyme that get reduced by accepting electrons released by dehydrogenases.
- Derivative of vitamin Niacin.

- $NAD^+ + 2e^- + 2H^+ \rightarrow NADH + H^+$

hydride acceptor
oxidizing agent)



NAD^+
$NADP^+$

hydride donor
(reducing agent)

NADH
NADPH

NAD/ NADP accepts two electrons and one Proton at a time.

2) **Flavoprotein**:

- It is another class of enzymes that catalyse oxidation reduction using flavin nucleotides (FMN or FAD).
- Tightly bound to enzymes (Prosthetic group).
- Derived from Riboflavin.
- Both FMN and Fad posseses same active sites capable of undergoing reversible oxidation reduction.

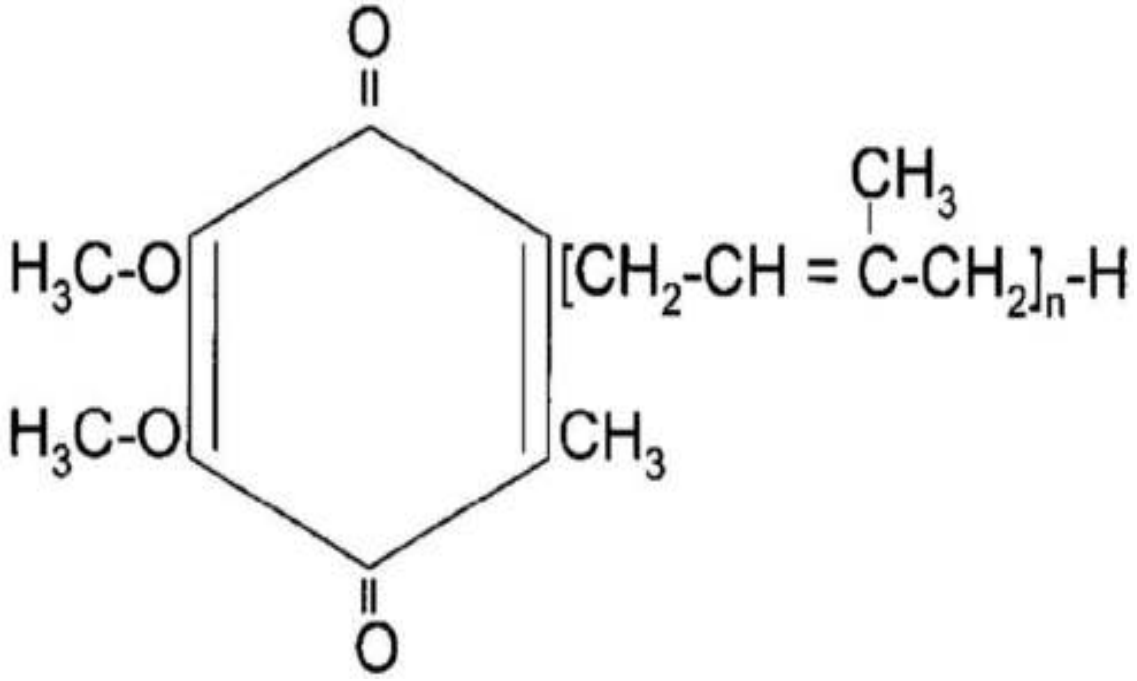
- $FAD + 2H^+ + 2e^-$ ⟶ $FADH_2$

- Oxidized flavoprotein- Absorption maxima 570nm
- Reduced flavoprotein- Absorption maxima 450nm

3) Coenzyme Q

- Hydrophobic quinone, fat soluble.
- Ubiquitous
- Contains long, non-polar side chain composed of isoprenoid ring.
- Different types of Co-Q based on no. of isoprenoid ring.

e.g. Mammalian mitochondria- 10 isoprenoid ring, Bacteria- 6-9

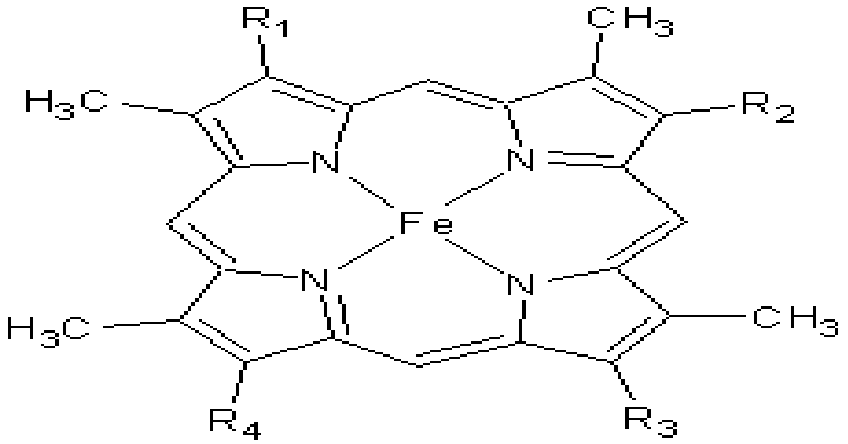


4) Cytochrome:

Initially discovered by McMunnin in animal cells.

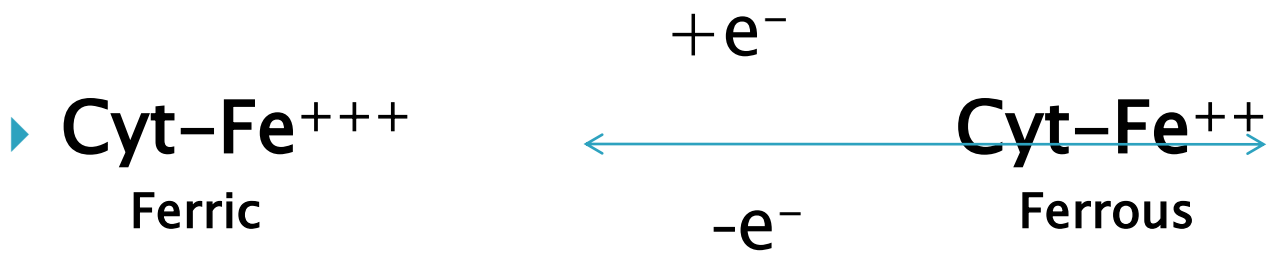
Keilin carried detail work.

It is Heme containing protein complex with four Porphyrin rings.



Four N of porphyrin rings are joined to central iron (Fe).

On the basis of absorption spectra classification as- Cyto-c & c1, Cyto-b & b1, Cyto a & a3

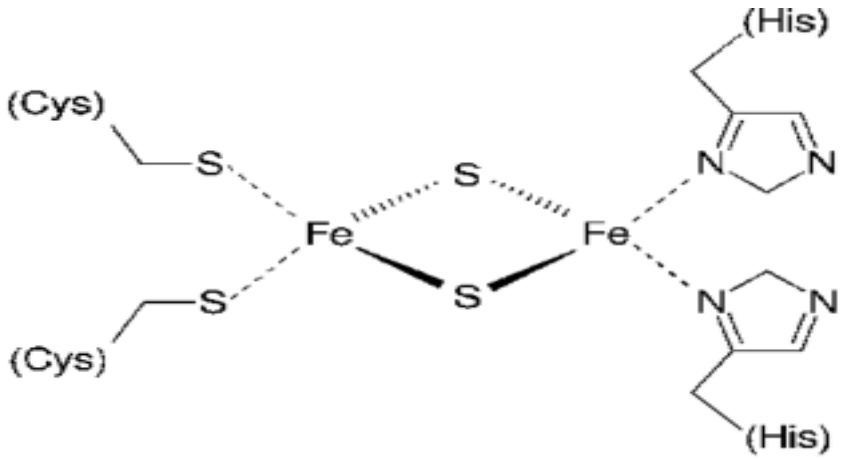


The central iron either exists as Fe^{2+} in reduced form or as Fe^{3+} in oxidised form.

- Undergoes reversible oxidation-reduction reactions.

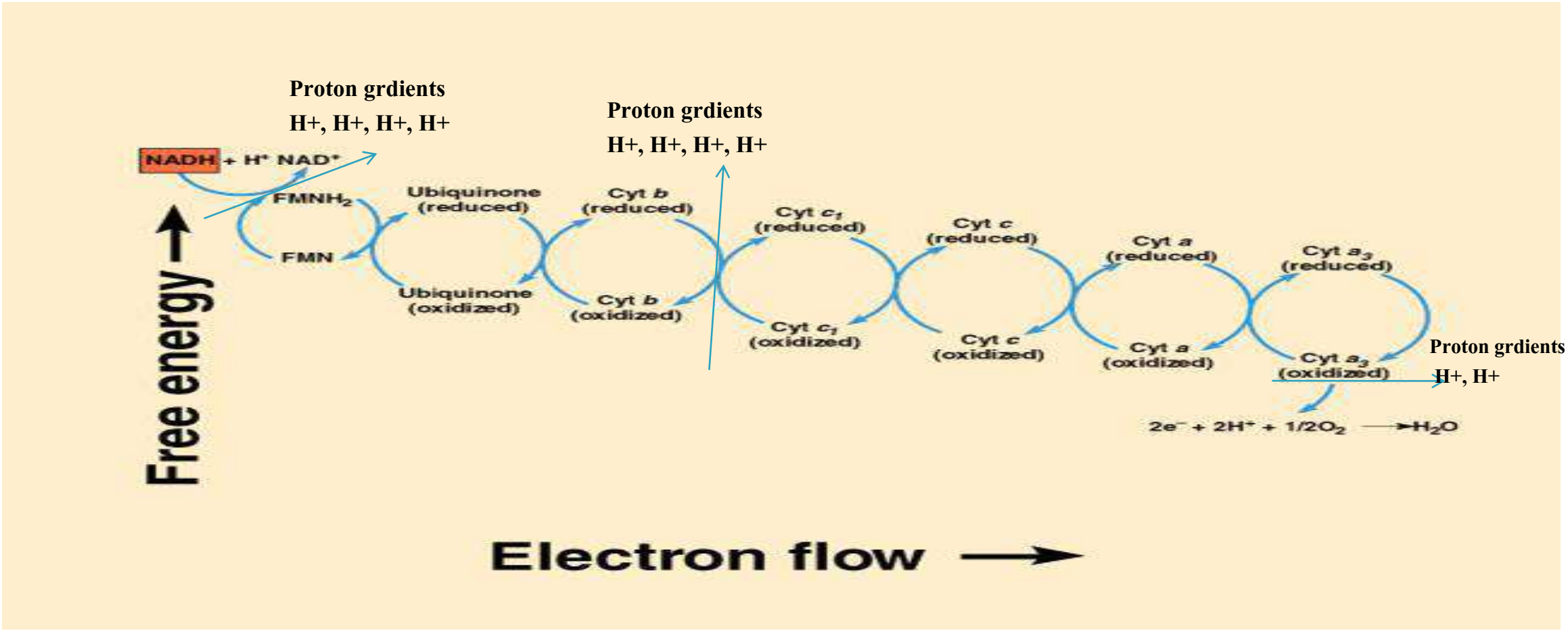
5) Iron-Sulfur protein:

- These are the proteins that binds iron atoms in a lattice of sulfur atoms.
- Grouped in several clusters as- 2Fe-2S, 4Fe-4S, 8Fe-8S.



- Carry only one electron.
- Discovered by Beinert. Later studied extensively by Reiske.

Sequence of Respiratory Electron Transport Chain



Mechanism Of ETC

- It is a series of reactions that results in re-oxidation of reduced carriers.
- The flow of electrons begins with reduction of NAD+ to NADH with the help of enzyme dehydogenase which catalyses oxidation of substrate.
- The hydrogen atoms are then transferred to FMN and Co-Q via FeS proteins.
- The Hydrogen atom undergoes ionization and splits to electrons (e-) and protons (H+).
- Electrons moves further to Oxygen through Cytochrome b, c, a, a3. While protons transported to inter-membrane space.
- After transfer from Cyto-a3 electrons and protons are brought together to produce hydrogen. Finally O2 combines with hydrogen to form Water.

Protonmotive Force:

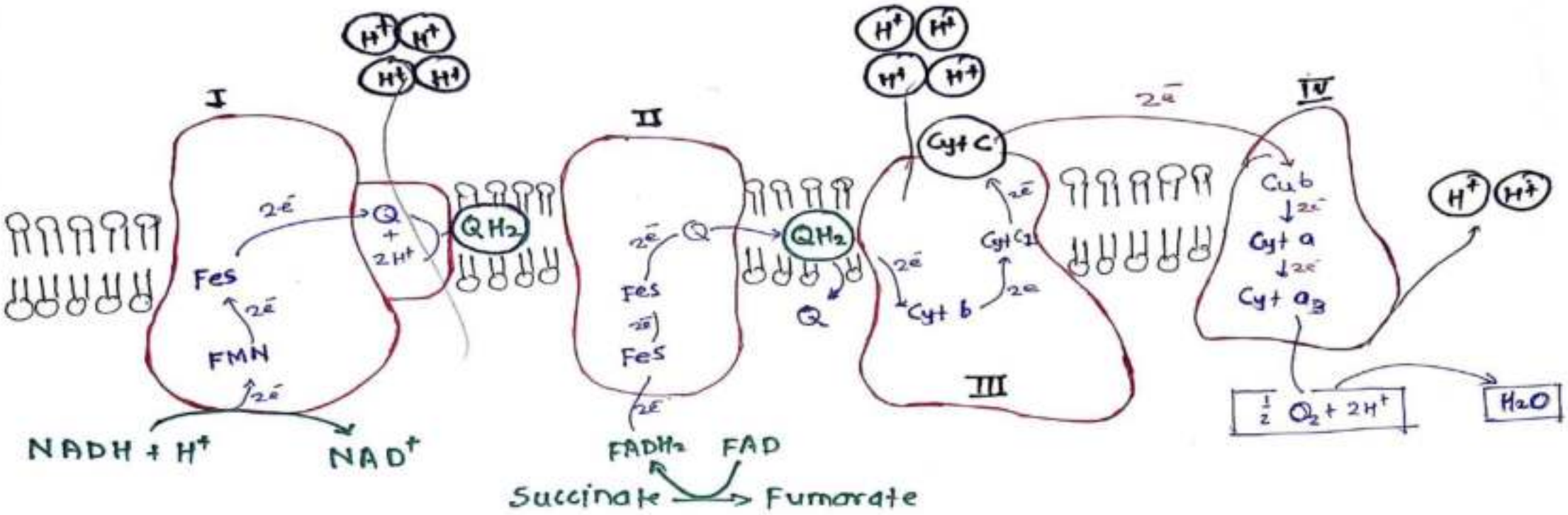
- Various hypothesis have been proposed to explain mechanism of ATP generation.
- Most widely accepted model is "**Chemiosmotic hypothesis**" proposed by Peter Mitchell 1978.
- According to this hypothesis, flow of electrons through the carriers releases energy which drives protons outside plasma membrane.
- Transfer of protons outside generates **Proton gradient** (Difference in charge)
- Thus, energy released during transfer of electrons is conserved as '**Proton motive Force**'.
- Proton motive Force is a form of potential energy that can be used for:

  a) Permease systems

  b) Syntheis of ATP from ADP and Pi.

  c) Rotation of flagellar motor

The carriers of ETC functions as Complexes as:



➢ Complex I:

Receives 2 electrons from NADH and passes to CoQ via FMN and Fe S protein. During this process 4H+ are pumped out of the matrix.

$$NADH + 5H^+ + Q \longrightarrow NAD^+ + QH_2 + 4H^+$$

➢ Complex II:

Succinate is oxidized which releases elctrons. Released electrons are transferred to FAD which gets reduced to FADH2. Finally electrons from FADH2 are transferred to Co-Q via Fe-S proteins.

$$Succinate + FAD \longrightarrow Fumarate + FADH_2 \qquad FAD^+ + QH_2 \longrightarrow$$

➢ Complex III:

Passes electrons from CoQH2 to Cyto-C via Cyto-b, c1 and FeS protein.

During this process 4H+ are pumped out of the matrix.

$QH_2 + 2\ \text{Cytochrome c}\ (Fe^{3+}) \longrightarrow Q + 2\ \text{Cytochrome c}\ (Fe^{2+}) + 4H^+$

➢ Complex IV:

Receives 2 electrons from Cyto-c and via Cyto-a and a3 passes them to molecular oxygen which is reduced to form water. During this process 2H+ are pumped out of the matrix.

$0.5O_2 + 4H^+ + 2e^- \longrightarrow H_2O + 2H^+$

Anaerobic Respiration

- ✓ Final electron acceptor is an inorganic substance, ther than Oxygen(O2).
- ✓ Inorganic substance vary from organism to organism.

e.g. Pseudomonas, Bacillus- Nitrate (NO3)

Desulfovibrio- Sulfate (SO4)

- ✓ Does not involve all electron carriers of ETC.

ATP yield is low.

Median

Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

Median

- It is that value, which **divides the group of data in two equal parts.**
- One part consists all value greater and other values lesser than median.
- Equal number of values on both sides of median.
- Represented as **‘Md’.**
- Data should be arranged in either ascending or descending manner.

❑ **General formula for median:**

1. For Odd number of values:

$$Md = N\left(+\frac{1}{2}\ th\right)$$

❑ **Example**: The given data is.....

18, 35, 19, 26, 28, 30, 20, 24, 41, 33, 32, 22, 13, 27, 31

Arrange the data in ascending or descending manner.

13, 18, 19, 20, 22, 24, 26, 27, 28, 30, 31, 32, 33, 35, 41

$$Md = N\left(+\frac{1}{2}\ th\right) = 15 + \frac{1}{2} = \frac{16}{2} = 8^{th} = \mathbf{27}$$

56, 39, 44, 49, 46, 51, 42, 38, 30, 52, 35, 48, 62, 36, 60, 50, 53, 37, 33, 41, 65

Arranging data in ascending manner:
30, 33, 35, 36, 37, 38, 39, 41, 42, 44, 46, 48, 49, 50, 51, 52, 53, 56, 60, 62, 65

$$Md = \frac{N+1}{2}^{th}$$

$$Md = 21 + \left(\frac{1}{2}\right)$$

$$Md = \frac{22}{2} = 11^{th} = \mathbf{46}$$

95, 67, 74, 88, 82, 76, 92, 65, 70, 83, 60, 98, 54, 66, 59, 63, 62, 71, 90, 84, 61, 69, 50

$$Md = \left(\frac{N+1}{2} \right)^{th}$$

1. **For even number of values:**

$$Md = \frac{\left(\frac{N}{2}\text{th} + \frac{N+2}{2}\text{th}\right)}{2}$$

Example:

18, 35, 19, 26, 28, 30, 20, 24, 41, 33, 32, 22, 13, 27

13, 18, 19, 20, 22, 24, 26, 27, 28, 30, 32, 33, 35, 41

$$Md = \frac{\left(\frac{N}{2}\text{th} + \frac{N+2}{2}\text{th}\right)}{2}$$

$$= \frac{\left(\frac{14}{2}\text{th} + \frac{14+2}{2}\text{th}\right)}{2} = \frac{7\text{th} + 8\text{th}}{2} = \frac{26 + 28}{2} = \frac{+ 54}{2} = 27$$

56, 39, 44, 49, 46, 51, 42, 38, 30, 52, 35, 48, 62, 36, 60, 50, 53, 37, 33, 41

95, 67, 74, 88, 82, 76, 92, 65, 70, 83, 60, 98, 54, 66, 59, 63, 62, 71, 90, 84, 69, 50

❑ **Desirable properties:**

i. Uniqueness
ii. Simplicity
iii. It is a positional average
iv. Not drastically affected by extreme values

❑ **Undesirable properties:**

i. For large ungrouped data, calculations becomes tedious
ii. Each and every value is not considered.

Mode

MODE

- It is the most common item of series.
- **Definition**: The value of the variable, which occurs most frequently in the population.
- It is repeated highest number of times in the series.
- It is positional average.

Examples

The given data is.....

56, 39, 44, 49, 46, 51, 37, 42, 38, 39, 30, 52, 35, 48, 62, 36, 60, 50, 53, 39, 37, 33, 41

The mode of given data is **37**

95, 69, 67, 74, 88, 82, 76, 74, 92, 69, 65, 70, 83, 60, 98, 54, 66, 74, 59, 63, 62, 71, 90, 84, 74, 69, 50

18, 24, 35, 19, 26, 28, 30, 20, 19, 24, 41, 33, 32, 22, 13, 27

13, 18, 19, 20, 22, 19, 24, 26, 27, 28, 30, 24, 32, 33, 35, 41

❑ **Desirable properties:**

i. Easy to calculate and understand

ii. Not affected by extreme values

iii. Can be determined graphically

❑ **Undesirable properties:**

i. Not rigidly defined.

Applications of Biostatistics

Mr. Suraj D. Gabale

1. In physiology and medicine:

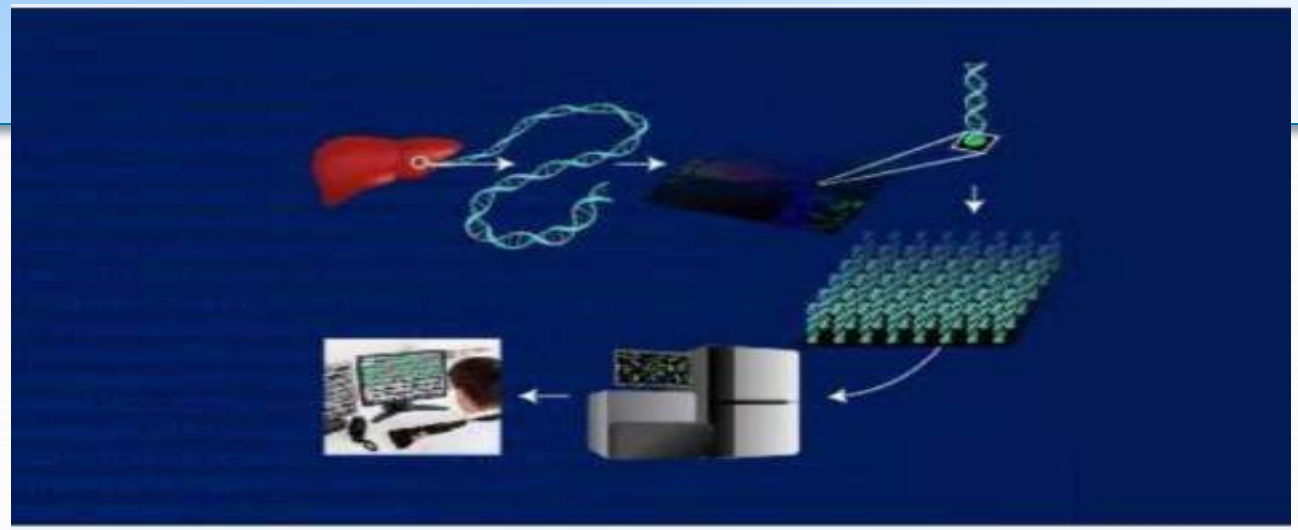
- To define what is normal or healthy in a population.
- To find **limits of normality** in variables such as weight, pulse rate etc. in a population.
- To find an association between two attributes such **as cancer and smoking** or **dengue and social class.**

2. In phamacology:

- To find out the **action of drug**.
- A drug is given to animals or humans to see the changes produced are due to the drug or by chance.
- To compare the action of two different drugs or two successive dosage of the same drug.
- To find out relative potency of a new drug with respect to a standard drug.

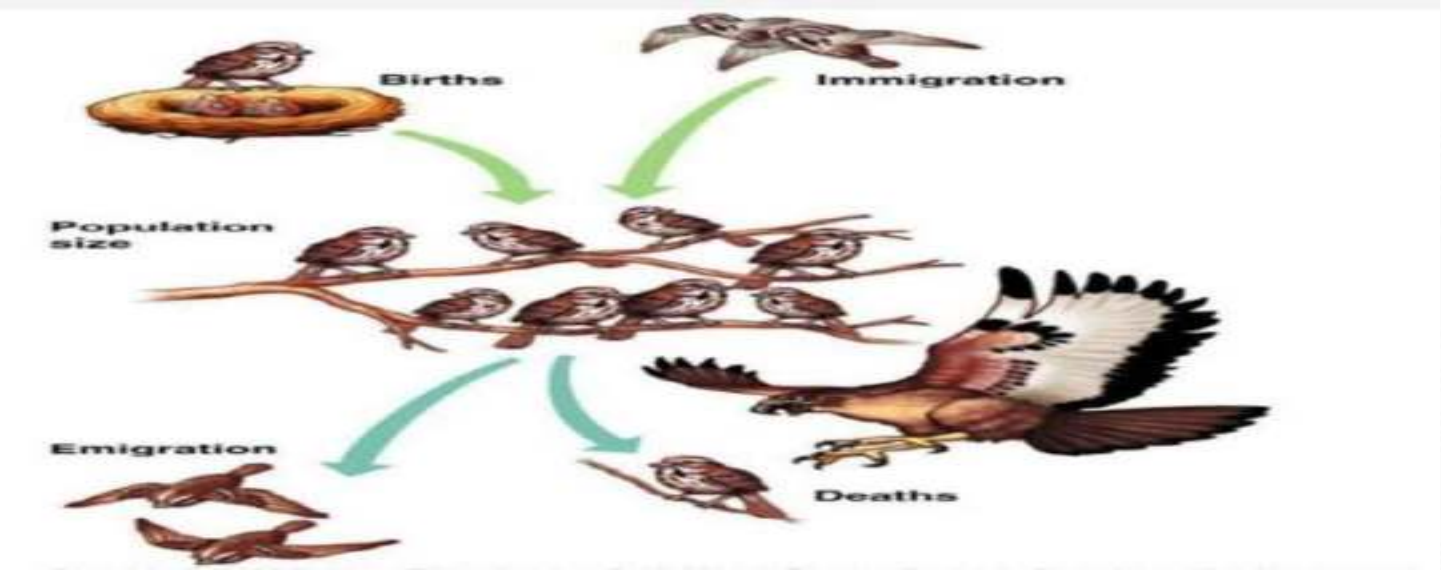
3. In Genetical studies:

•Studying genetic structure o population and changes occurring over generations due to mutation, migration and selection.
•To study small population size and its consequence on breeding.
•Most common areas where statistical methods are used-
  a. Human genome project
  b. Linkage analysis
  c. Sequencing



4. Statistical Ecology:

- To predict total number of species in the whole population from the observed number of species in the sample
- To study **mortality/natality rate** in a population
- To study **species richness** or **species abundance.**



5. In Environmental management:

- To understand relationship between processes in environmental biology and its management.
- Regular monitoring to detect **changes in environment**.
- To conclude effectiveness of **biological waste treatment** techniques.

Antigens

Antigens

❖ **Definition**: Any substance which can stimulate host immune system and causes production of antibodies or sensitized cells and reacts specifically these products.

❖ Also known as "**immunogen**".

❖ Two important properties of antigens:

a. Immunogenicity

b. Immunological specificity

A. Immunogenicity:

- Ability of antigen to **stimulate immune response**.

B. Immunological specificity:

- Ability of an antigen to **react specifically** with antibodies or sensitized cells.
- Also known as ‘reactivity’.

❖ Antigenicity:

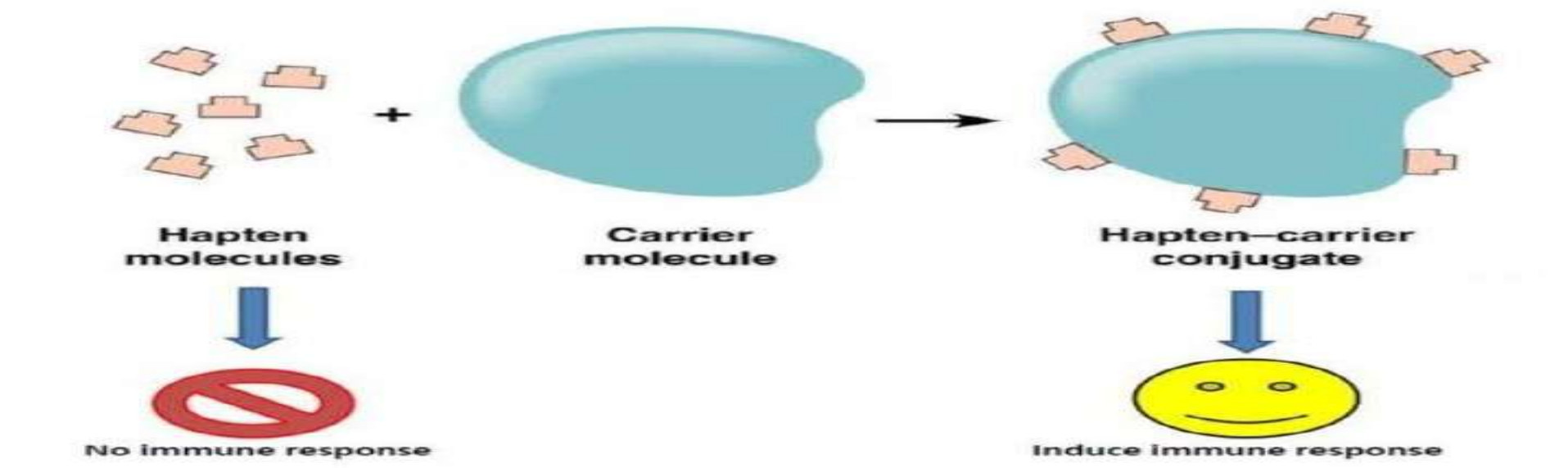
- Ability of antigen to **stimulate immune response** and are **react with the products** of immune response.

A. Complete antigen

- ✓ A substance which shows both properties of antigens like immunogenicity and immunological specificity.

B. Hapten

- The substances which are **incapable of inducing antibody formation** but can react specifically with antibodies.
- Also known as **incomplete antigen** or **partial antigen**.
- Haptens become immunogenic on combining with large molecules called ‘carrier’.



e.g. **Dinitrophenol:**

- Dinitrophenol = Incomplete Ag
- Dinitrophenol + serum albumin = Complete Ag

❖Some substance acts as hapten in one animal but complete antigen in others.

e.g. Pneumococcal polysaccharide

It acts as complete Ag in mice but hapten in rabbits.

Chemical nature of antigens

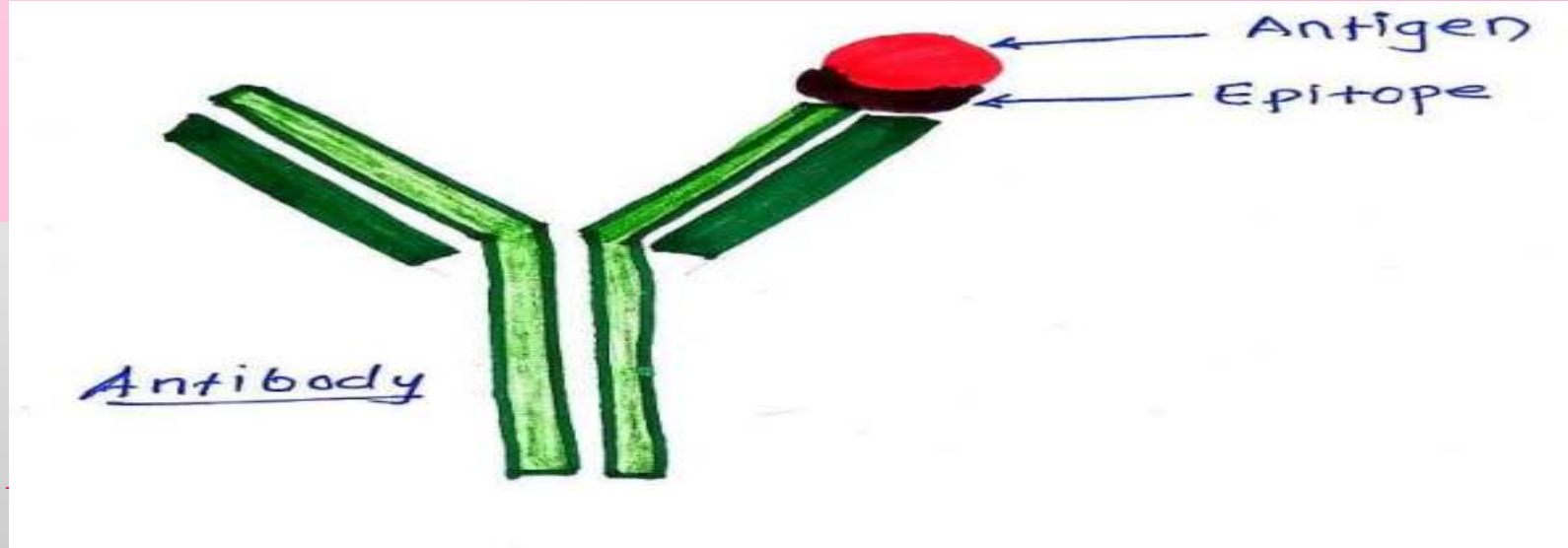
- Chemically Ag's are **proteins** or **polysaccharides**.
- Molecular weight: More than 10,000 Daltons.
- They may be nucleo-proteins, lipoproteins or glycoprotein's.

- Many naturally occurring substances acts as Ag.

e.g. Bacteria, viruses, other micro-organisms, pollens, egg white and metabolic products of micro-organisms

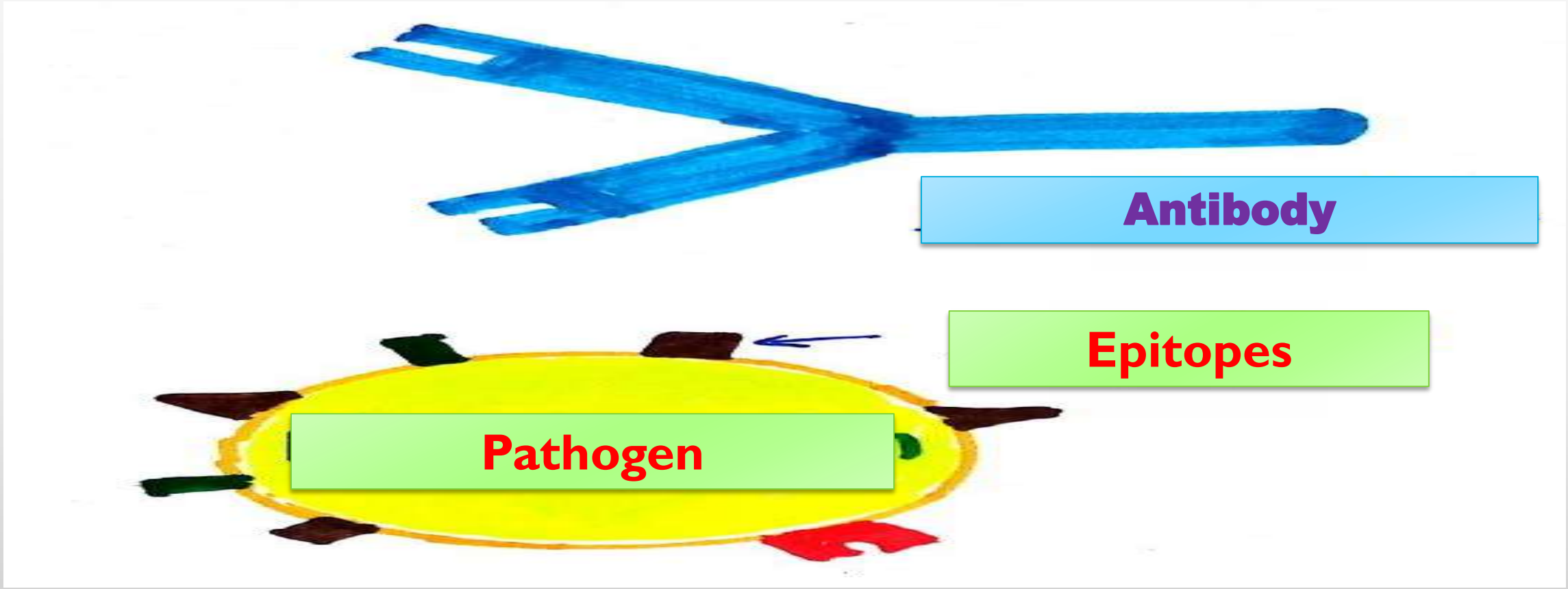
Epitope:

- The part of antigen responsible of binding wiith Ab's.
- Also known as '**antigenic determinant**'.
- Present at the surface of antigen.
- Binding between Ag – Ab may be similar to '**lock & key fit'**.
- Each Ag usually contains more than one epitope.



Functional valency of Ag's:

The **number of antigenic determinants** per molecule of antigen.



Types of Antigens

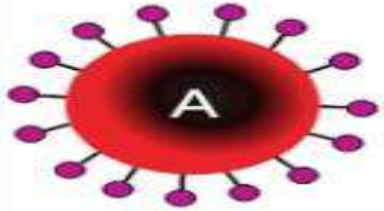
Types of antigens

1. Isoantigens
2. Auto-antigens
3. Heterophile/ cross reactive antigens
4. Histocompatibility antigens
5. Microbial antigens
6. T cell dependent and T cell independent antigens

1. Isoantigens

❖**Antigens that are found in some but not in all members of a species**.

e.g. Blood group antigens

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in red blood cell	 A antigen	 B antigen	 A and B antigens	None

2. Autoantigens

- Also called **self** or **autologous** antigens.
- These are not recogized as self and immune respose developed.
- e.g. **Eye lens proteins**
- Some Ag's are absent during embryonic life but develop later.
- e.g. **Sperms**

- Immune respose against autoantigens results into **'autoimmune disorders'.**
- e.g. **Rheumatid arthritis**

3. Heterophile antigens

- Also called '**cross reactive antigen**'.
- Antigens that are present on the **surface of unrelated plants, animals and bacteria but are identical**.
- Antibodies to such antigen can cross react with the others.
- Mostly these are **polysaccharides**.

e.g. **Forssman Ag:**

- It is glycolipid antigen.
- Present on RBC's of horse, sheep, dog, cat, toad, whale, turtle, chicken and other organisms.
- Also found on tissues of Guinea pigs and some human beings

4. Histocompatibility antigens

- The antigens that are **specific to each individual** of a species.

- These are antigens are used **as markers of tissue compatibility** during grafting or transplantation of cellular material.

e. g. **Human leukocyte antigen (HLA) or Major histocompatibility complex (MHC)**

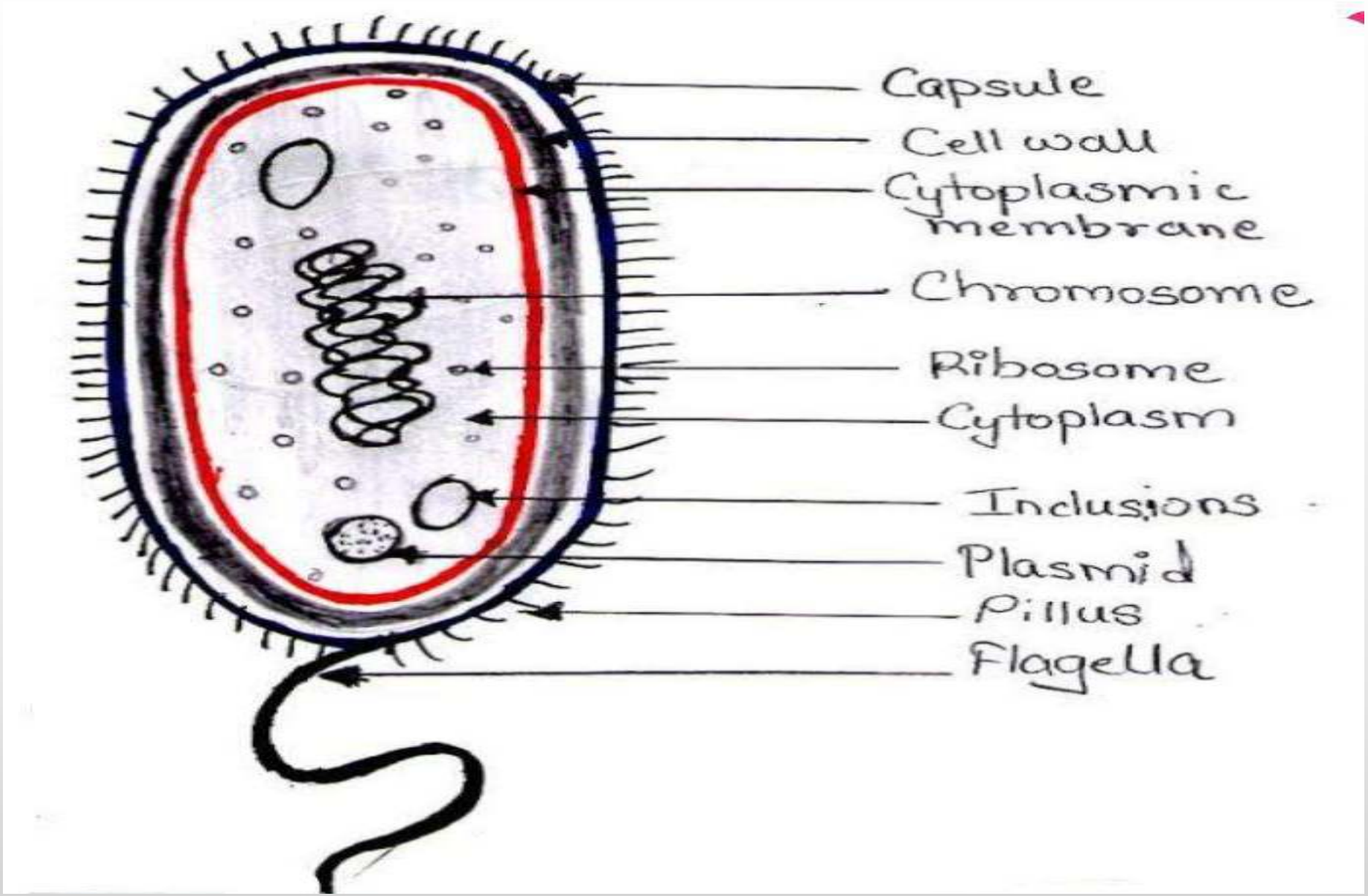
5. Microbial antigens

- Antigen that is of microbial origin.
- **Exotoxins** of many pathogens are potent antigen.
- Bacterial cells contains many antigenic components.

e.g. 1. **'O' antigen** (cell wall)

2. **'H' antigen** (flagellar)

3. **Vi antigen** (Capsular)

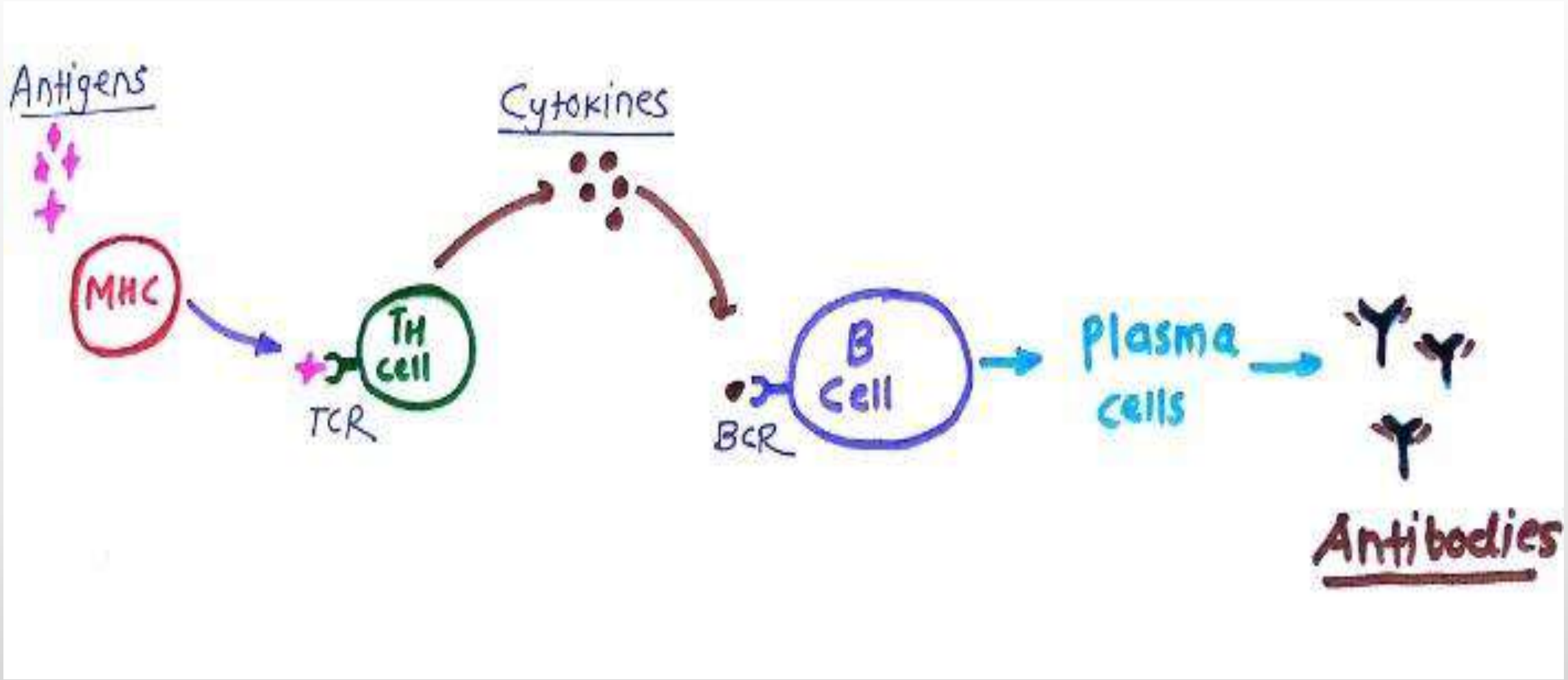

Capsule
Cell wall
Cytoplasmic membrane
Chromosome
Ribosome
Cytoplasm
Inclusions
Plasmid
Pillus
Flagella

6. T cell dependent & T cell independent Ag

❖T cell dependent Ag:

- ✓ Requires T cell co-operation for induction of antibody production by B cells.
- ✓ Structurally more complex

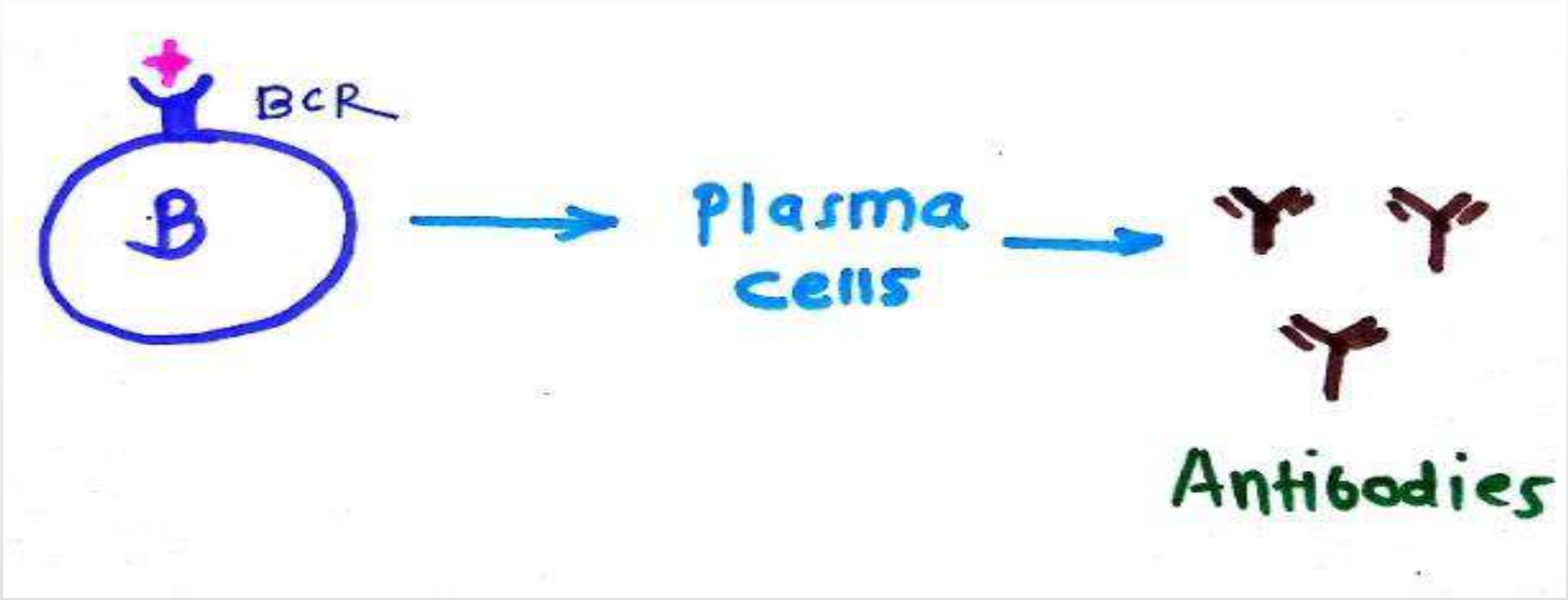
e.g. **Erythrocytes**, **Serum proteins**.


Antigens
MHC
TH cell
TCR
Cytokines
B Cell
BCR
Plasma cells
Antibodies

❖T cell independent Ag:

- ✓ Do not require T cell for induction of antibodies by B cells.
- ✓ Simple in structure.

e.g. **Pneumococcal capsular polysaccharide, bacterial LPS and flagellar proteins**


BCR
B
Plasma cells
Antibodies

Factors affecting antigenicity

I. Size

- Size of antigen has direct relation with antigenicity.
- **Very large molecules are excellent antigens.**

  e.g. Hemocyanin (60,00,000 D mol.wt.), thyroglobulin (6,69,000 D) and tetanus toxin (55,000 D)

- Molecules of **low molecular size are poor antigens.**

  e.g. Insulin protein (5,700 D) and histones (6,000 D)

2. Chemical nature

- Most of naturally occurring antigens are chemically proteins and polysaccharides.
- These are comparatively less antigenic than lipids & nucleic acids.
- Proteins are better antigenic than polysaccharides of same size.
- In polypeptide antigens amino acid **tyrosin** (aromatic a.a.) enhances antigenicity.
- **Synthetic polymers are less antigenic than naturally occurring polymers**.

3. Solubility

❖Synthetic polymers are less antigenic as they are insoluble in body fluids.

❖ Any substance which is **not converted to soluble form** by tissue enzymes is **non-antigenic.**

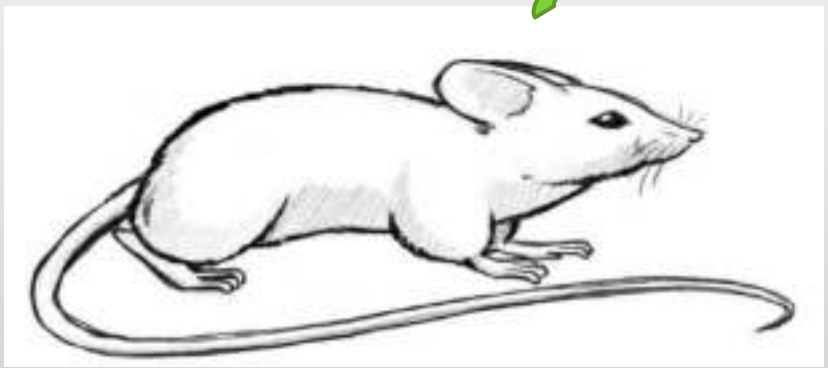
e.g. **Pneumococcal polysaccharide:**

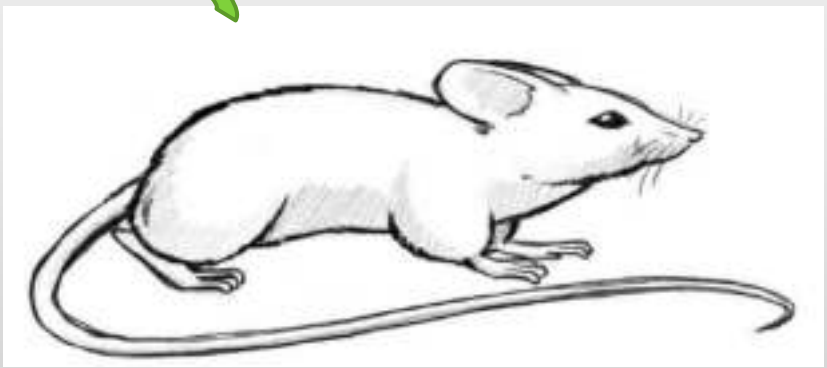
- It is greater immunogenic in mice than rabbits
- Because in mice it is converted to soluble form by liver enzymes

4. Foreignness

- Antigenicity of substance is related to degree of it's foreignness.
- Antigens from related species are less antigenic than from unrelated species.

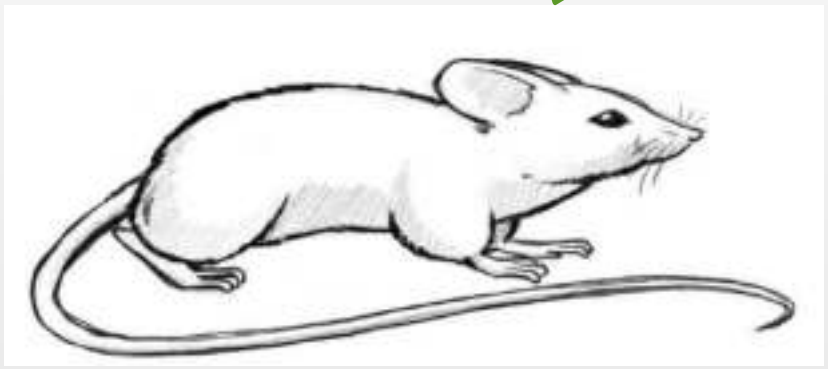
Antigens

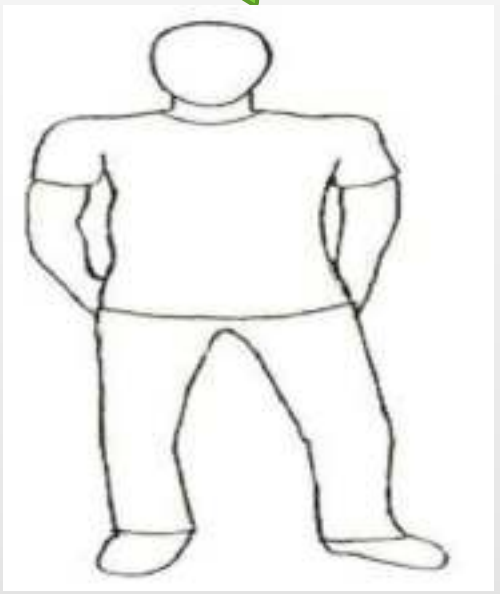




No immune response

Antigens





Immune response

5. Structural rigidity

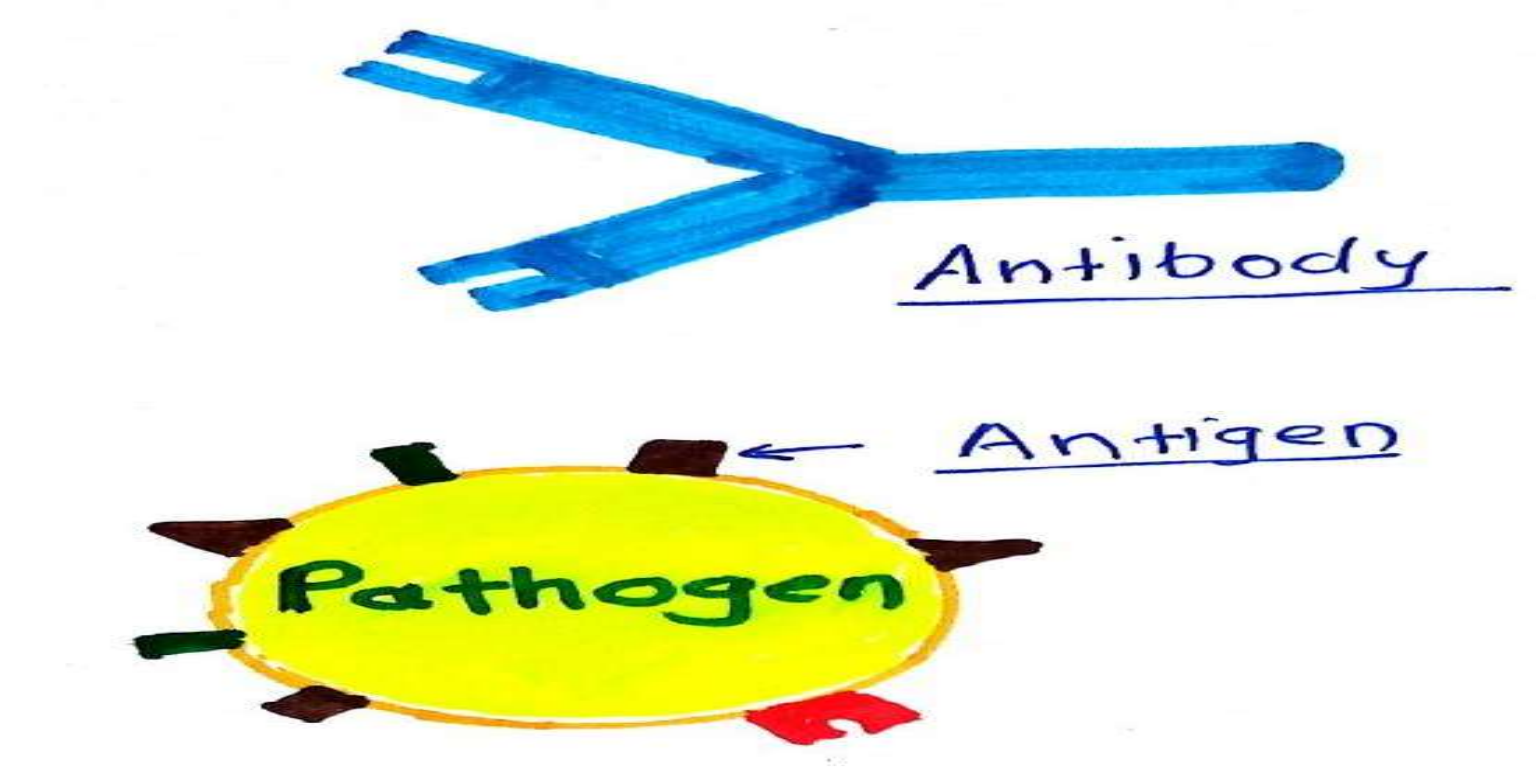
❖ **Antigenic determinants must be correctly exposed to lymphocytes.**

❖ Molecule having enough molecular size but not having structural rigidity can be poor antigenic.

e.g. **Polymerized flagellin** (flagellar filament) is more immunogenic than monomeric flagellin

6. No. Of antigenic determinants (epitope)

- More the number of epitopes, more will be the immunogenicity.



7. Route of entry of antigen

❖Antigens are much effective if injected via parentral routes such as intradermal, subcutaneous, intravenous, intramuscular or intraperitoneal than when injected orally.

❖It is because, if given orally it may be destroyed by digestive enzymes.

8. Dose of antigen

- **Antigenicity also depends upon the no. of antigen administered.**
- Less no. of antigen is insufficient to induce immune response.
- Large no. of antigens may inhibit immune response.
- Thus optimum dose of antigen is necessary for normal immune response.

9. Adjuvants

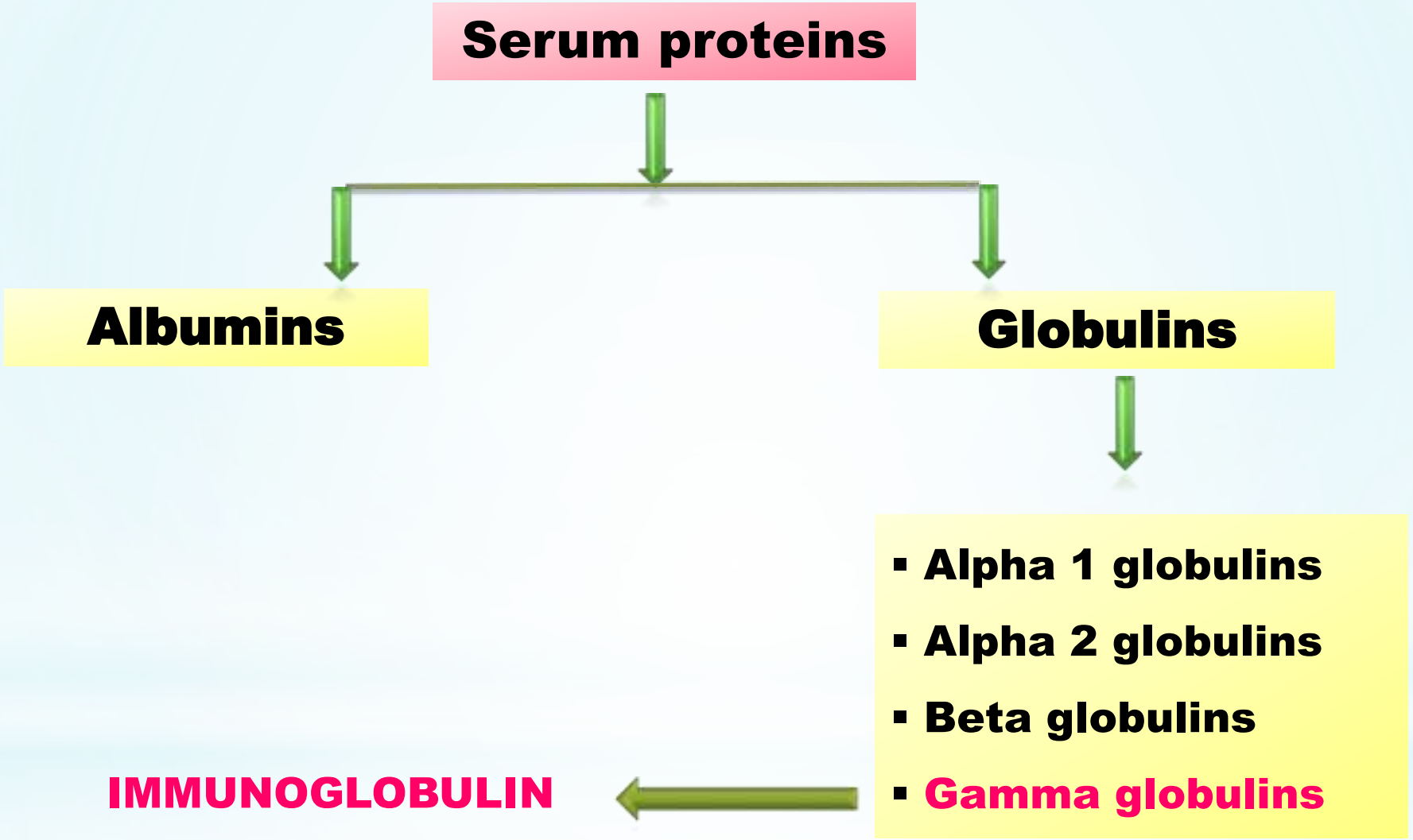
- **Substance which enhances immunogenicity is known as adjuvants.**
- Adjuvants increase persistance of antigen into body and thus can keep antigenic stimulus persistance.
- **Examples of adjuvants:**

Silica particles, mineral oils, aluminium hydroxide, Freund's incomplete and Freund's complete antigens.

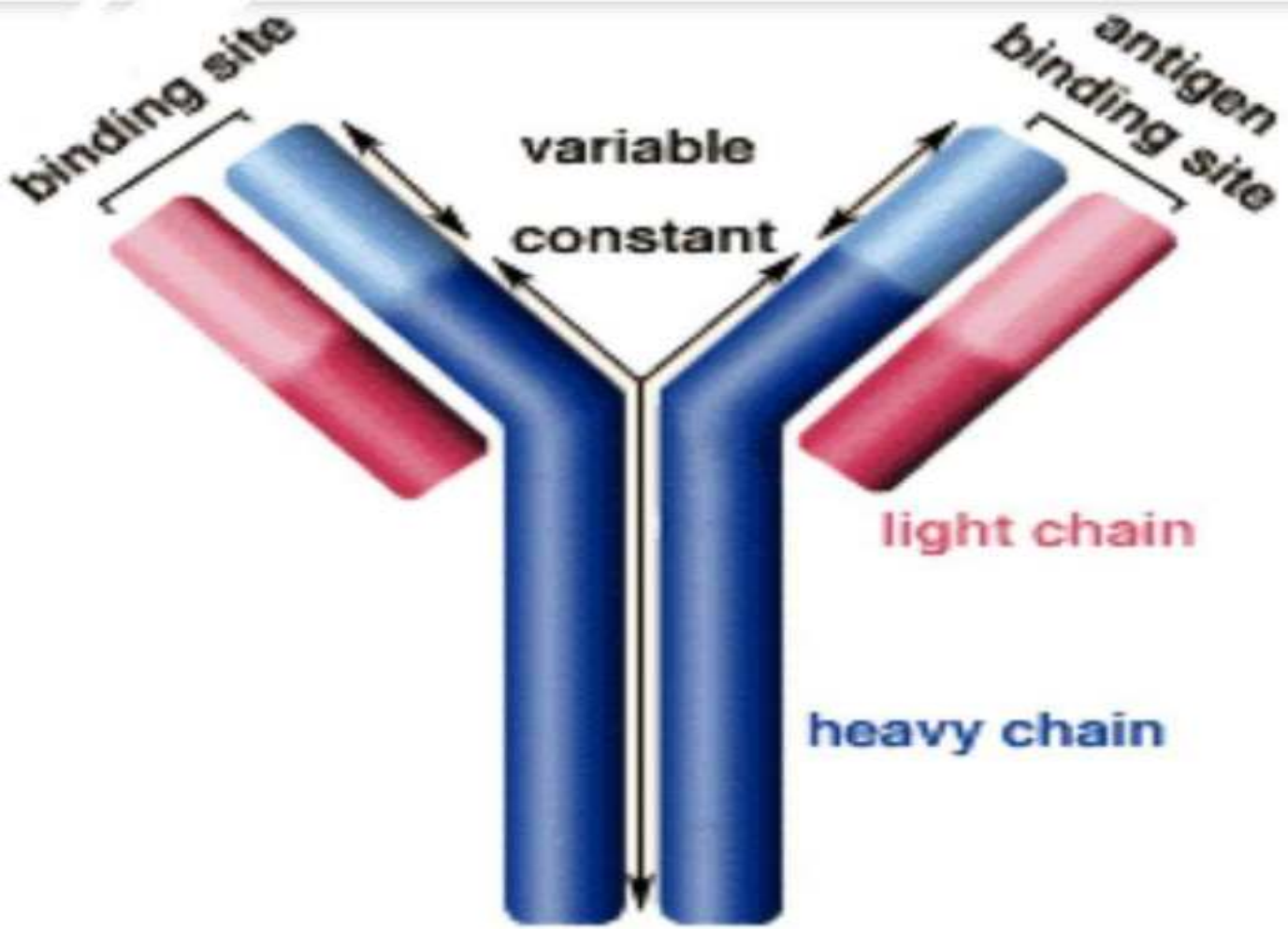
Antibody

ANTIBODY

- **Antibody is a protective protein produced by immune system in response to the presence of antigen.**
- It is plasma protein produced by **B lymphocytes**.
- Chemically it is glycoprotein in nature.
- It have property of combining with specific antigens.


Serum proteins
Albumins
Globulins
Alpha 1 globulins
Alpha 2 globulins
Beta globulins
Gamma globulins
IMMUNOGLOBULIN

Structure of Antibody



Light chain:

214 amino acids

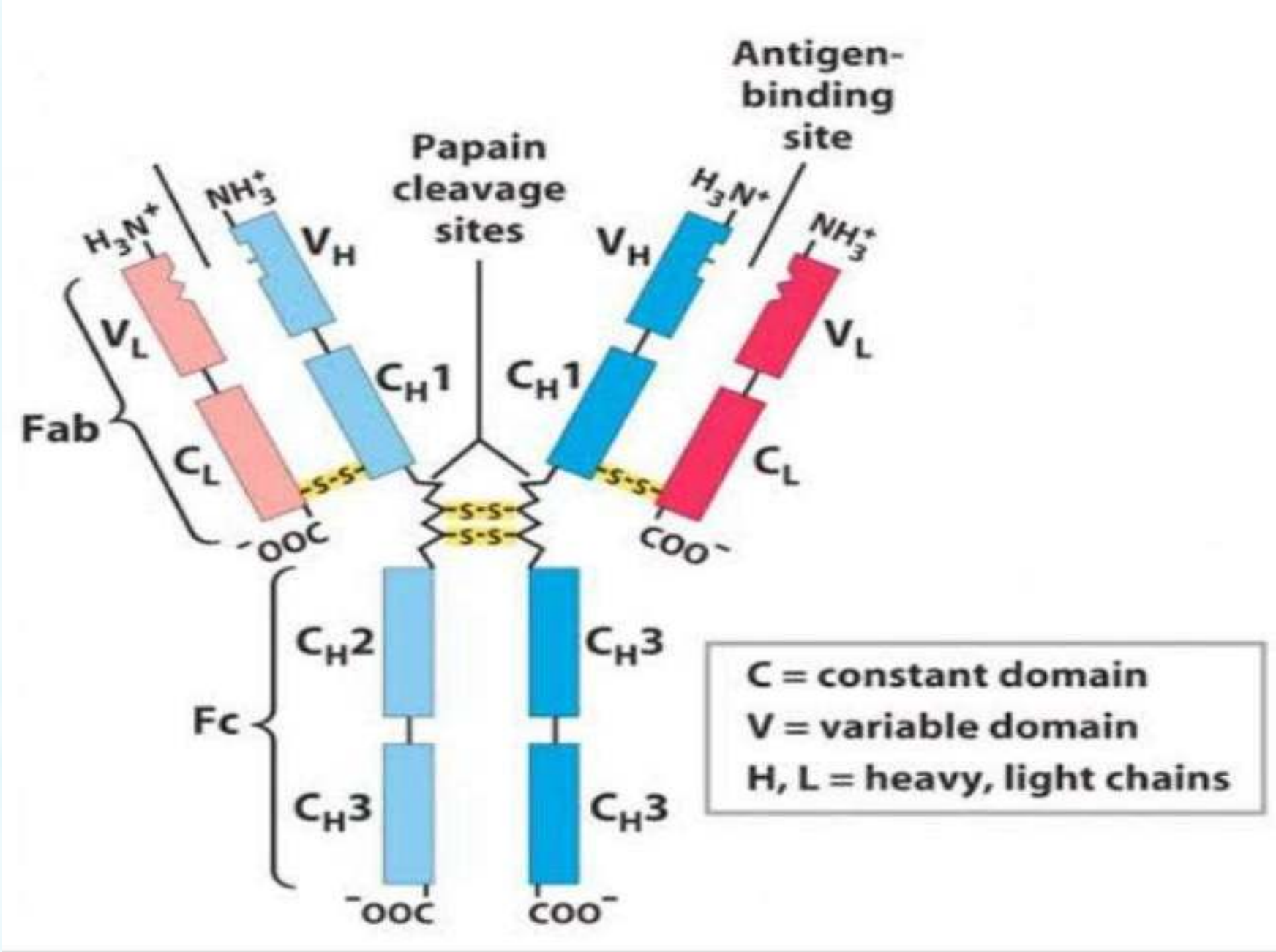
25,000d

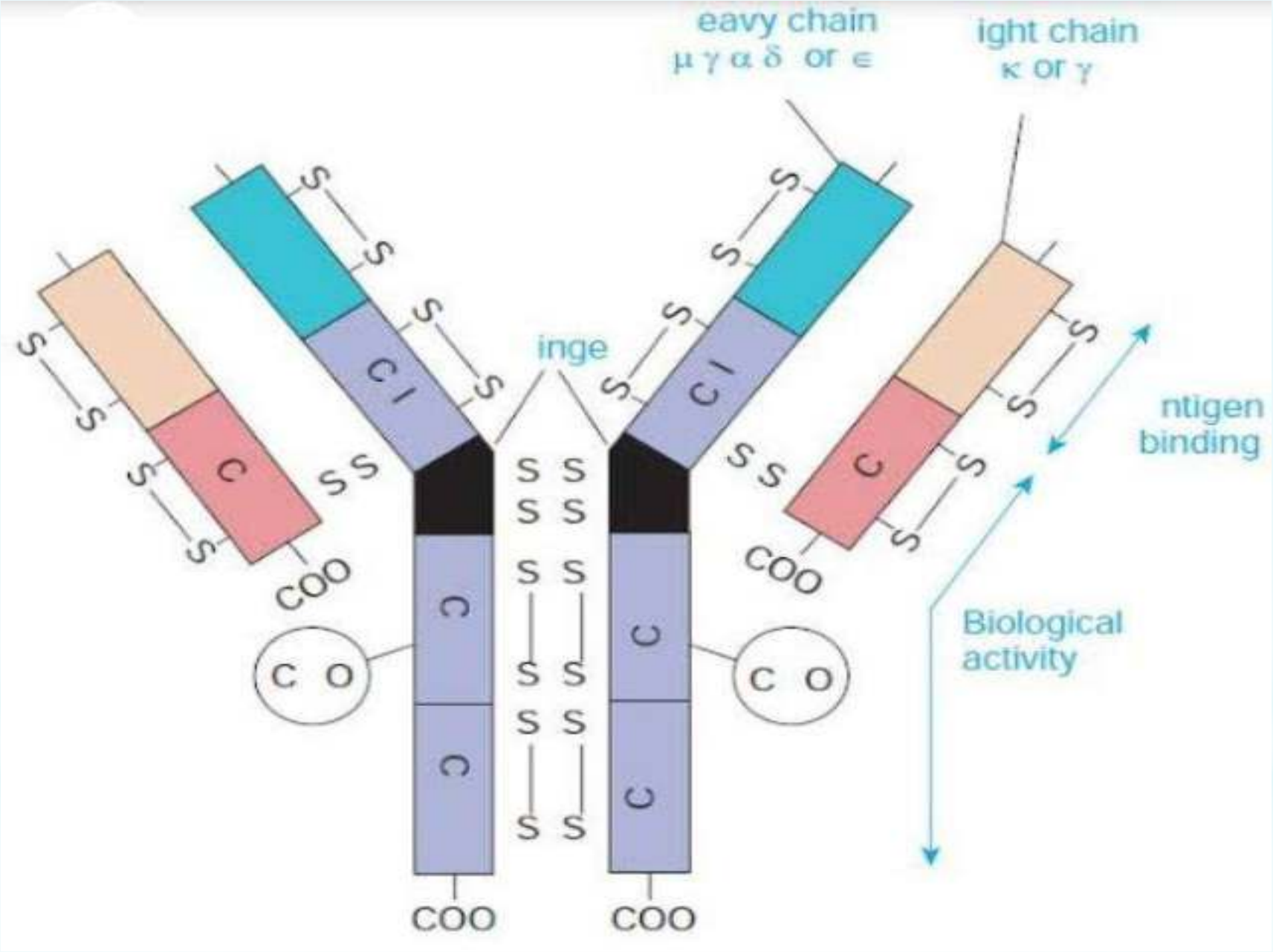
Kappa (k) & Lambda (λ)

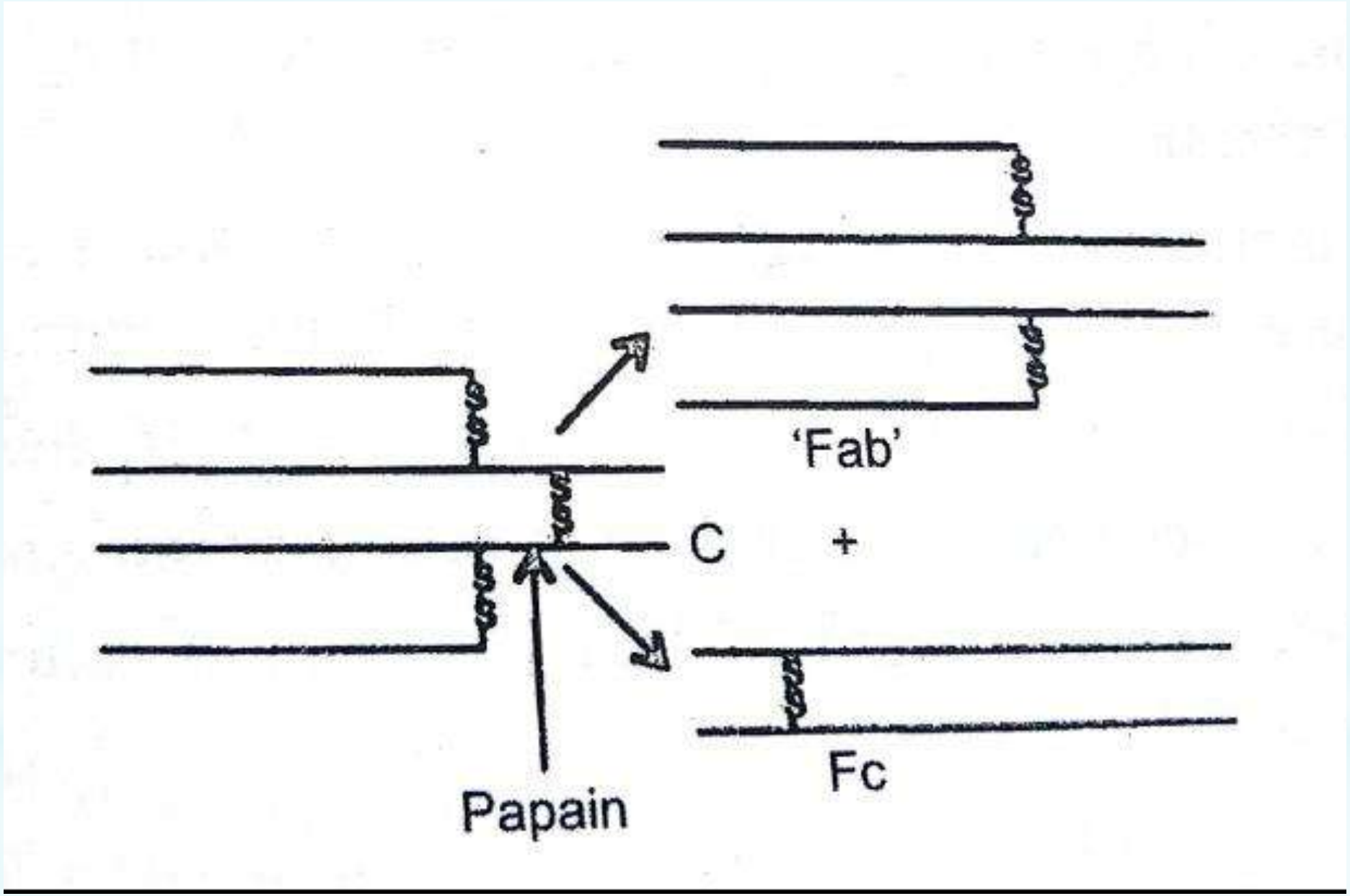
Heavy chain:

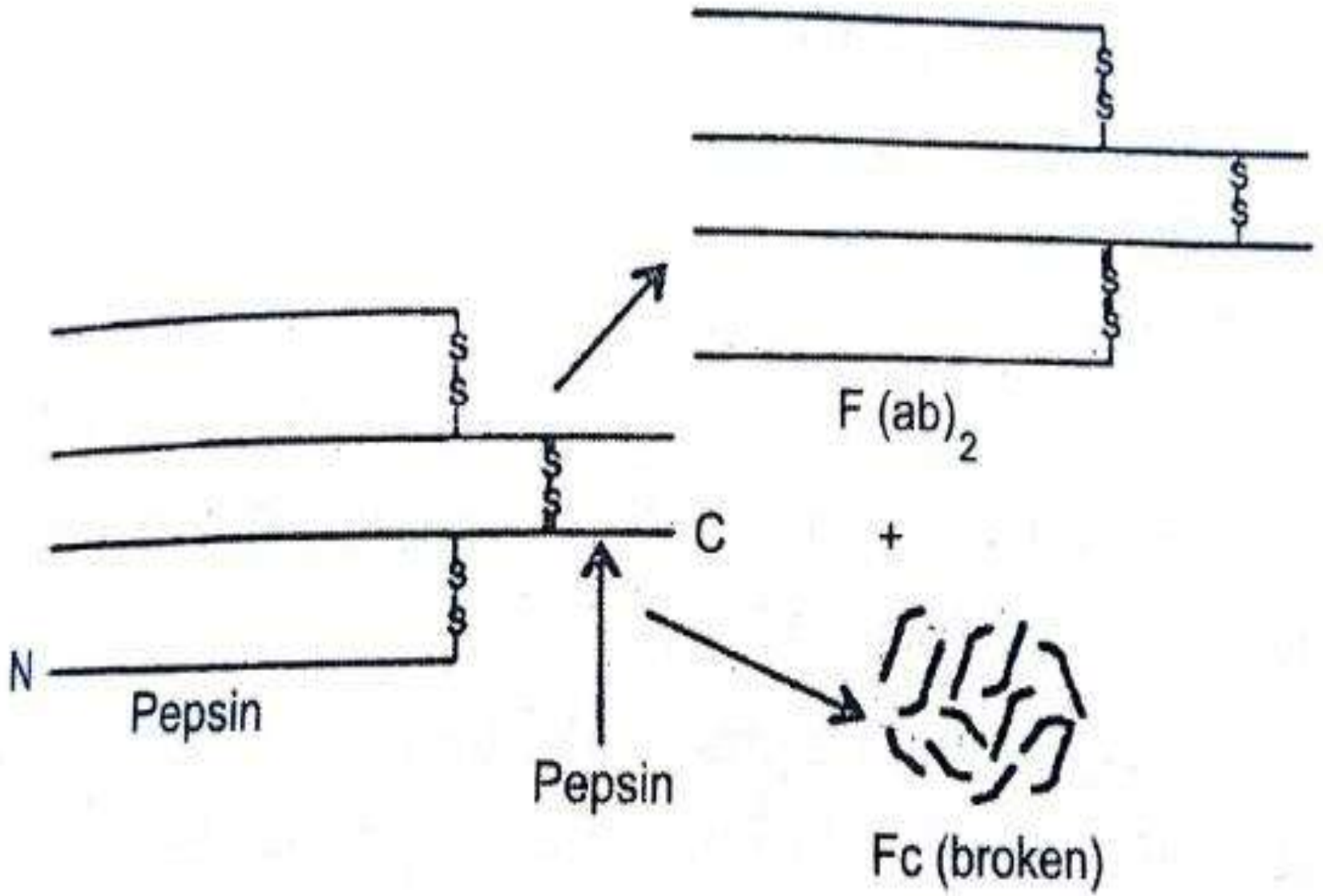
50,000d

α, β, μ, Y, d


Antigen-binding site
Papain cleavage sites
H_3N^+
NH_3^+
V_H
V_L
C_H1
C_L
Fab
^-OOC
COO^-
C_H2
C_H3
Fc
C = constant domain
V = variable domain
H, L = heavy, light chains


eavy chain
μ γ α δ or ∈
ight chain
κ or γ
inge
ntigen
binding
Biological
activity
C
C I
COO
C O
S
S S


'Fab'
C
+
Fc
Papain


F (ab)2
C
+
N
Pepsin
Pepsin
Fc (broken)

Types of Antibodies

Types of antibodies

Based on structural differences and biological properties types of antibodies are:

1. Ig G
2. Ig A
3. Ig M
4. Ig D
5. Ig E

Immunoglobulin G

- It is a typical antibody consisting **2 heavy** chains and **2 light chains**.
- Accounts for 70% of total immunoglobulin in human serum.
- It's concentration in normal serum is about **8-26 mg/litre**.
- Produced during secondary immune response.
- Molecular weight: **1,50,000 d**.
- Sedimentation coefficient is '**7's**.
- Carbohydrate content is 3%.
- There are four antigenically distinct subclasses of IgG as **IgG1, IgG2, IgG3, IgG4 and IgG5.**

Biological Functions of IgG

1. It is only immunoglobulin that crosses the human placenta.

   Thus it gives protection to newborn for about 6-9 months.

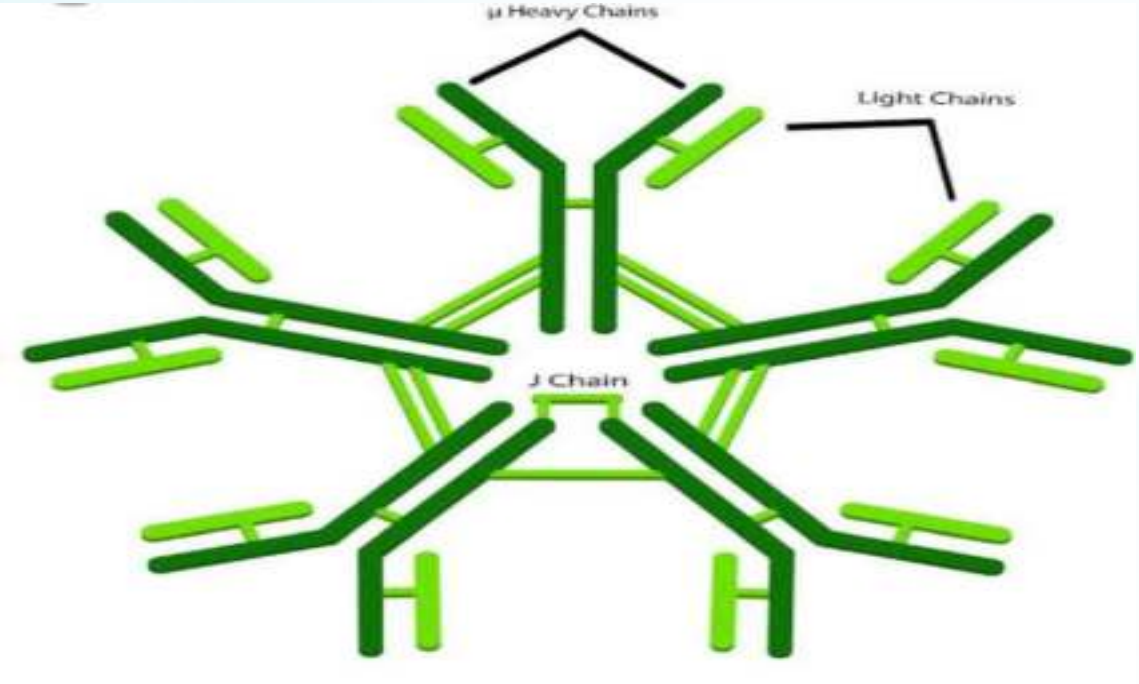
2. It activates classical complement pathway during Ag-Ab reactions.

3. It neutralizes toxins and viruses.

4. Works as opsonins during phagocytosis.

Immunoglobulin M (IgM)

- It is largest immunoglobulin.
- Molecular weight: 9,50,000 d (Macroglobulin).
- It is pentameric, each with four polypeptide chain.



- Concentration in serum is about 2-5 mg/ml.
- Half life period: 5 days
- Carbohydrate content: 10-12%.
- Sedimentation coefficient: 19s
- No subclasses of IgG.
- **It is earliest immunoglobulin to be synthesized by foetus.**
- The number of antigen binding sites on IgM are only 5.
- However, human and rabbit IgM contains 10 sites.

Biological Functions of IgM

1. It is the first antibody to appear in primary immune response but have short half life period.

   So it is used as indicator of recent infection.

2. Human foetus can synthesize IgM antibodies, if it's B cell are antigenically stimulated.

3. It has high functional affinity for multivalent antigens.

   e.g. It can react with polyvalent antigens as RBC's or ***E. coli*** cells.

4. Also shows properties such as opsonization, complement fixation, agglutination etc.

5. Because of its macromolecule size, it is located in blood and thus gives protection against blood infections.

6. ABO blood group antigens are of Ig M type.

Immunoglobulin A (IgA)

❖IgA is found only to some extent in blood serum, but predominantly in tears, saliva, colostrum and in secretions of respiratory, gastrointestinal and urinogenital tracts.

❖**Hence also known as secretory antibodies.**

❖ Second in abundance i.e. 10-15%.

❖ There are two subclasses of IgA as IgA1 and IgA2.

❖ Serum IgA is a monomer having structure similar to IgG.

❖ Molecular weight: 1,60,000d.

❖ But human secretary IgA is dimer linked by ‘J’ chain with mol.wt. 5,00,000d.

- IgA is synthesized by plasma cells in lamina propria of mucous membrane.
- Carbohydrate content: 10%
- Sedimentation coefficient: 7s
- Serum concentration: 0.6 to 4.2 mg/litre.
- Half life period: 6-8 days.
- Have 2 subclasses as IgA1 and IgA2.

Biological functions of IgA

1. Secretary IgA is termed as **mucosal paint or antiseptic paint of mucosal membrane.**
2. Neutralizes toxins and promotes phagocytosis.
3. **Found to produce immunity against tapeworms.**
4. **IGA is present in colostrum** and thus protect the baby from intestinal pathogens.

Immunoglobulin D (IgD)

- Has typical antibody structure with 4 polypeptide chains.
- It is slightly larger than Ig G with mol.wt. 1,80,000d.
- **Serum concentration**: 0.03 mg/litre.
- **Sedimentation coefficient**: 7s
- **Carbohydrate**: 12%
- Rate of synthesis is 0.4 mg/Kg body weight/ day.
- **Half life is 2-3 days**.
- There are 2 subclasses of IgD as IgD1 and IgD2.

Biological functions of IgD

1. IgD has not been shown to have antibody activity. So no direct role in the specific defence mechanism.
2. Acts as a receptors for antigens on the surfac of B lymphocytes.

Immunoglobulin E (IgE)

- It is a monomer with typical immunoglobulin structure.
- **Molecular weight**: 1,90,000d.
- It is heat labile immunoglobulin.
- It is found only in trace amount in serum, average serum level is 0.00004 mg/litre.
- But in persons with allergic conditions, it's level may be 50-100 times higher.
- **Half life period:** 2-3 days.
- **Sedimentation coefficient**: '8' s

- It has binding affinity for mast cells and basophilrs, which posses receptors for Fc region of IgE.
- Carbohydrate content: 12%
- It is usually known as '**skin sensitizing antibody**'.
- It is also known a "**reagin antibody**", as it is significant in allergic reactions.

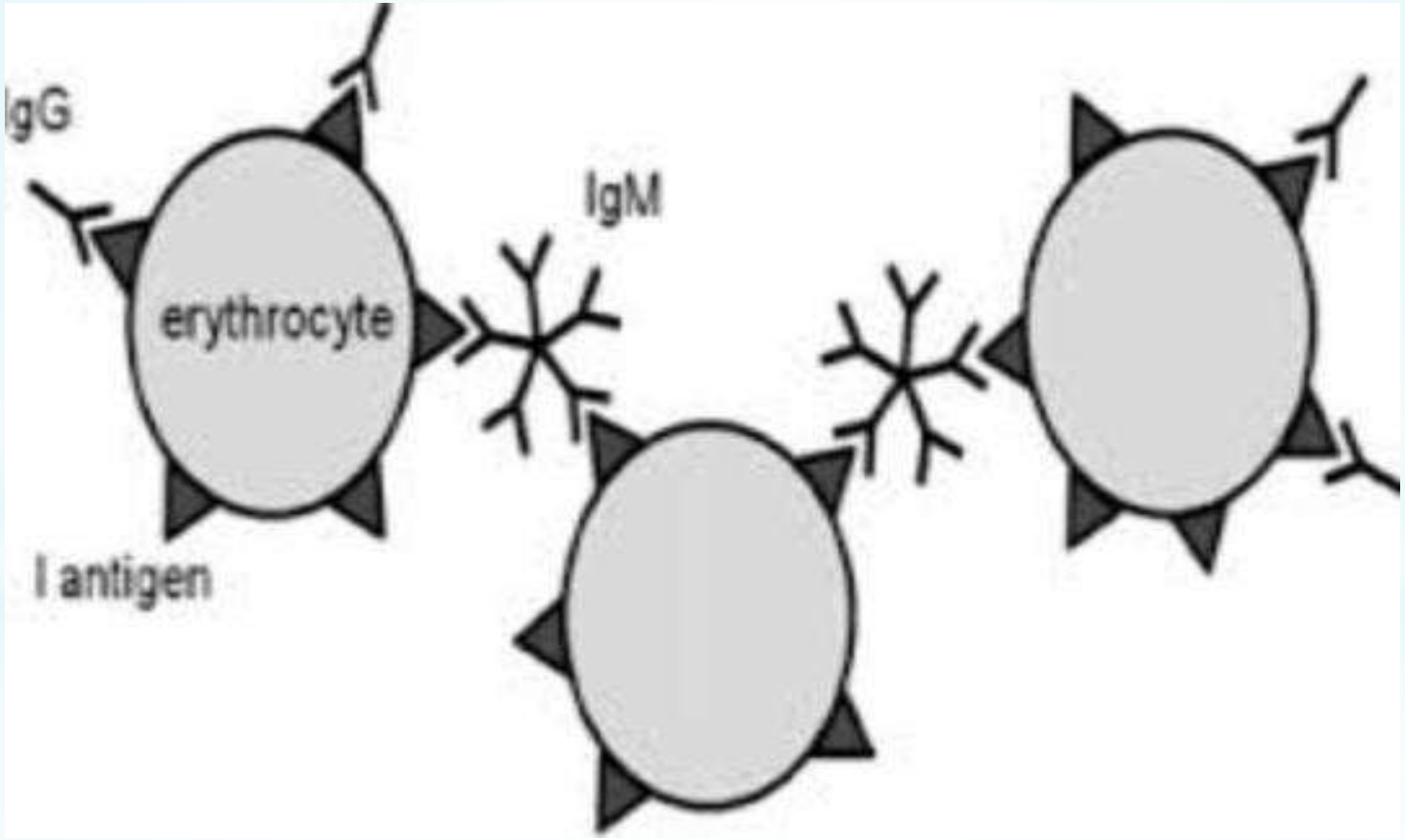
Biological functions of Ig E

1. Protective role in childrens with parasitic infections in intestine.

2. IgE mediates reaginic hypernsitivity (Type I hypersensitivity).

Antibodies can also be categorized on the basis of their functions:

1. Agglutinins:



2. Precipitins:

❖They combine with soluble antigens like tetanus toxins and this complex becomes insoluble, gets precipitated and toxin is inactivated.

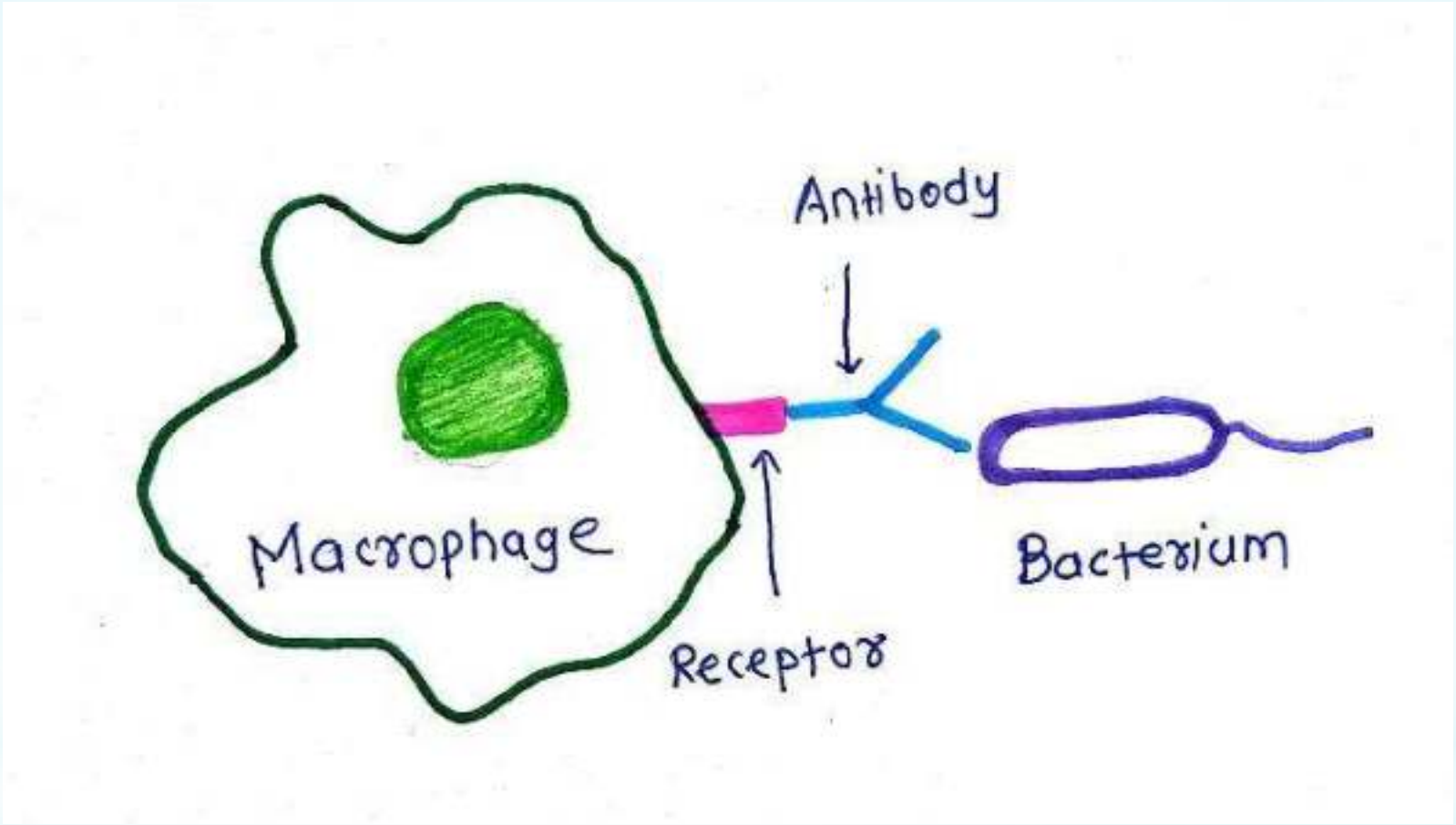
3. lysins:

Some antibodies can bind to cellular agents and causes their lysis.

4. Antitoxins:

Antibodies that neutralize the toxins of organisms.

5. Opsonins:



6. Complement fixing antibodies:

IMMUNE RESPONSE

Immune response

❖ When any foreign substance enter the body, the body reacts or responds to it and this response is called immune response.

Immune response is of two types:

1. **Humoral immune response**
2. **Cell mediated immune response**

1. Humoral immune response:

- It involves production of antibodies in response to specific antigen.
- It provides **primary defence** against infection.
- Also participates in **hypersensitivity reactions** and **autoimmune disorders**.

Cell mediated immune response:

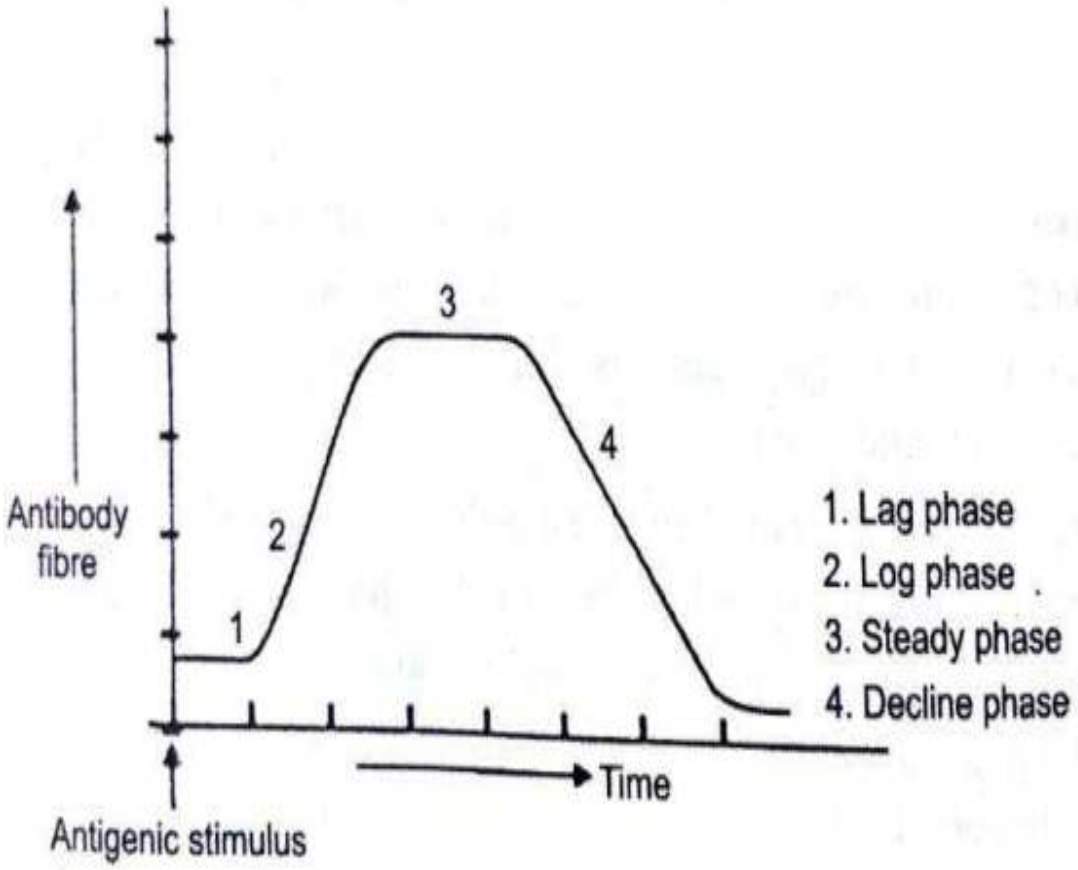
- Large number of activated lymphocytes (T cells) are produced to destroy foreign organisms.
- It provides defence against **fungi, viruses** and **intracellular bacterial pathogens.**
- Also participates in delayed hypersensitivity and autoimmune diseases.

Primary immune response

The humoral immune response produced by body to the initial antigenic stimulus (i.e. primary dose of antigens).

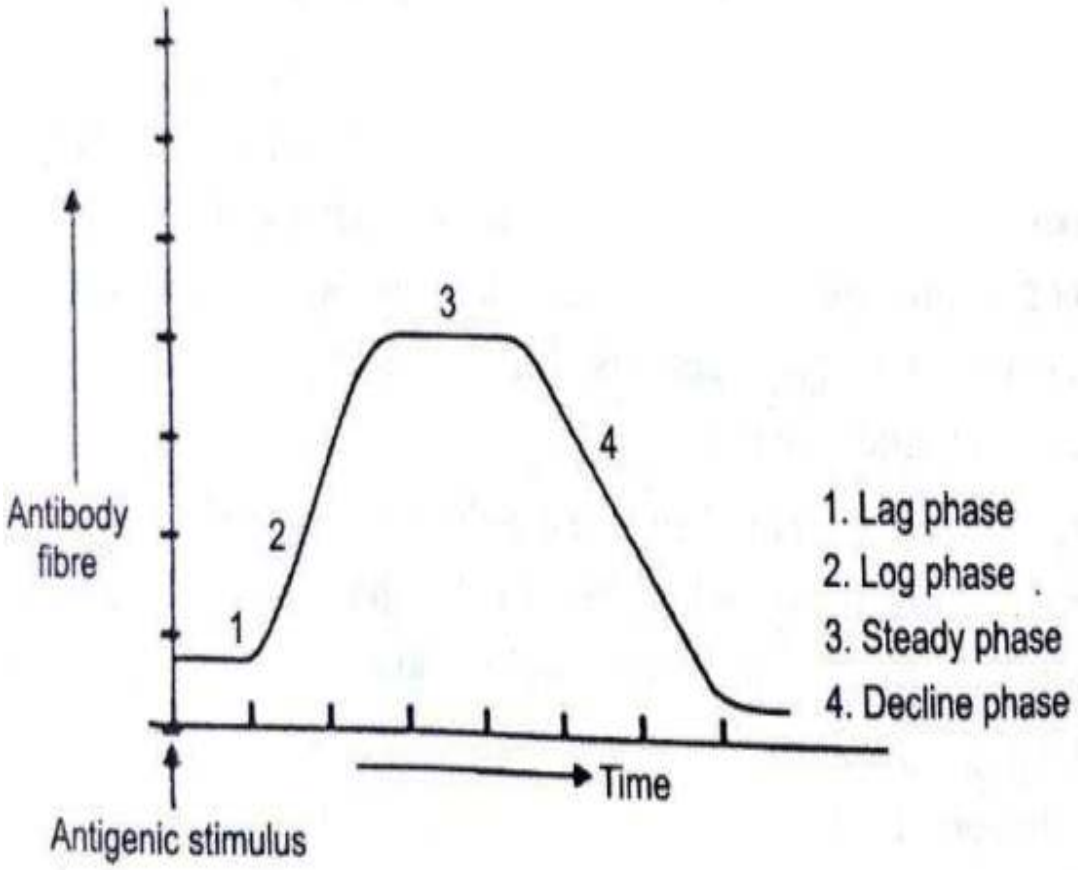
It is characterized by 4 phases:

1. **Lag phase**
2. **Log phase**
3. **Plateau phase**
4. **Decline phase**



1. Lag phase:

- Immediately after injection of antigen.
- No antibody production.
- Also known as latent or induction period.
- Duration varies from several hours to days.
- It depends upon **nature and amount of antigen, route of administration of antigen, species and health of animal.**



2. Log phase:

- **Steady rise in the titre of antibodies.**

3. Plateau:

- **Antibody titre is steady.**

4. Decline phase:

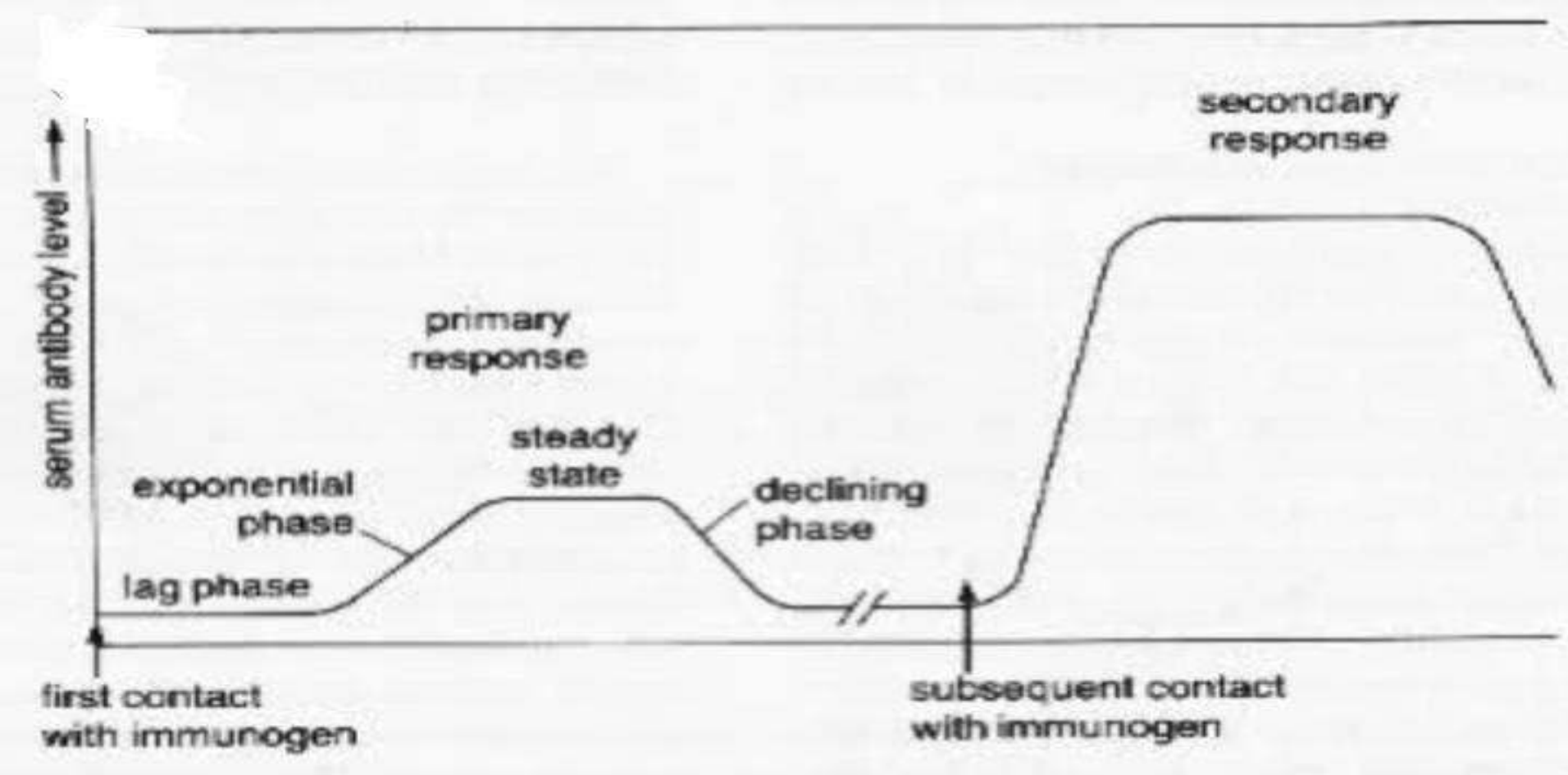
- **Catabolism of antibodies is more than production of antibodies.**

Secondary immune response

- **Immune response against same pathogen that previously infected the same animal body.**
- It is subsequent immune response.
- It differs from primary immune response both qualitatively and quantitatively.

Characteristics of Secondary Immune response:

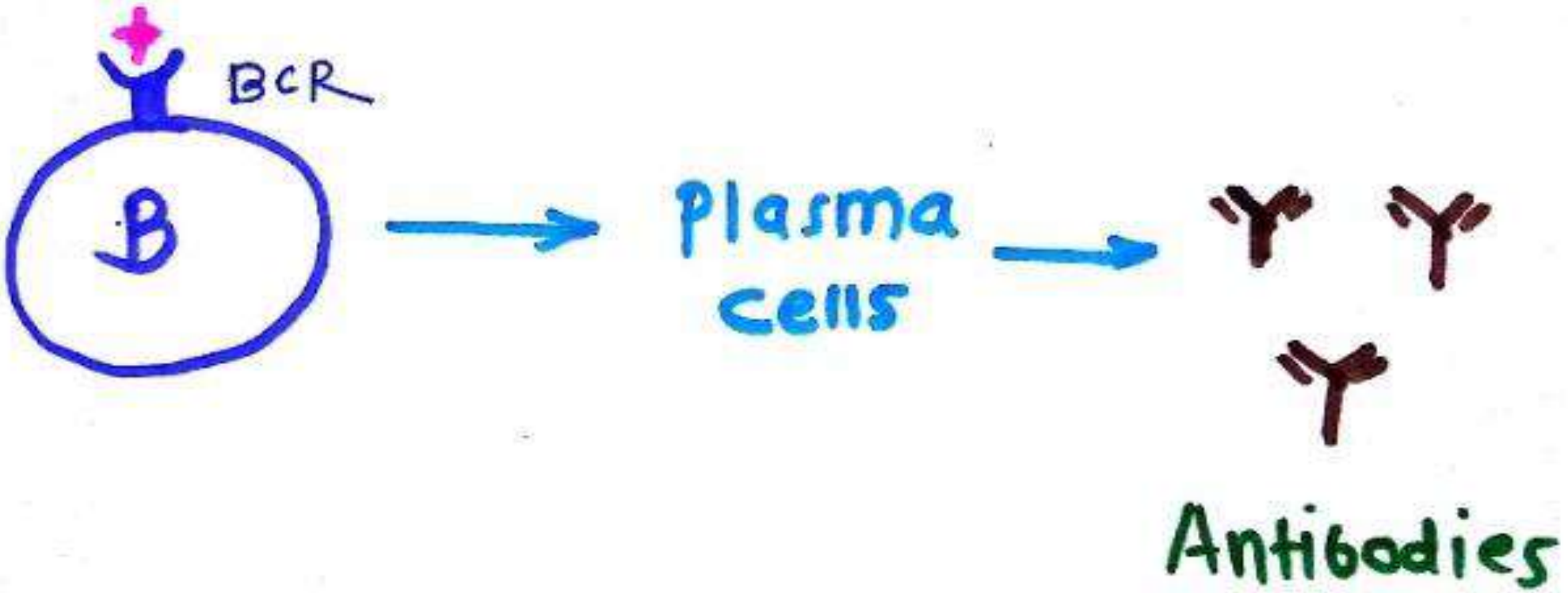
1. Animal **responds very quickly**.
2. **Very short or negligible lag phase**.
3. Following lag phase, there is **sharp increase in antibody** content of blood than prim. immune response.
4. Much **extended plateau period**.
5. Plateau period followed by decline phase.

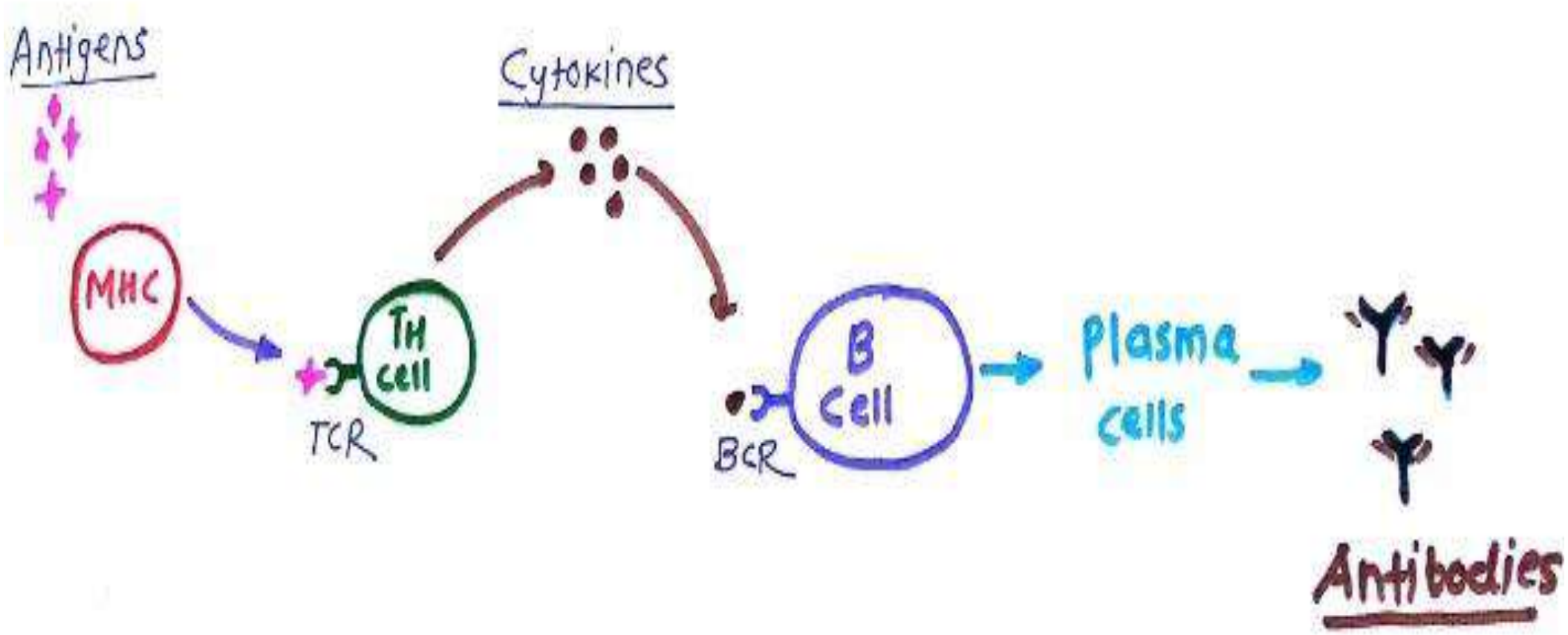

serum antibody level
secondary response
primary response
steady state
exponential phase
declining phase
lag phase
first contact with immunogen
subsequent contact with immunogen

Difference between primary and secondary immune response

Primary Immune response	Secondary immune response
Slow and short lived	Rapid and long lived
Long latent period	Negligible latent period
Antibody tire is less than secondary.	Antibody tire is more than Primary.
Decrease in antibody titre is less gradual in decline phase.	Decrease in antibody titre is more gradual in decline phase.
Negative phase absent	Negative phase present
There is induction of immuno-competent cells for production of antibodies.	There is induction of large amount of antibody production.

Formation of antibodies




Antigens
MHC
TH cell
TCR
Cytokines
BCR
B Cell
Plasma cells
Antibodies

Factors affecting immune response

1. Feedback inhibition:

Passive administration of antibodies for that antigen simultaneously, before or after injection of antigen, suppresses the immune response.

2. Nutritional status:

3. Age:

4. Genetic factor:

- Person who responds to particular antigen is called '**responder**'.
- Person who do not responds is called '**non-responder**'.

5. Dose of antigen:

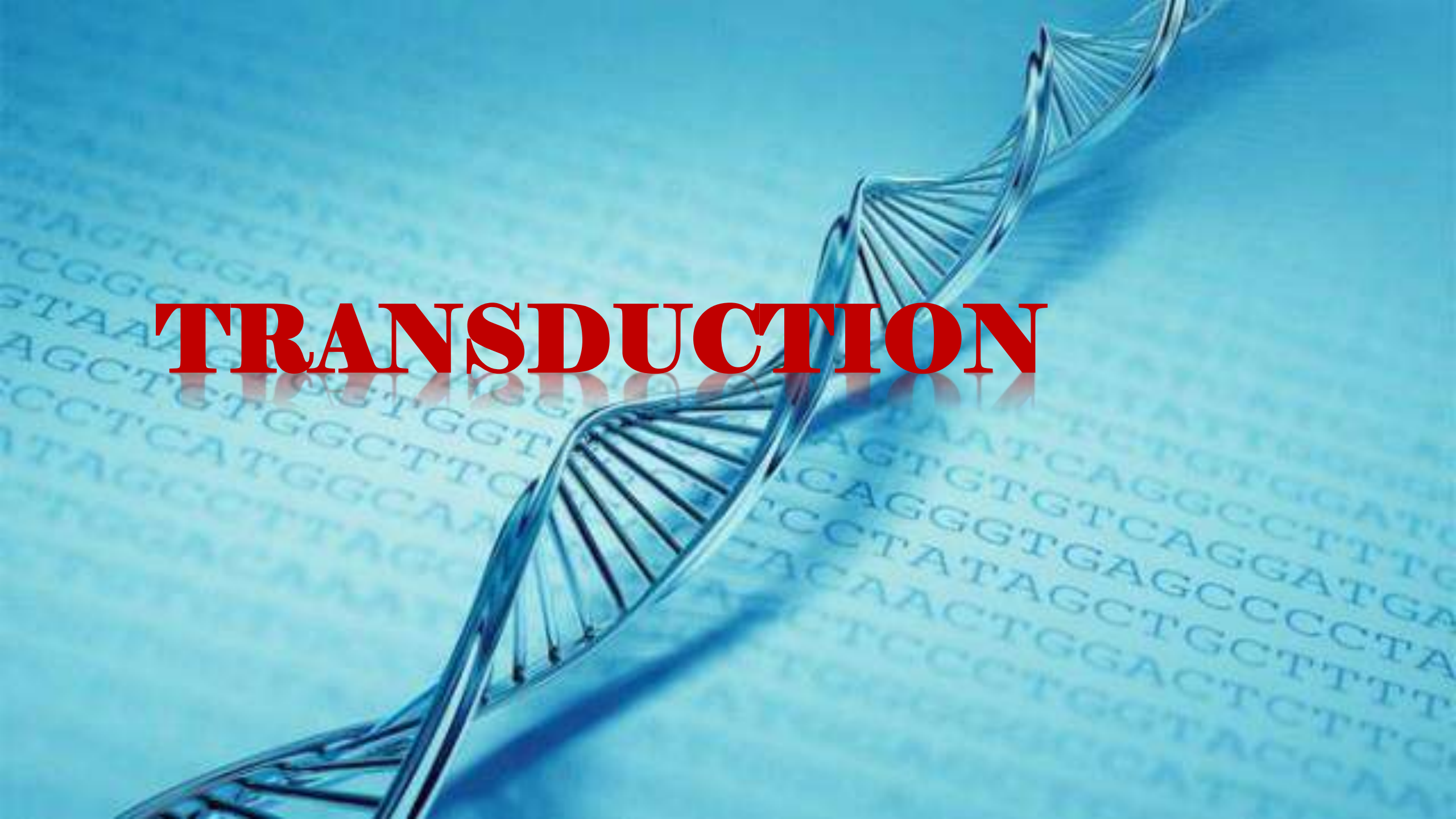
6. Route of administration:

7. Nature of antigen:

8. Multiple antigens

Prof. Suraj Dipak Gabale

(Assistant Professor)
Department of Microbiology,
Vivekanand College, Kolhapur.


TRANSDUCTION

- **Definitions**:
- 1) The transfer of genetic material from one bacterium to another through bacteriophages is called **Transduction**.

- **2)Transduction** is the process by which foreign DNA is introduced into a cell by a virus or viral vector.

HISTORICAL

Lederberg & Zinder

- Transduction was first discovered in 1952 by Joshua Lederberg and Norton Zinder

Joshua Lederberg

Norton Zinder

- **Discovery:**
- N. Zinder and J. Lederberg (1952)
- Auxotrophic strains of *Salmonella typhimurium*

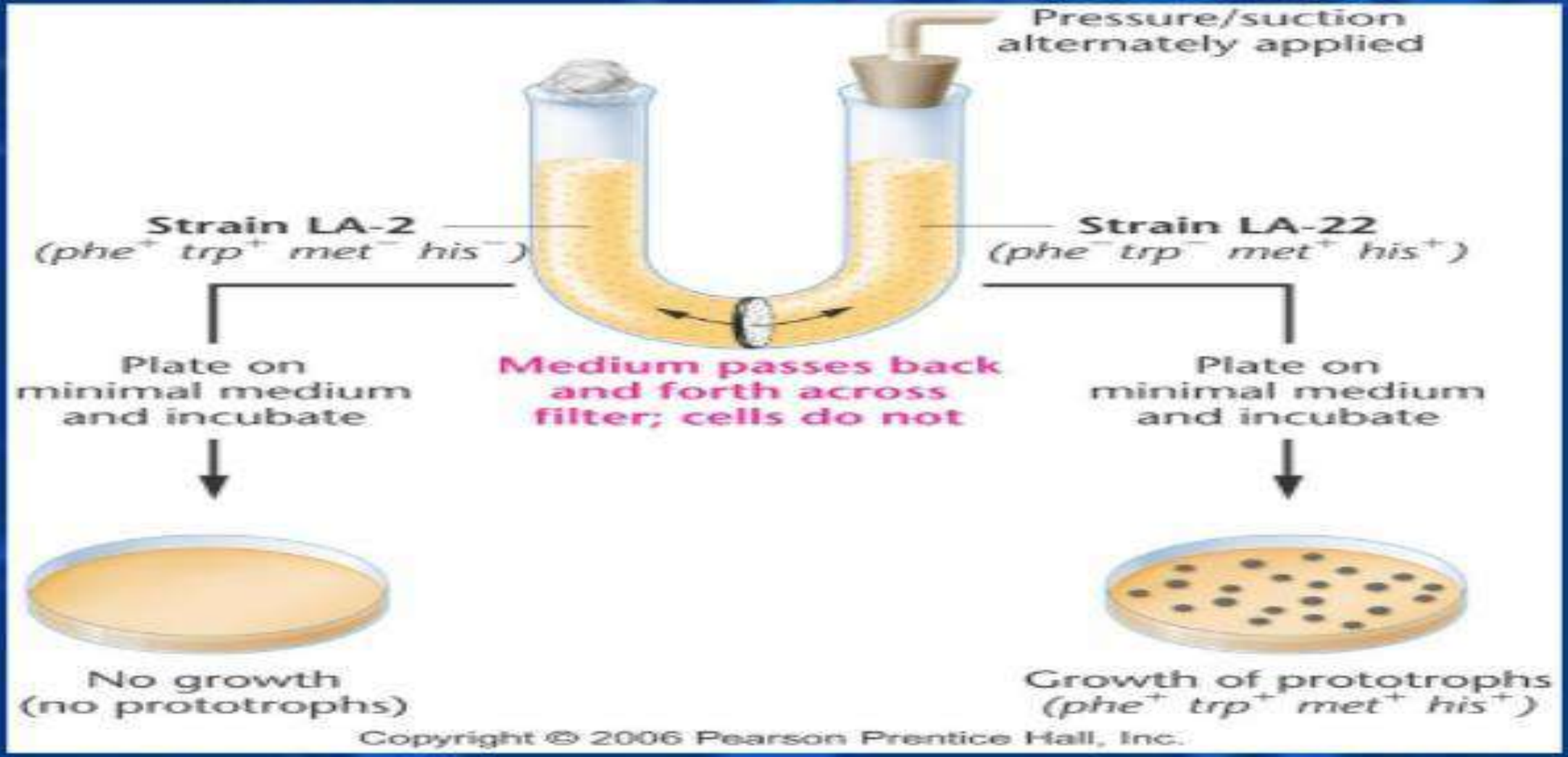
  LA_{22}: Phe^{-} Trp^{-} Met^{+} His^{+}

  LA_{2}: Phe^{+} Trp^{+} Met^{-} His^{-}
- *Davis U tube- Bacteria proof filter*

  -Prevents transfer of bacterial cells

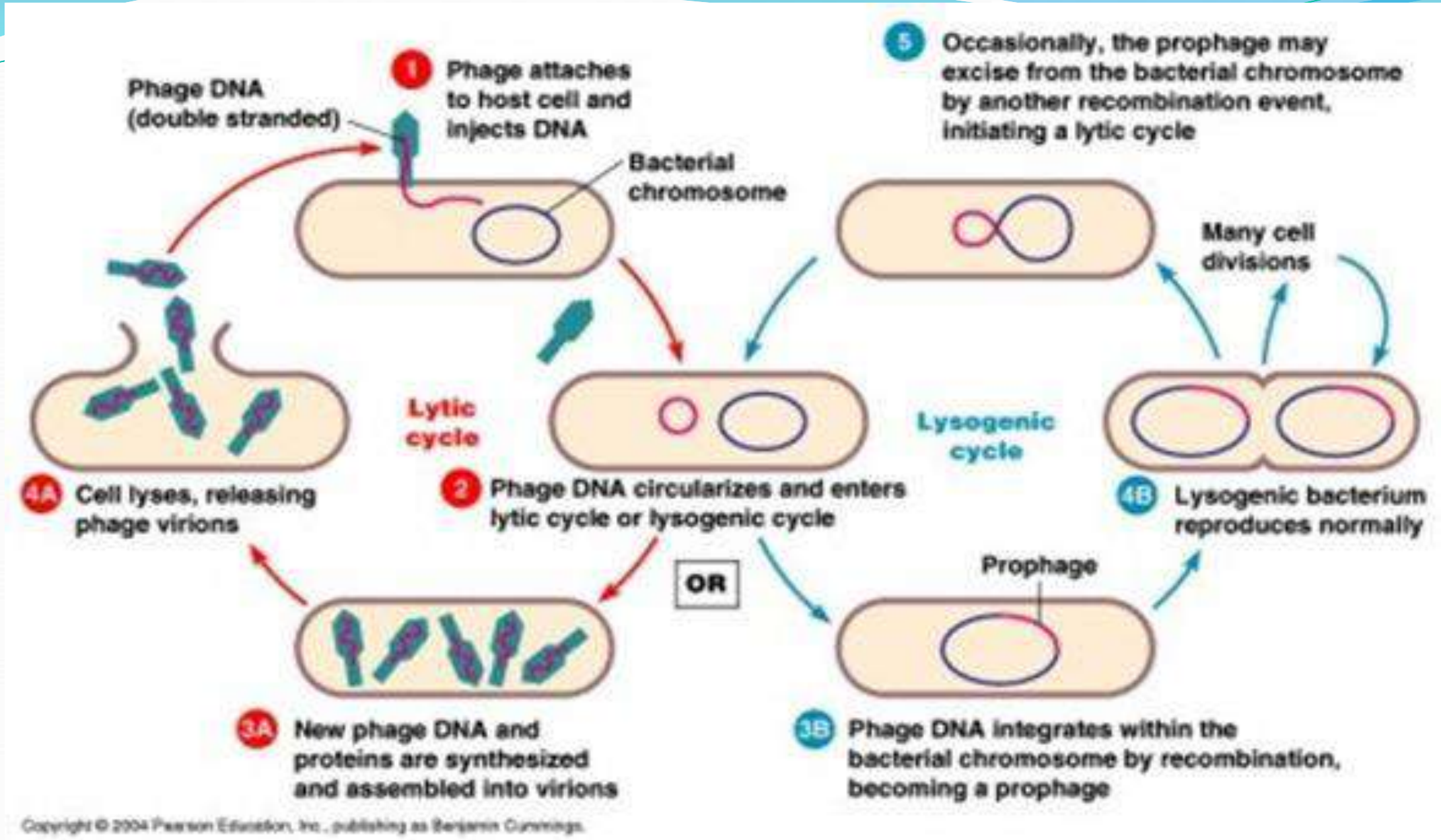
  -Allows passage of nutrients(smaller than 0.1 μm)

U-TUBE EXPERIMENT

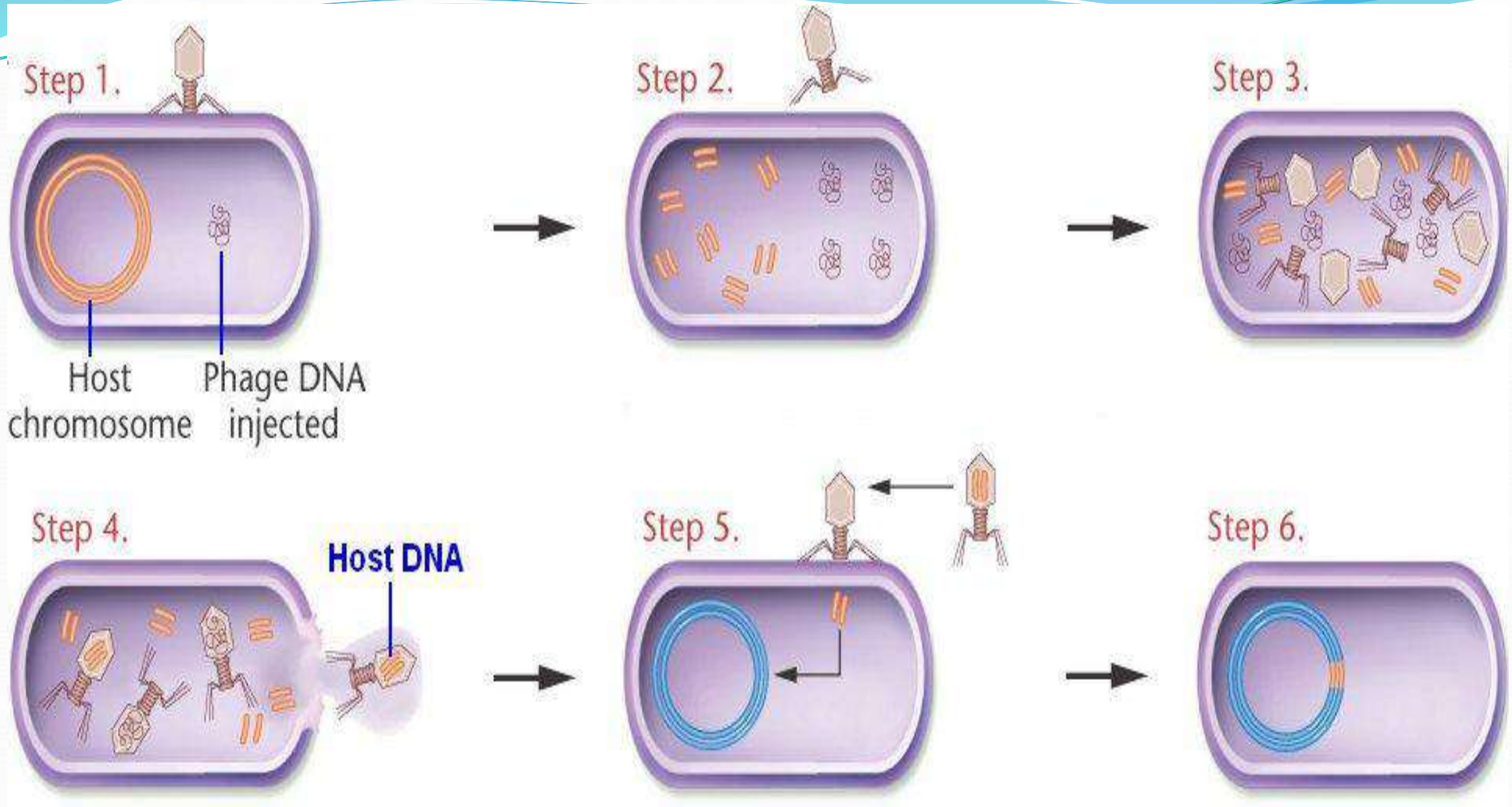


P22 pahge- Lysogenic/ temperate phage of *S. typhimurium* strain $LA_{22.}$

- **Lysogeny**- Stable relationship between bacteriophage and host cell. (No lysis of host)
- **Lytic phage**- Phage which causes lysis of host upon infection.



- P22 phage-
- Lysogenic state -*S. typhimurium* strain LA22 (rarely undergoes lytic cycle)
- Lytic (Non-lysogenic) - LA2.
- This phage passes through filter and infect LA2 strain, where they undergo another lytic cycle and produces new phage particles.
- During assembly instead of viral DNA, fragment of bacterial chromosome is packed.
- Such phage containing fragments of host chromosome pass through the filter and inject DNA into LA22 strain.


Step 1.
Host chromosome
Phage DNA injected
Step 2.
Step 3.
Step 4.
Host DNA
Step 5.
Step 6.

❖Occurance:

-Various members of enteric group of bacteria

-*Pseudomonas*, Staphylococcus, Bacillus

-Temperate phages:

Ex- λ- *E. coli*, P22-*S. typhimurium*,

- **Types of Transduction:**
- 1. Specialised/restricted transduction:

  This is the transduction in which phages transfer only a few restricted genes from one cell to another cell.
- 2.Generalized transduction:

  This is the transduction in which phages transfer any fragment of bacterial DNA from one cell to another cell.

Specialized Transduction

- Also called Restricted transduction as only restricted genes are transferred from one cell to another.
- Example- λ and Ø 80 (Lysogenic phages)
- These phages integrates their genome in host genome at specific sites. But sometimes under certain inductions they detaches from host genome.
- During detachment they take some gene portion of host genome and leaves some of their own genes into host genome.
- Such phage is called Transducing Phage.

❖When such transducing phage containing host gene infects another host then it transfers those restricted genes to secondary host.

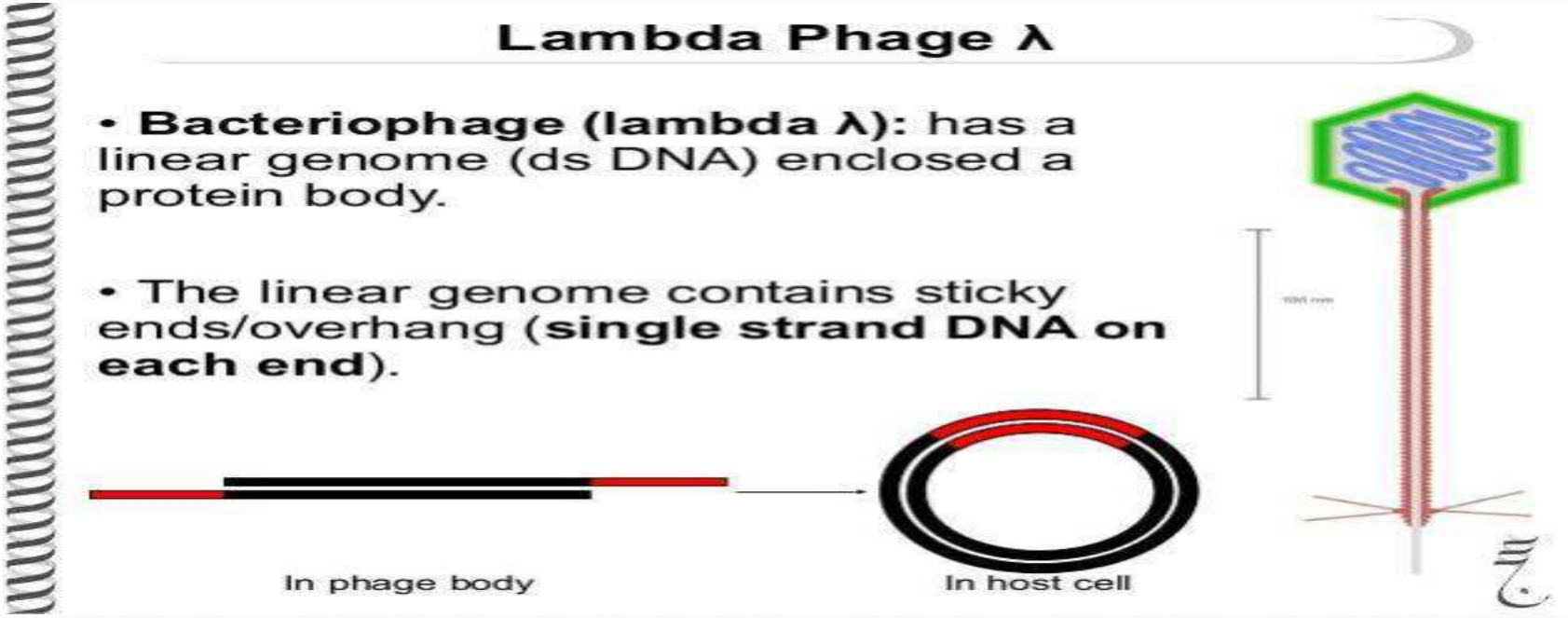
SPECIALIZED/ RESTRICTED TRANSDUCTION IN λ PHAGE:

It is a temperate phage of *E.coli k12.*

λ pahge :

Genome- Linear, ds DNA with single stranded projections(12 nucleotides) at each ends.

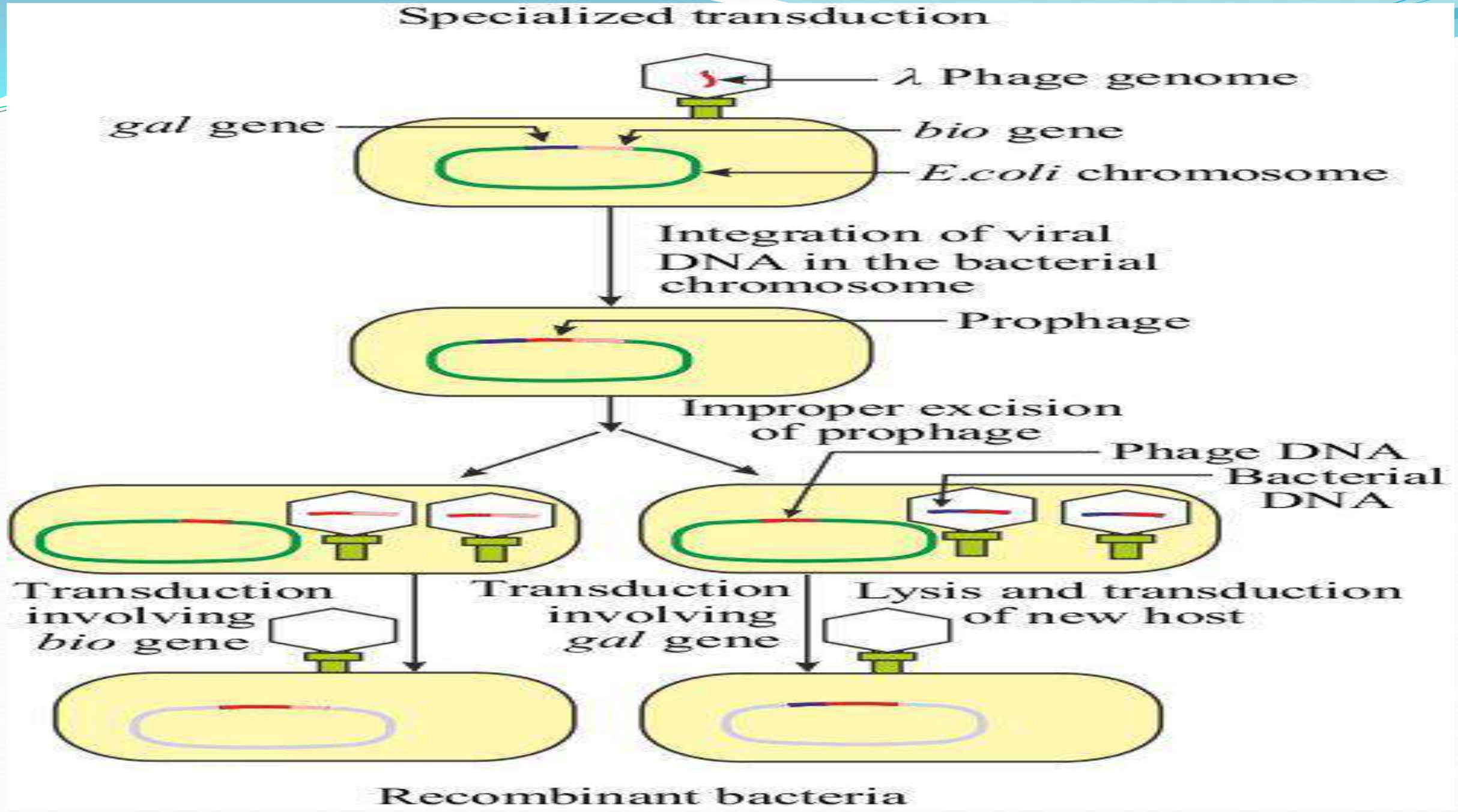
12 Nbp sequence-GGGCGGCGACGT



•After penetration lambda genome becomes circularised using its cohesive ends.

Circular Lambda phage integrates in host chromosome by crossing over.

site of integration is highly specific i.e. integrates between Biotin synthesis and Galactose utilizaton genes.


Specialized transduction
λ Phage genome
gal gene
bio gene
E.coli chromosome
Integration of viral DNA in the bacterial chromosome
Prophage
Improper excision of prophage
Phage DNA
Bacterial DNA
Transduction involving bio gene
Transduction involving gal gene
Lysis and transduction of new host
Recombinant bacteria

❑Excision of phage DNA(Detachment) :

*Under certain inductions prophage enters lytic cycle.

*Detaches from host genome. But during detachment it takes either Galactose or Biotin gene along with it and leaves some of its genes on E.coli chromosome.
Such phage is called Defective phage.

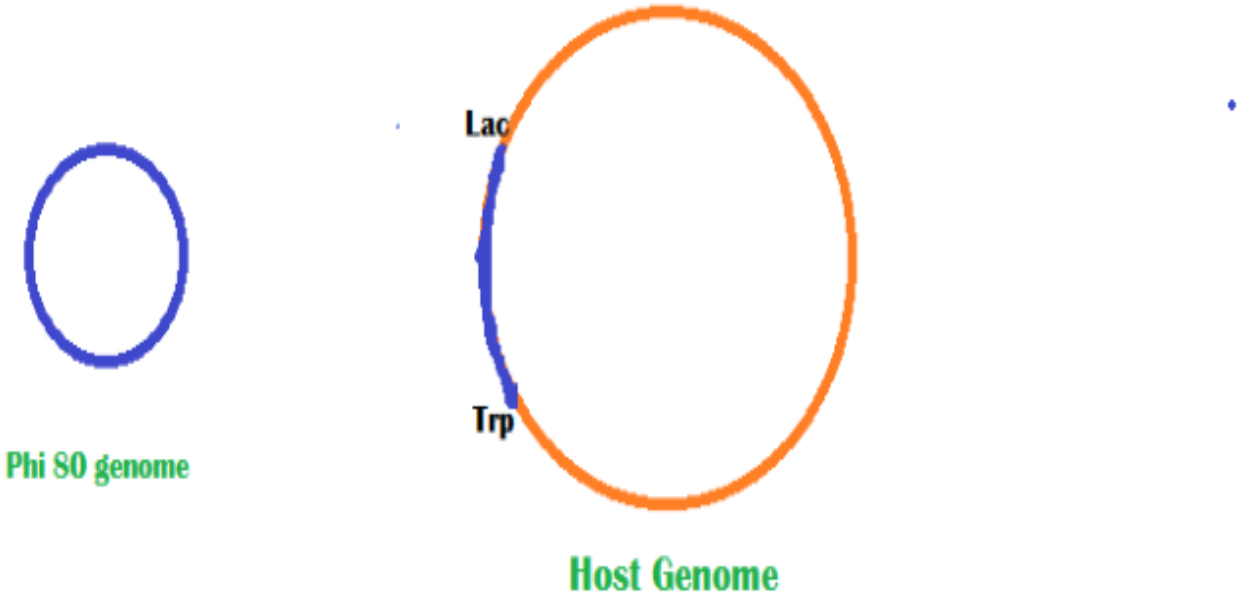
*If defective phage carry gene for galactose utilization, they are designated as λ d gal whereas if they carry Biotin gene, they are designated as λ d bio.

*The size of DNA packed in a phage head is fixed.

*When defective phage infects another host cell, they cannot replicate until super infection by same phage. So, super infecting normal phage is called "Helper" phage.

Specialized transduction in Ø80 phage-

Host- *E. coli*



Integration site- Between Lactose utilization genes or Tryptophan synthesizing genes. Resulting phage is either λ d trp or λ d lac.

GENERALIZED TRANSDUCTION:

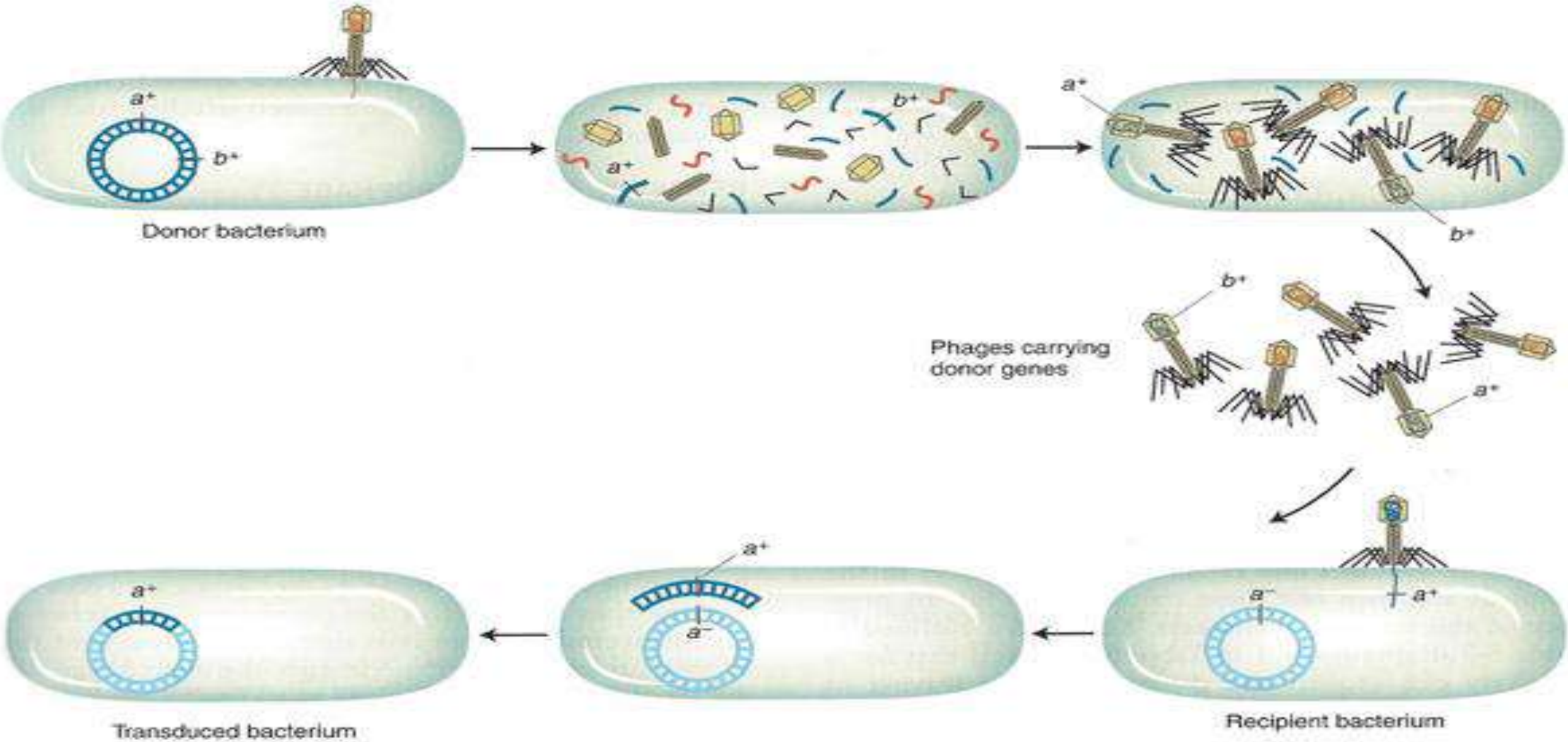
- This is the transduction in which phages transfer any fragment of bacterial DNA from one cell to another cell.
- Example- P1 and P22 phage

- It occurs only during Lytic cycle of Virulent as well as temperate phage.
- After infection viral genome may or may not integrate in host chromosome. If integrate then site of infection may not be fixed.
- It means each and every gene has equal chance of getting transferred from one cell to another.

GENERALIZED TRANSDUCTION IN P1 PHAGE:

- Host: *E. coli*
- Genome: dsDNA
- Many times does not integrate genome in host chromosome but multiplies separately.
- After penetration of DNA in host it degrades host genome into fragments.
- Phage DNA replicates and also transcribed to produce mRNA for capsid protein production.
- During assembly (Maturation) phage DNA is packed in head, but sometimes it packs host gene fragment.
- Such phage(carrying host genes) when infects another host then it transfer the genes from previous host.
- Such phage is called as "Generalized transducing phage"
- Frequency of Generalized Transduction: One in 10^6 TO 10^7 cells.

Phage P1 can cause generalized transduction



GENERALIZED TRANSDUCTION IN P22 PHAGE:

- Host: *Salmonella typhimurium*
- Genome: dsDNA
- Integrates its genome in host genome, but integration site is not fixed.
- After induction detaches from hot genome and takes adjacent gene sequence from host.
- Such phage(carrying host genes) when infects another host then it transfer the genes from previous host.

ABORTIVE TRANSDUCTION:

- Transduction in which the Phage genome has no capacity of integration into the host genome and also lacks genes for its own replication then it remains as it is in the host cell.
- When host cell divides then it will be transferred to only one of the daughter cell. In this way it gets diluted from generation to generation.
- This is known as Abortive Transduction.

PHAGE CONVERSION

- Temperate phage induces change in the phenotype of infected bacteria. This is referred as Phage conversion or Lysogenic conversion.
- lysogenic conversion lasts only as long as phage or prophage is present in the host cell.
- Some lysogenic phage carry genes that can enhance the virulence of the bacterial host.
- For example, some phage carry genes that encode toxins. These genes, once integrated into the bacterial chromosome, can cause the once harmless bacteria to release potent toxins that can cause disease.

Bacteriophages: Lysogenic Conversion

Examples of Virulence Factors Carried by Phage

Bacterium	Phage	Gene Product	Phenotype
Vibrio cholerae	CTX phage	cholerae toxin	cholera
Escherichia coli	lambda phage	shigalike toxin	hemorrhagic diarrhea
Clostridium botulinum	clostridial phages	botulinum toxin	botulism (food poisoning)
Corynebacterium diphtheriae	corynephage beta	diphtheria toxin	diphtheria
Streptococcus pyogenes	T12	erythrogenic toxins	scarlet fever


QUESTIONS?


Thanks!

BACITRACIN

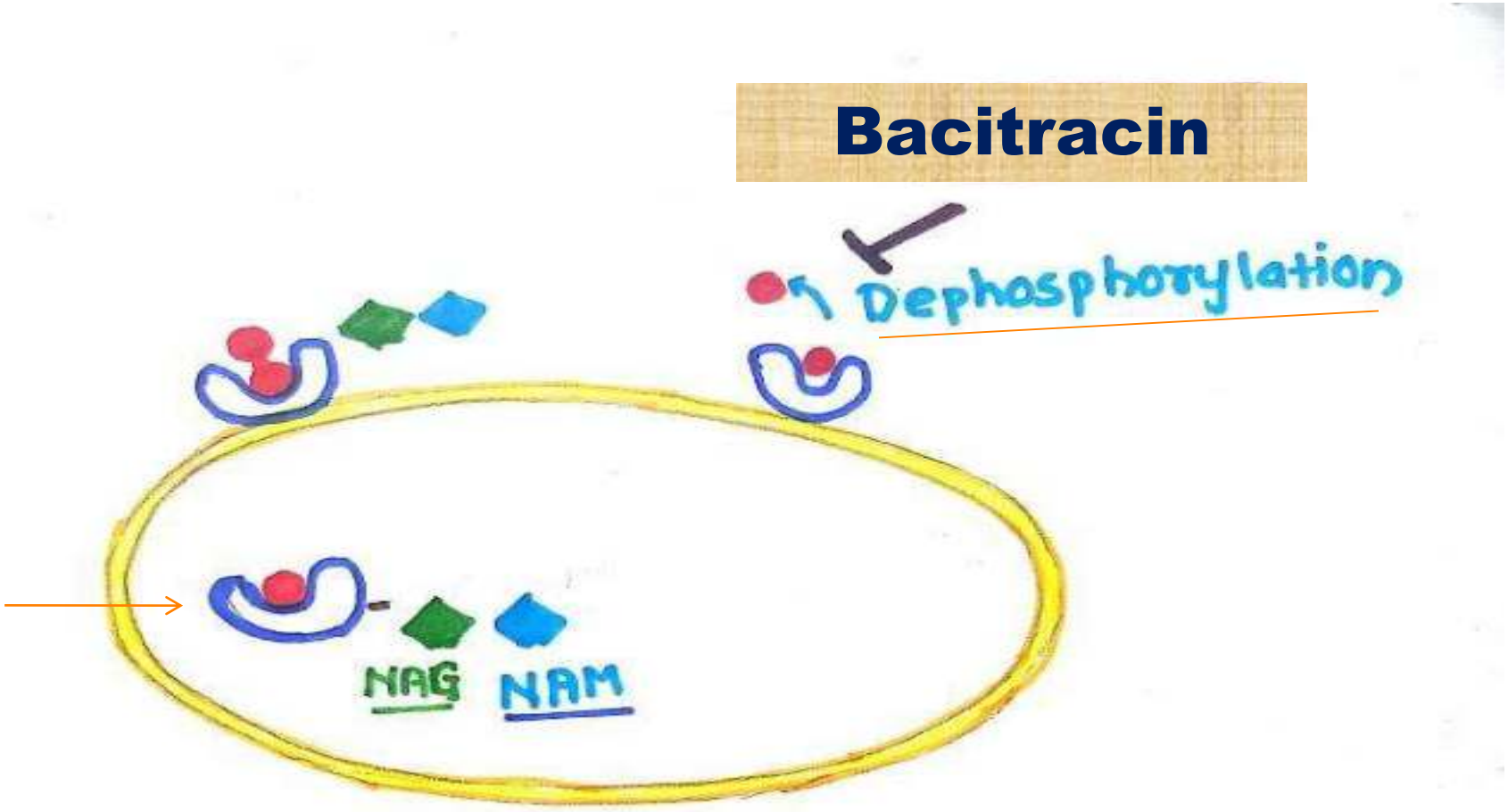
Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

Bacitracin

- It is a cyclic peptide antibiotic.
- Produced by ***Bacillus subtilis.***
- First isolated in 1945.
- Inhibits synthesis of the bacterial cell wall by preventing the **transport of peptidoglycan precursors** through the cell membrane.

Mechanism of action

Bacitracin



Bactoprenol

Uses of Bacitracin

- In combination with other antibiotics (Like polymyxin B and neomycin) used as an ointment.
- For treatment o skin and eye infection.

Side effects

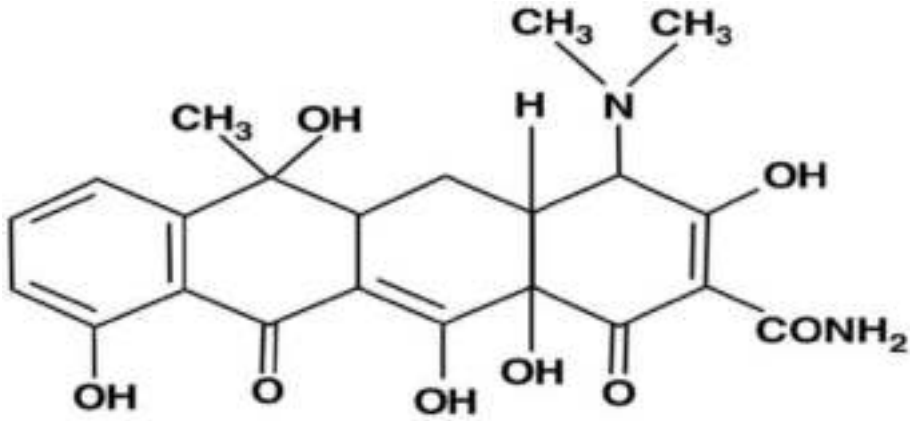
- Potent allergen
- Nephrotoxic

TETRACYCLINE

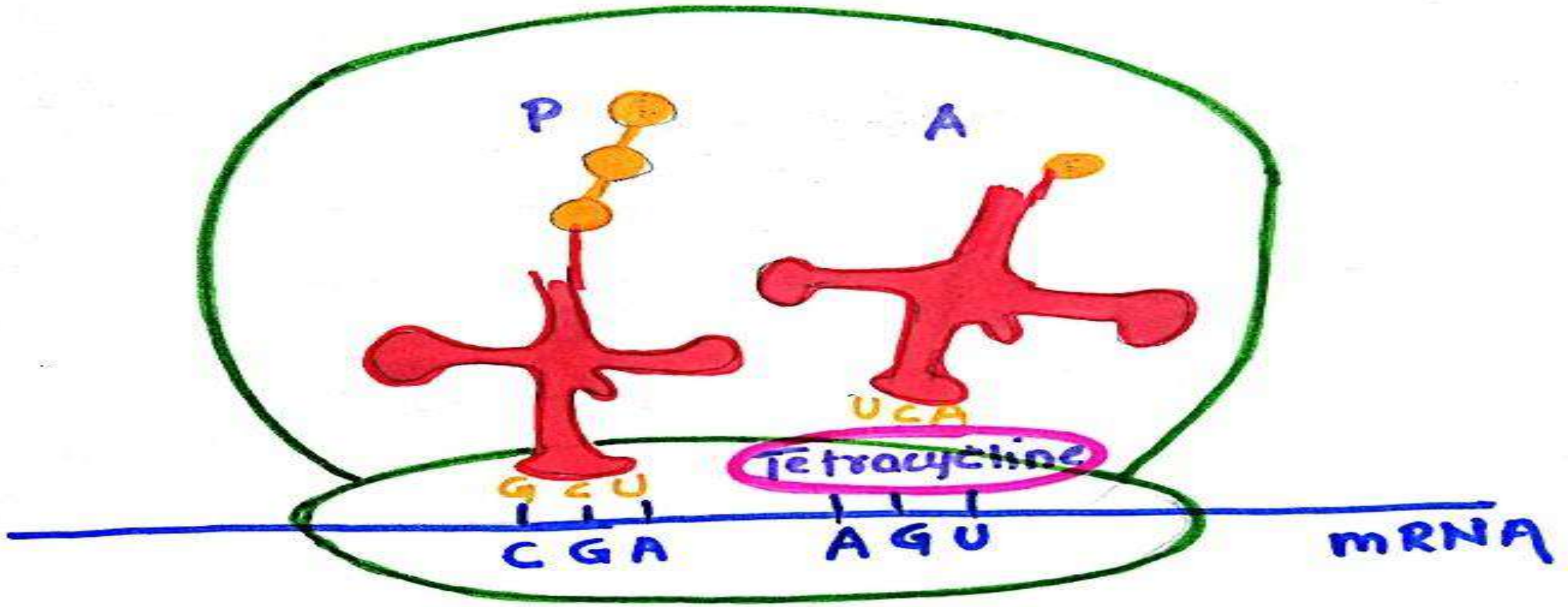
Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

Tetracycline

- It is an antibiotic used for treatment wide variety of bacterial infections.
- **Inhibits protein synthesis.**
- **Bacteriostatic**
- Taken by mouth
- Originally made from Streptomyces species.
- Brand name '**Sumycin**'.



Mechanism of action



Uses of Erythromycin

- Used to treat infections of the skin, intestines, respiratory tract, urinary tract, genitals, lymph nodes and other body organs.

Adverse effects

- Nausea, vomiting, diarrhea
- Fever, chills, body aches
- Swollen tongue

THANK YOU!!

DRUG RESISTANCE

Drug resistance

❖ **Definition**: The ability of bacteria and other micro-organisms to withstand (resist) a drug that would kill it or limit its growth

Paul Ehrlich (1907)- First time observed drug resistance.

❖ **Cross resistance**:

Resistance shown by organism towards structurally or chemically related drugs

Ex- Resistance to Chloramphenicol and Erythromycin

❖ **Multidrug resistance**:

Resistance shown by organism towards number of drugs (structurally or chemically unrelated)

Ex- *Shigella flexneri- R*esistant to 8 different drugs

Types of drug resistance

1) Pre-existing drug resistance:

Resistance to drug from beginning.

Ex- *Staphylococus aureus*: Resistance to penicillin at the introduction of drug
Now resistance has increased upto 85%

2) Transmissible drug resistance:

Several bacteria carries 'R plasmid'.
R plasmid- contains 2 components
a. Transfer factor (T)
b. Drug resistance factor (R)

Antibiotic resistance genes are also located on genetic element other than plasmid

Ex- Many composite transposons- contains one or more resistance genes

Table:Transposons carrying genes for antibiotic resistance

Sr. No.	Transposon	Antibiotics
1	Tn 5	Kanamycin, Bleomycin, Streptomycin
2	Tn 9	Chloramphenicol
3	Tn 10	Tetracycline
4	Tn 21	Streptomycin, Spectinoycin, Sulfonamide
5	Tn 551	Erythromycin
6	Tn 4001	Gentamycin, Kanamycin

3) Mutational drug resistance:

- Resistance acquired due to spontaneous mutations in bacterial chromosomes.
- Very rare

Mechanism of drug resistance

1) Blocking of drug transport/preventing entry of drug:
2) By pumping the drug out of the cell:
3) Alteration of target site:
4) Inactivation or detoxification of drug

1) Blocking of drug transport/preventing entry of drug:

Change in permeability of membrane makes organism resistant

Examples-

a. Mycobacteria:

High concentration of mycolic acid in cell wall makes many drugs impermeable.

b. Many Gram negative bacteria:

Unaffected by Penicillin G as it can not penetrate outer membrane

c. *Staphylococcus aureus*:

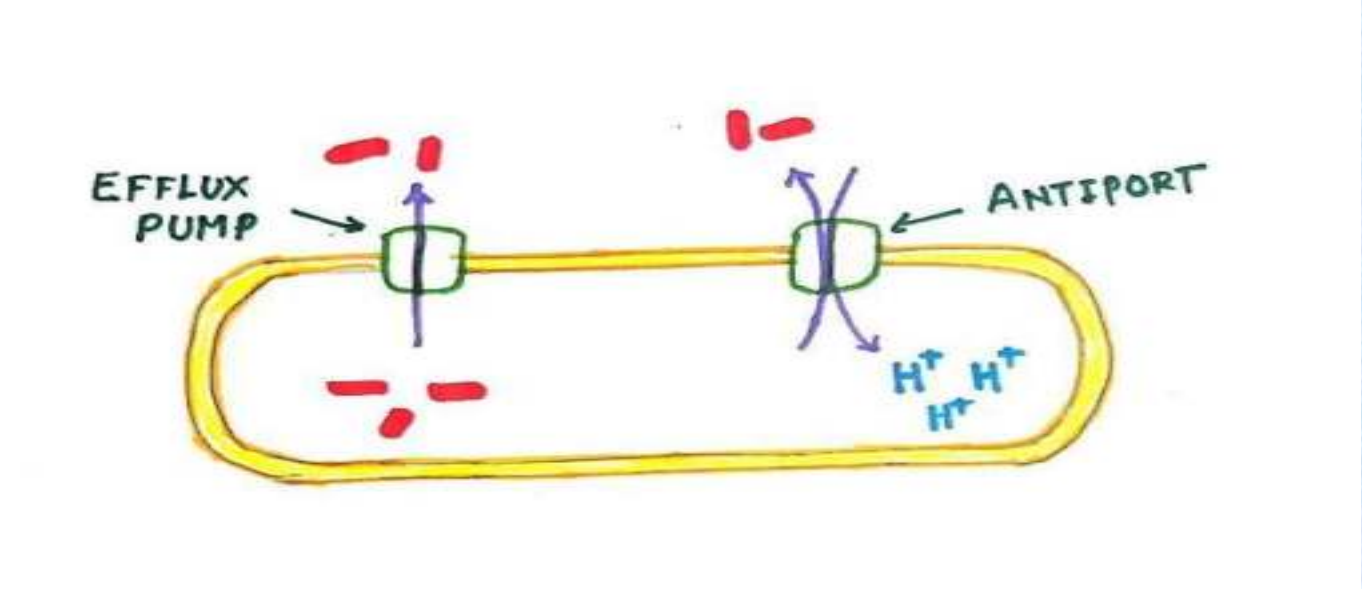
Produces **Tet** protein which binds to receptor site for tetracyclin

2) By pumping the drug out of the cell:

Some pathogens have plasma membrane translocase.

❖ **Translocase:**

- Also called Efflux pump that expel drug out.
- Nonspecific
- Multidrug resistance pump



Examples: *E.coli, Pseudoonas aeruginosa, Mycobacterium smegmatis, Staph. aureus* etc.

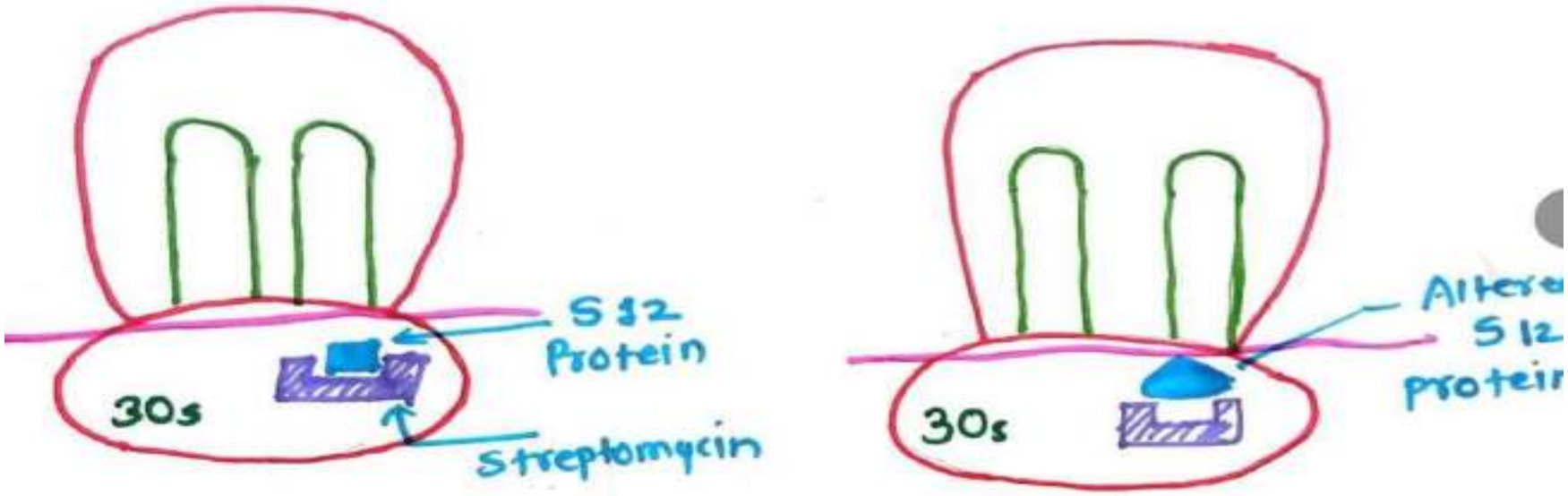
3) Alteration of target site:

Each drug have specific target site.

If target site is altered, then organisms becomes resistant to that drug.

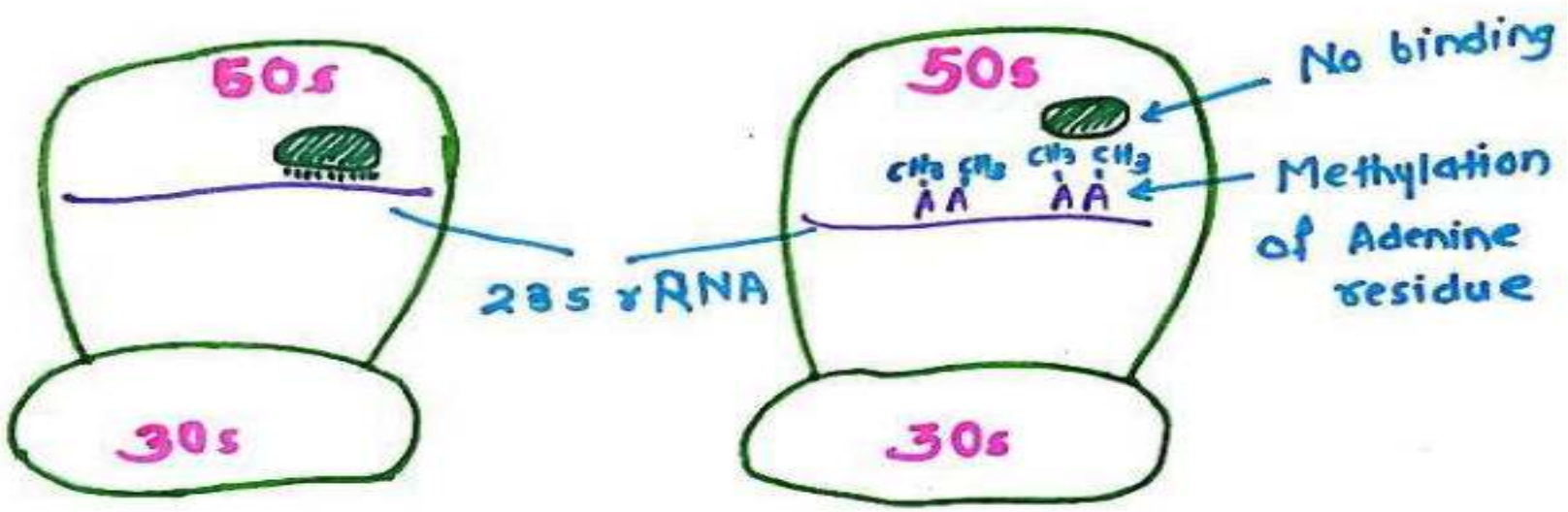
Examples:

a. Streptomycin resistance:



b. Erythromycin and Lincomycin:

- Acts on protein synthesis.
- Binds to 23s rRNA of 50s ribosomal subunit and inhibit protein synthesis.



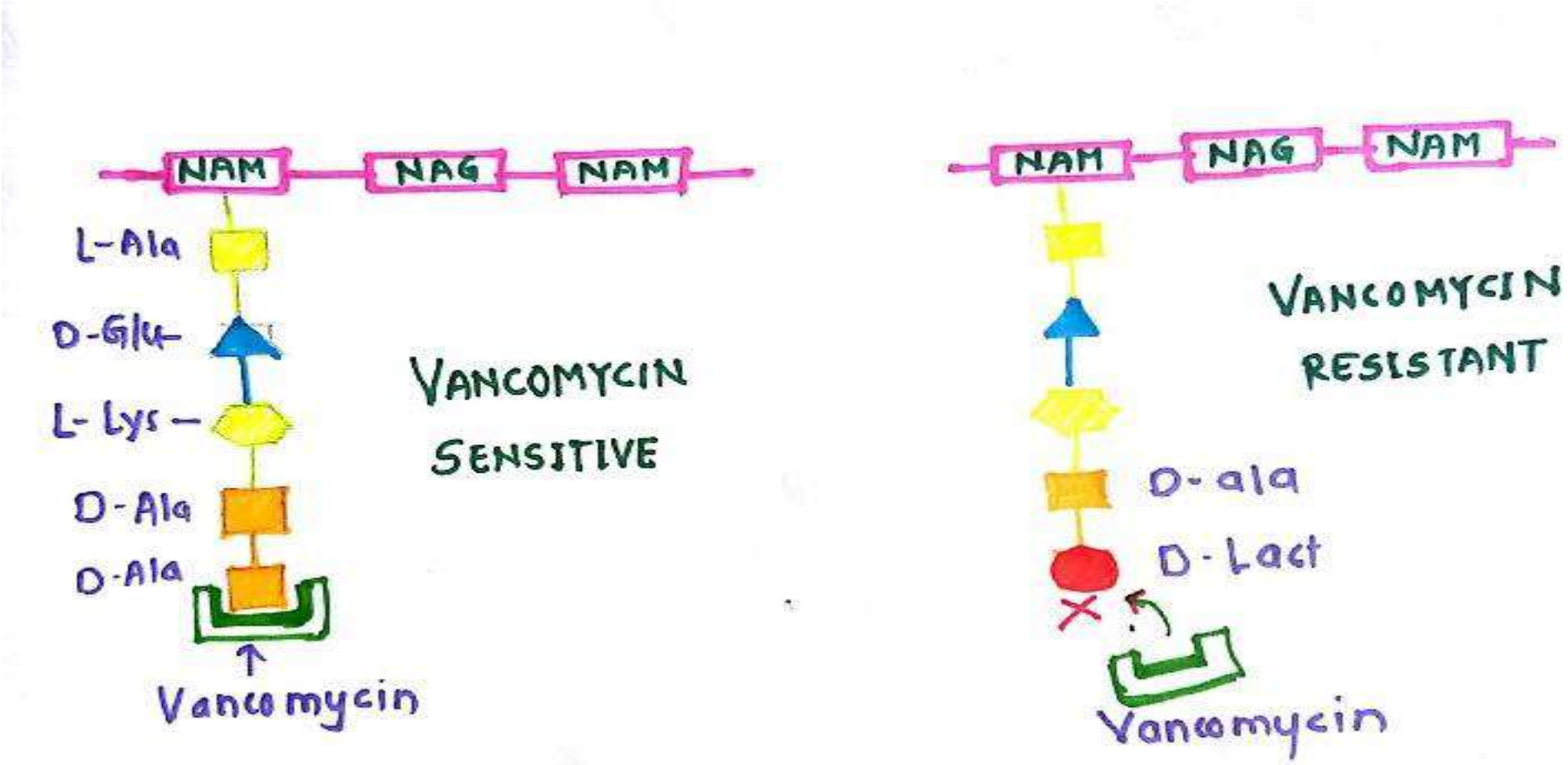
Erythromycin sensitive

Erythromycin resistant

Methylation carried by plasmid coded'methylases'.

c. Vancomycin:

Binding site: Terminal D-alanine-D-alanine residues

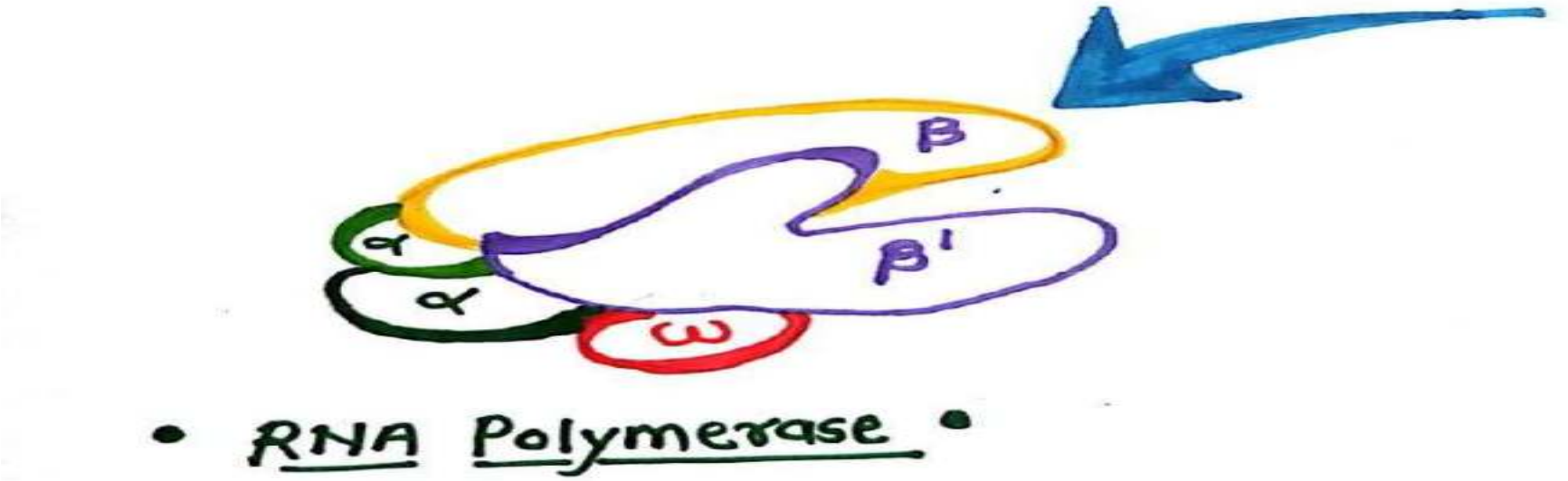


Examples: Enterococcus species

d. Rifampicin

Biding site: Beta subunit of RNA polymerase

Blocks Initiation of transcirption.



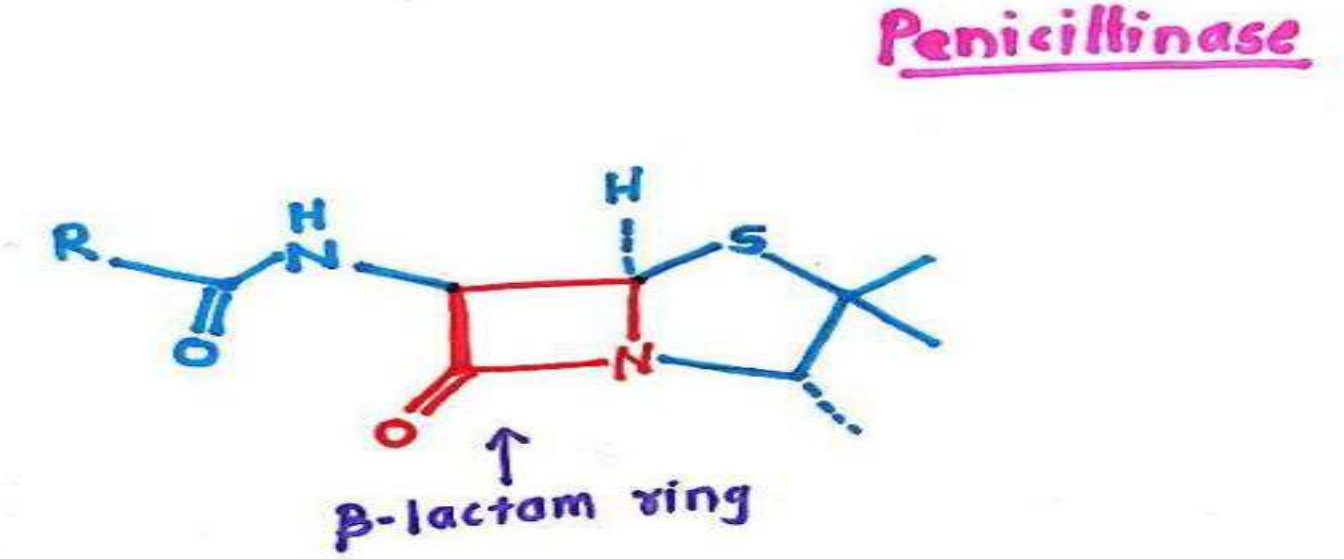
- Resistance is developed due to mutations that alters beta subunit of RNA polymerase
- **Example: *Mycobacterium tuberculosis***

4. Inactivation or detoxification of drug

- Inactivation of drug by chemical modifications.
- Specific enzymes are used for drug inactivation.

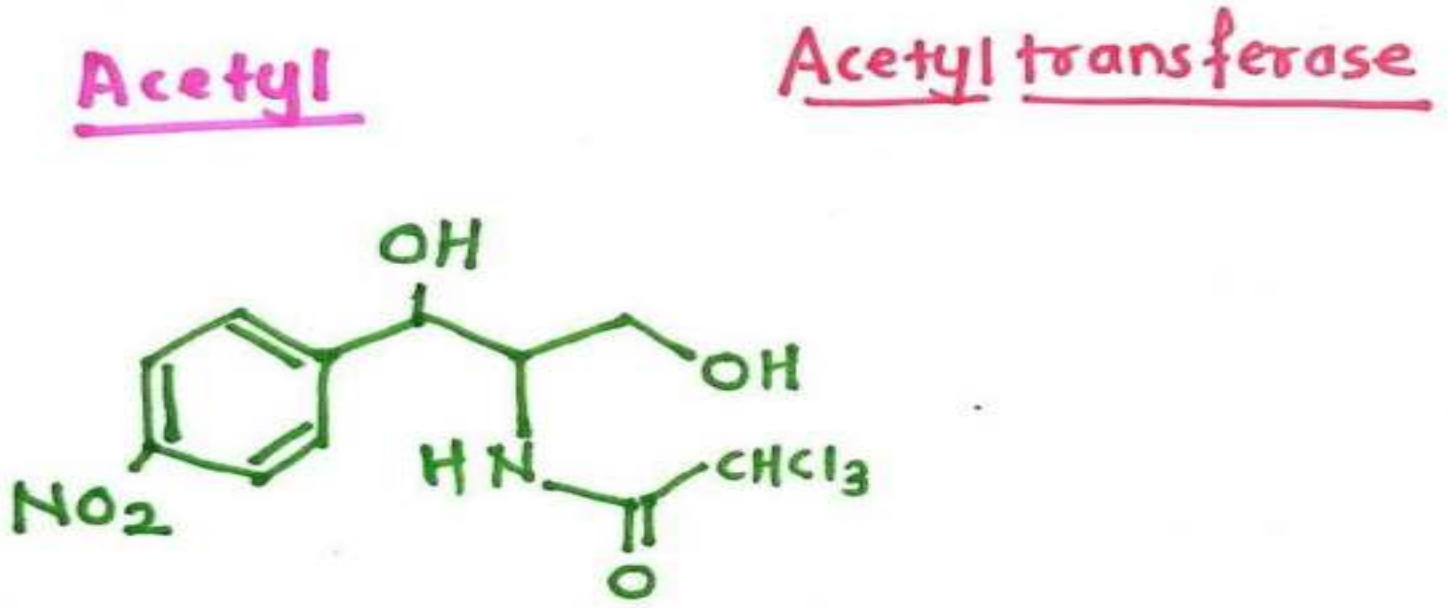
•Examples:

a. Penicillin resistance:



b. Chloramphenicol resistance:

•Plasmid coded enzyme '**acetyl transferase**' acetylates hydroxyl group of chloramphenicol and inactivates it.



c. Aminoglycosides resistance:

❖ **Aminoglycosides-**

• These are group of antibiotics which contains **amino sugars**.

• Inhibits protein synthesis.

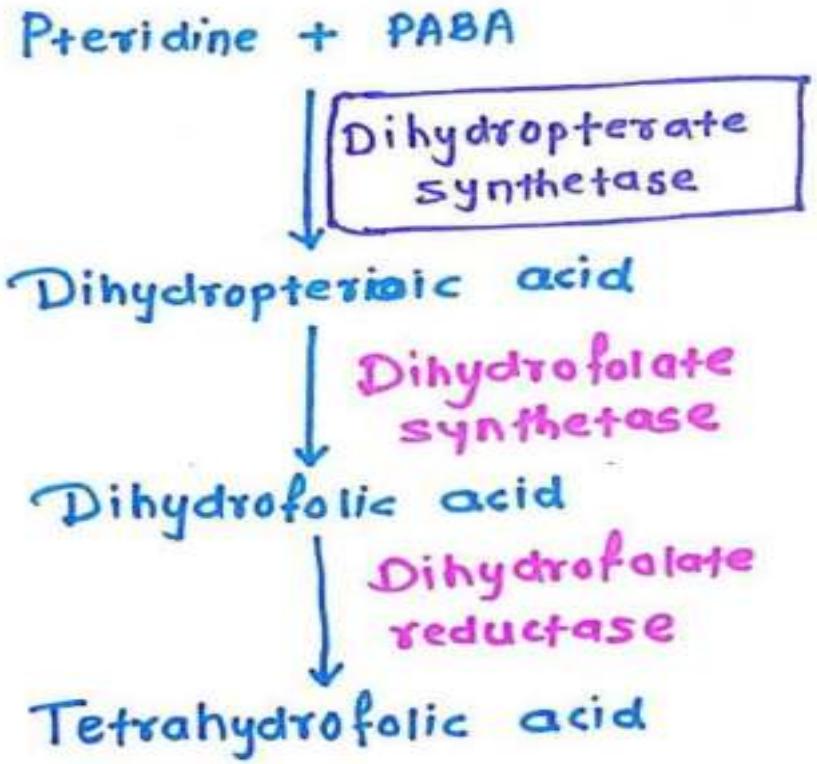
•Ex.- Streptomycin, Gentamycin, Tobramycin, Kanamycin, neomycin

• Inactivated by transfering **acetyl** (acetyl transferase) or **phosphate** (phosphotransferase) or **adenyl groups** (adenyl transferase) on hydroxyl groups.

5. Metabolic bypass:

• Metabolic step which inhibited by specific drug is altered or bypassed.

• Ex.- **Sulfonamide resistance:**
• Sulfonamide acts as a structural analogue of PABA (para amino benzoic acid)
• Inhibits pathway of folic acid synthesis .

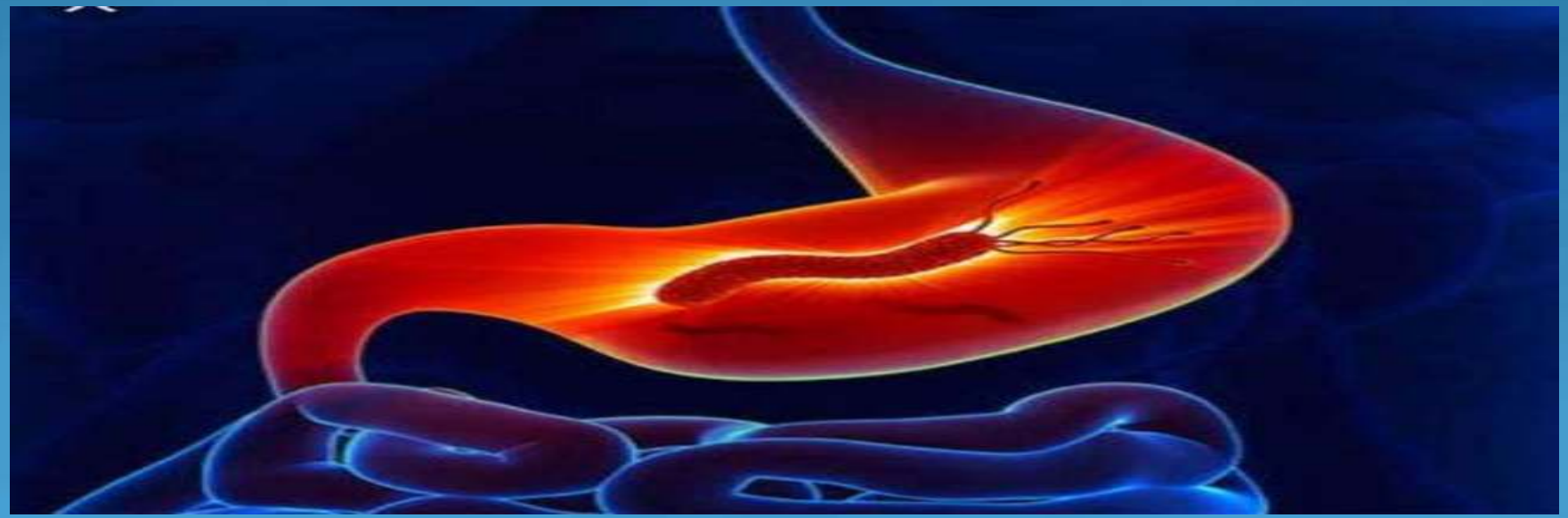


6. Accumulation of metabolite antagonistic to drug:

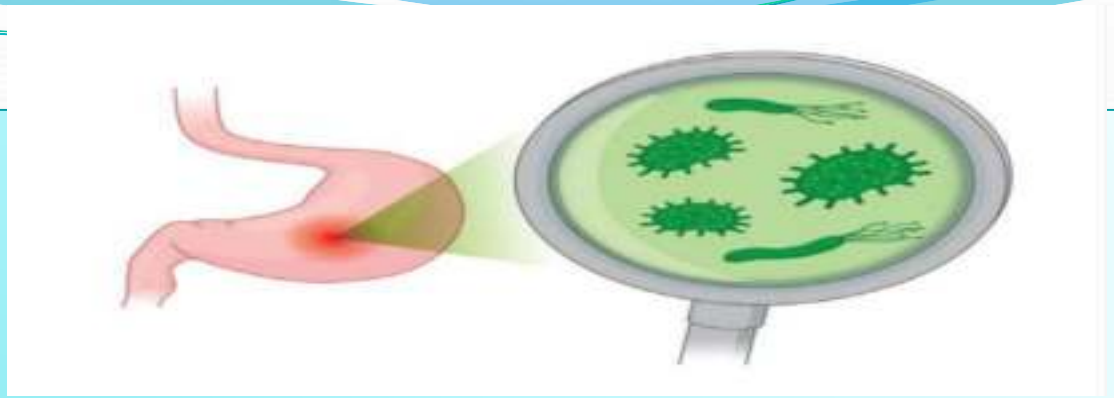
Ex- Pneumococci resist sulfonamide by overproduction of PABA

Helicobacter pylori

Mr. Suraj D. Gabale

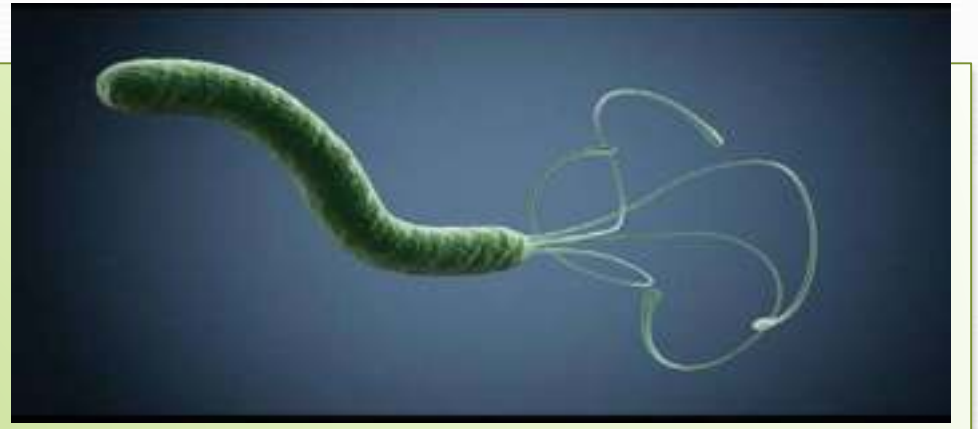


Helicobacter pylori



- Grows in digestive tract and attack stomach lining.
- Causes ulcer in stomach and duodenum.
- 1982- First time identified by Australian doctors Barry Marshall and Robin Warren
- Previously known as Campylobacter pylori.
- 2015- Estimated that 50% population across world has H. Pylori in upper GI tract.
- Can also infect monkeys.

Morphology



- Helix shaped, Gram negative bacterium.
- Size: 3µm long and 0.5µm in diameter
- Some members have either short or tapered rod shape.
- On culture media mostly assumes rod shape.
- Motile by lophotrichous flagella.
- Produces adhesins which helps in adhesion to epithelial cells of stomach.

Cultural characters

- Microaerophilic
- Requires 5-10 % CO2 and high humidity.
- Best growth at 37^0 C but not at 43^0 C and below 30^0 C
- Optimum pH: 6-7

- Grows well on **blood agar** and **chocolate agar**
- Colonies are convex, circular, translucent and bigger than 2mm

1. Columbia blood agar:

•Small dome shaped, translucent colonies

•Sometimes weakly hemolytic

2. Modified columbia urea agar:

- Small, round, creamy colonies
- Change in colour of slant from orange to pink.

3. Egg yolk emulsion agar:

- Large red colonies against yellow background

Biochemical characters

- Produces oxidase, catalase, phosphtase and H_2S
- **Produces abundant Urease**
- Negative nitrate reduction test

Virulence factor

- **Flagella:**

Helps in movement of bacteria to epithelial cells of stomach.

- **Urease:**

Neutralizes stomach acidity by producing ammonia.

- **Lipopolysachharide:**

Adheres to host epithelial cells.

- **Exotoxins:**

Cag A- Activates IL-8 to induce inflammation.
Induces host cell apoptosis

Vac A (Vacuolating toxin)

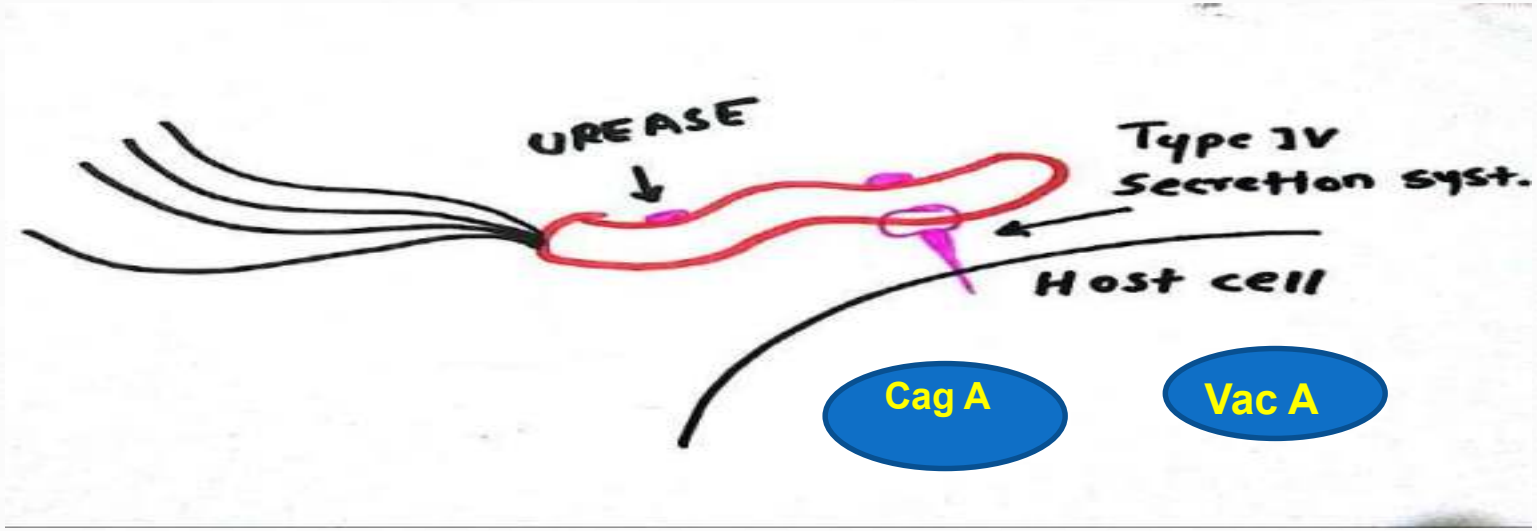
- **Secretary proteins:**

Mucinase, protease, lipases- Damages mucosal cells

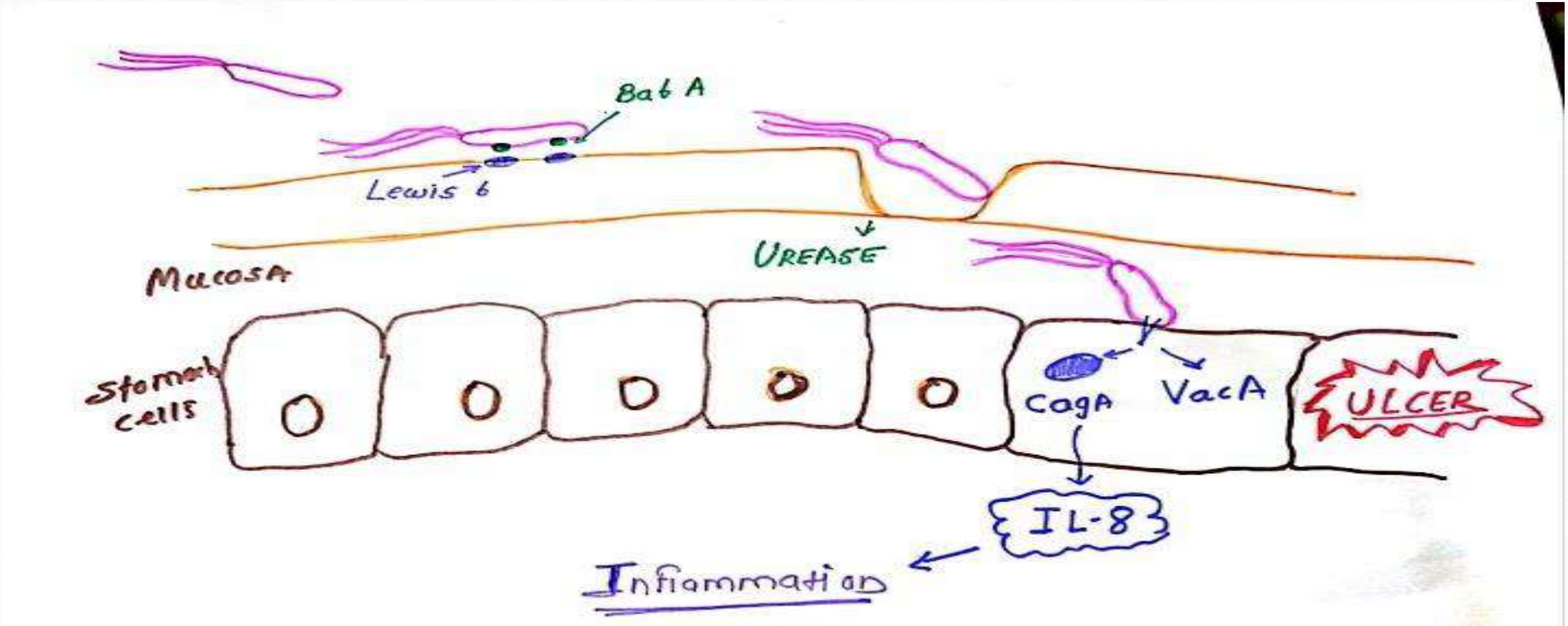
- **Type IV secretion system:**

Injection of exotoxins inside cells.

Pilli like structure



Pathogenesis

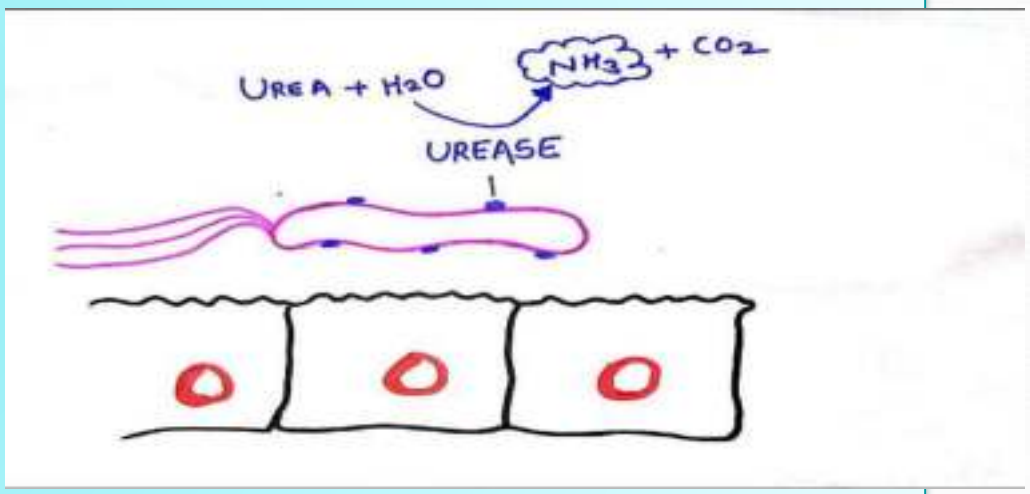


1. Adhesion:

H.pylori binds to membrane associated lipids and carbohydrates with the help of adhesins

Ex. Adhesin Bab A binds to Lewis b antigen

2. Reduction of stomach acidity:



Urea + H_2O $\xrightarrow{\text{urease}}$ **NH_3** + CO_2

3. Inflammation:

- Type IV secretion system releases Cag A inside the cells.
- Cag A removes peptidoglycan from its own cell wall.
- Peptidoglycan activates IL-8 which leads to inflammation.

➢**Other toxic factor released by pylori**

- The ammonia produced is toxic to epithelial cells
- Vac A: Forms vacuoles in epithelial cells
- Proteases, phospholipases: Damage the cells

Signs and symptoms:

- 80% individuals are asymptomatic
- Inflammation or damage to stomach lining may cause-
  - Stomach ache
  - Abdominal pain
  - Acid reflux
  - Nausea and vomiting
  - Black stool

If not treated for long time then may cause:
Gastro-esophageal eflux disease(GERD), Peptic ulcer, Cancer of duodenum and stomach

Lab diagnosis

- **Clinical samples: Blood, stool, biopsy specimen**
- 2 ways of diagnosis:

a) Non-invasive tests-

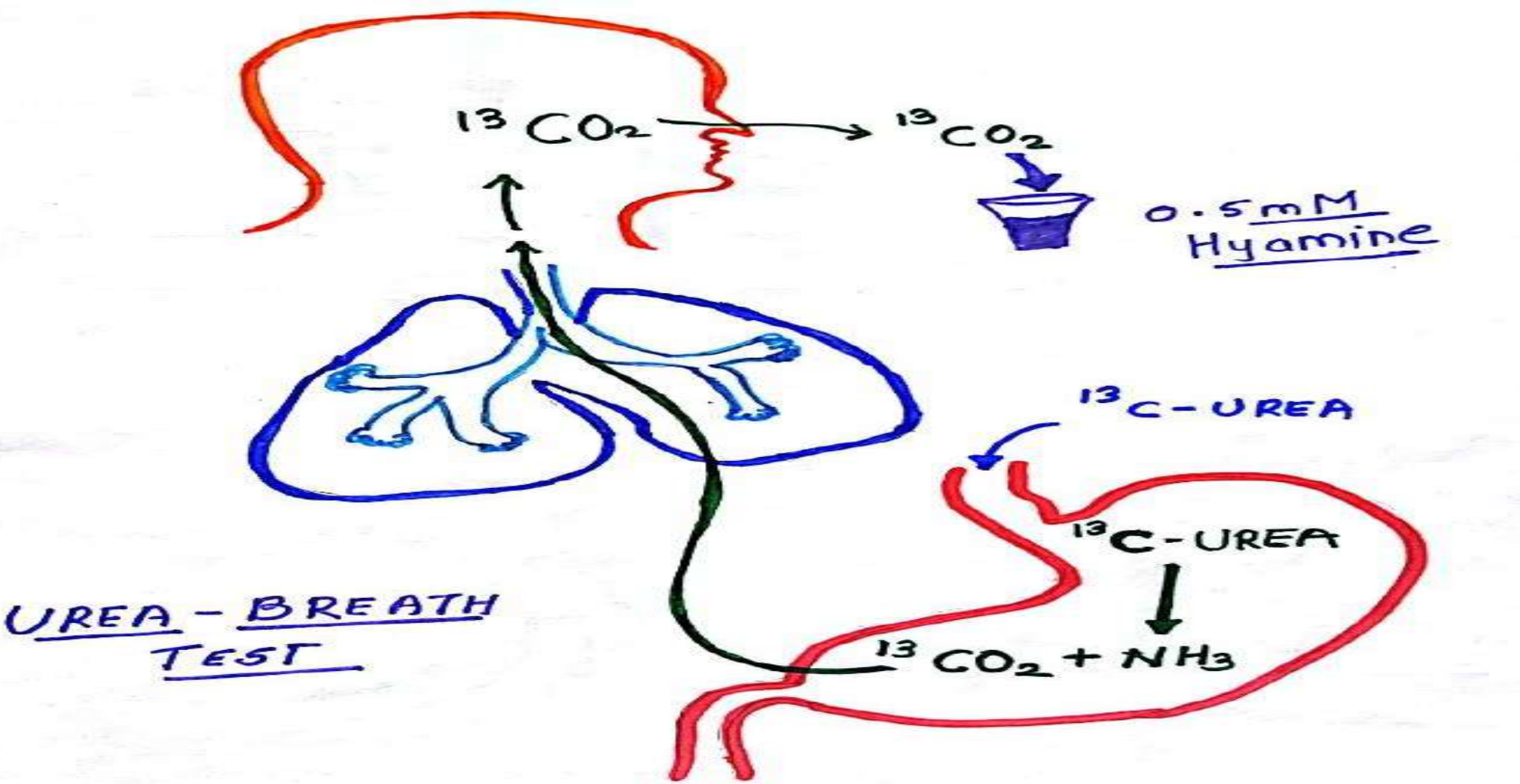
- Urea breath test
- Stool antigen test
- ELISA test

b) Invasive tests-

- Endoscopic biopsy
- Biochemical tests
- Biopsy urease test

Non-invasive tests

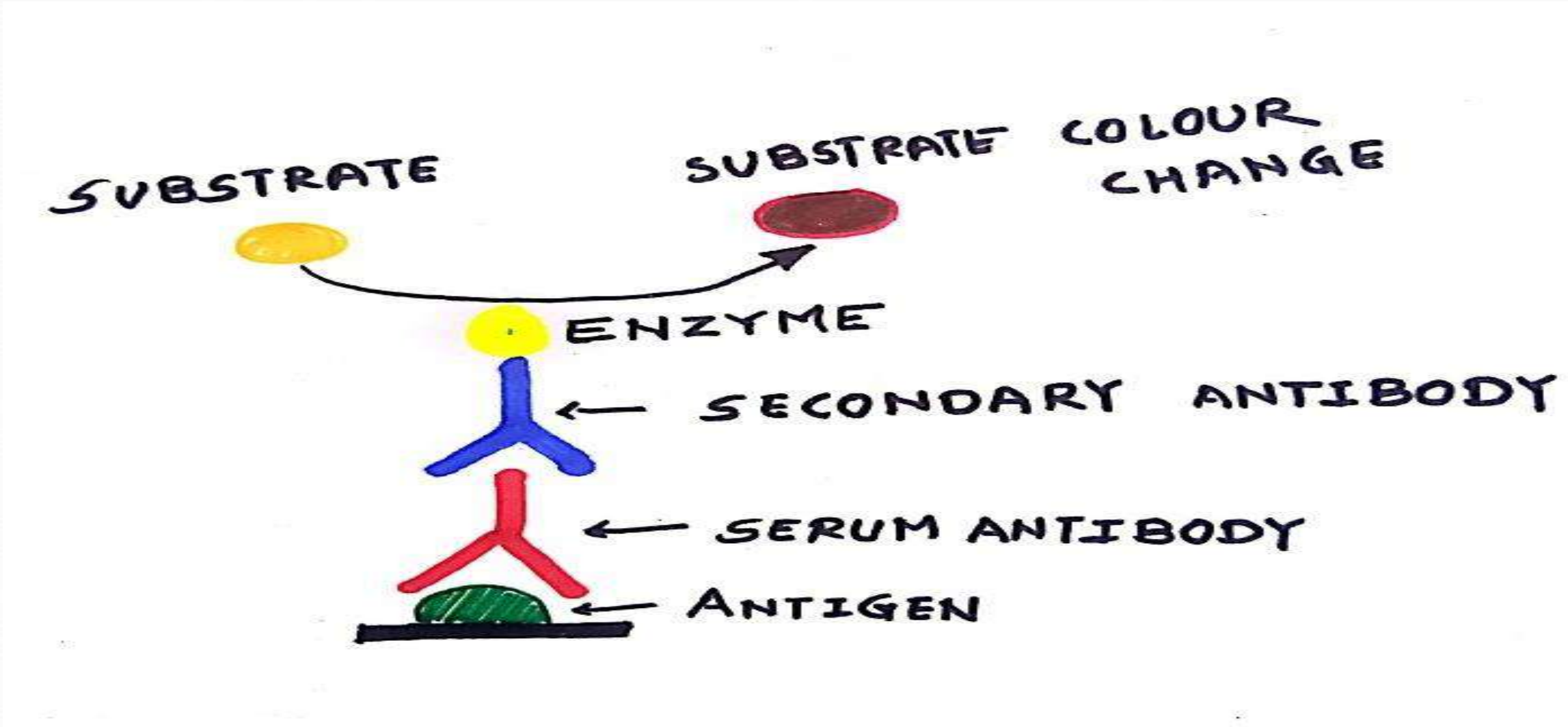
1. Urea breath test:



2. Stool antigen test:

- Detection of H. Pylori antigen in patient stool sample
- Mostly used to detect GI infection in childrens and sensitive peoples.
- ELISA based
- Indicates active infections

3. ELISA test


SUBSTRATE
SUBSTRATE
COLOUR CHANGE
ENZYME
SECONDARY ANTIBODY
SERUM ANTIBODY
ANTIGEN

Invasive tests

Endoscopy mediated multiple biopsies (tissue sections) from gastric mucosa are taken.

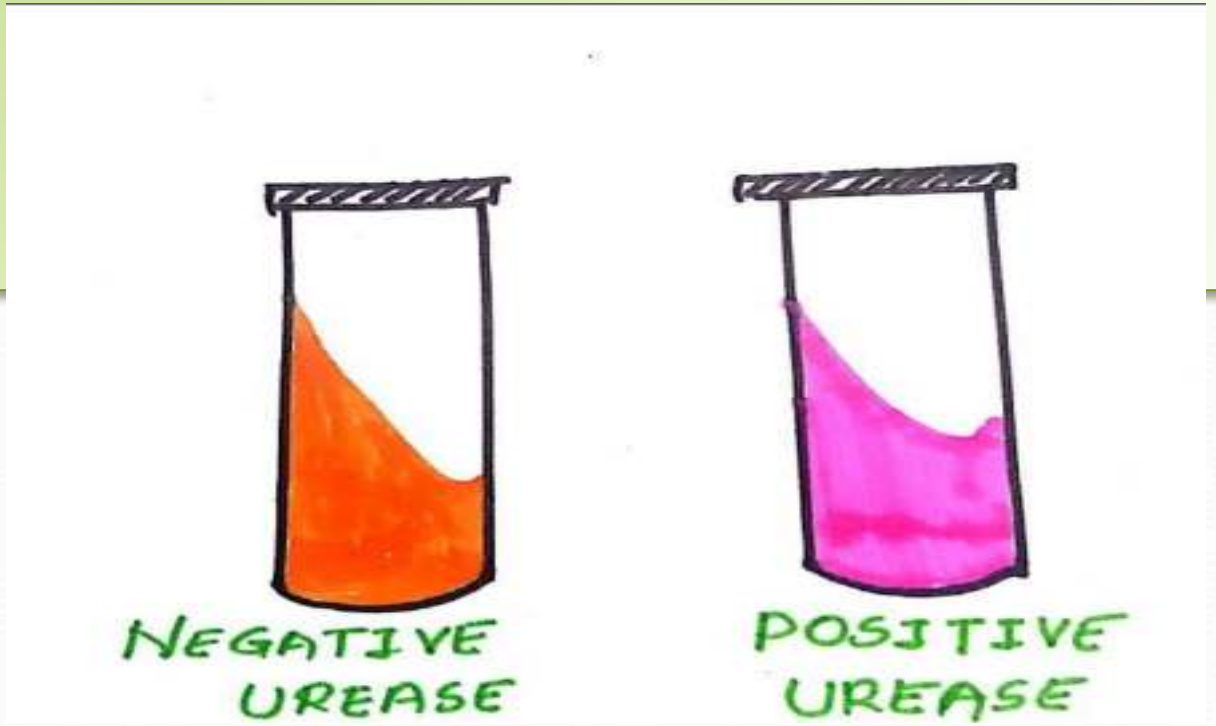
A) Microscopic observation:

- Biopsy section stained with silver stain.
- Observed for helical bacilli.

B) Biochemical tests:

Oxidase, Catalase, Usease

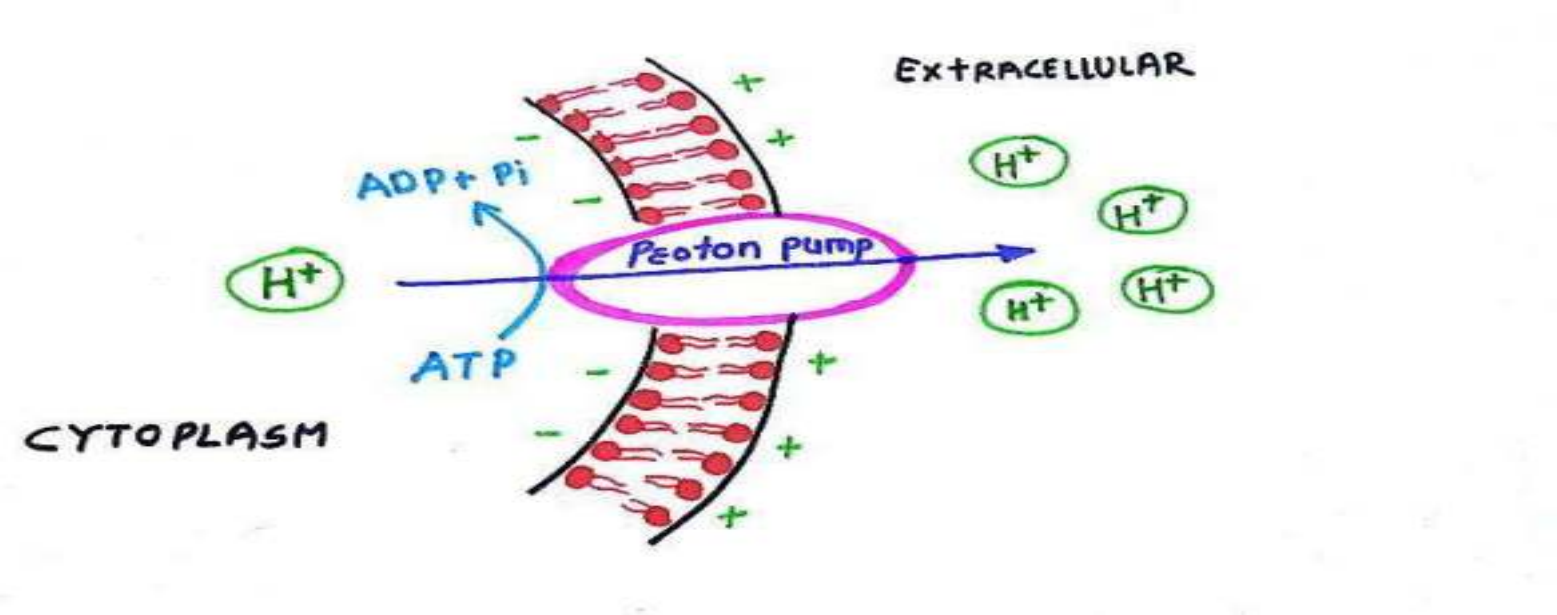
C) Biopsy urease test:



TREATMENT

❖ **Proton pump inhibitors:**

Dexlansoprazole, Esomeprazole, lansoprazole, Pntoprazole, Omeprazole, Rabeprazole



❖ **Antibiotics:**

PPi, Amoxicillin, clarithromycin

PPi, Metronidazole, Tetracycline and bismuth subsalicylate

Prophylaxis

- Washing hands
- Avoid food or water that is not clean
- Avoid under cooked food

THANK U!!

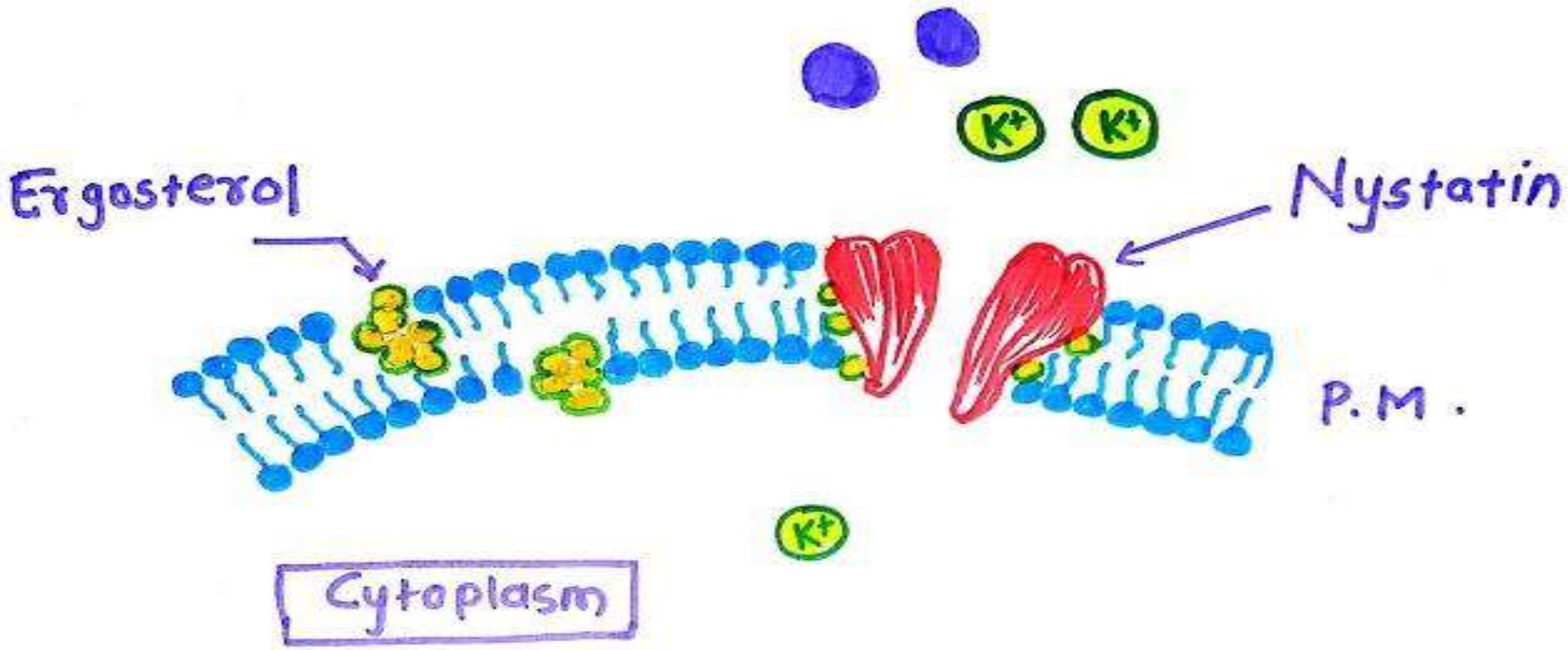
Nystatin

Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

Nystatin

- It is polyene antifungal drug
- Discovered by Rachel brown and Elizabeth Lee Hazen in 1950.
- Produced by ***Streptomyces noursei***.
- **Fungistatic and fungicidal**
- Similar to amphotericin B (antifungal agent) but has greater antifungal activity.
- It is **channel forming ionophore** which forms **pores in cell membrane** by binding to **ergosterol**.
- Very litte absorption
- Slightly soluble in water but more soluble in alcohol.
- Used orally.

Mechanism of action



Uses of Nystatin

- Used to treat Candida infections of skin including diaper rash, thrush, esophageal candidiasis and vaginal yeast infections.

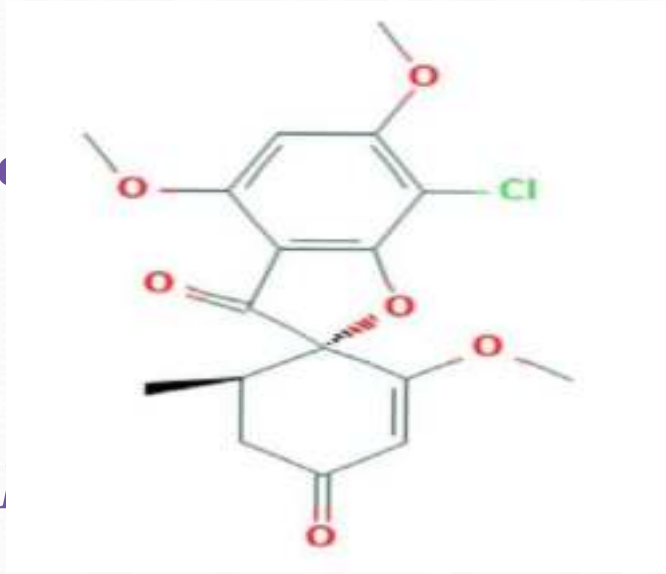
- Also used to treat fungal infections of stomach and intestine.

Side effects

- Diarrhea
- Nausea
- Vomiting
- Abdominal pains
- Rarely facial swelling, muscle aches
- Rashes, Intching, burning in applied area

GRISEOFULVIN

Griseofulvin



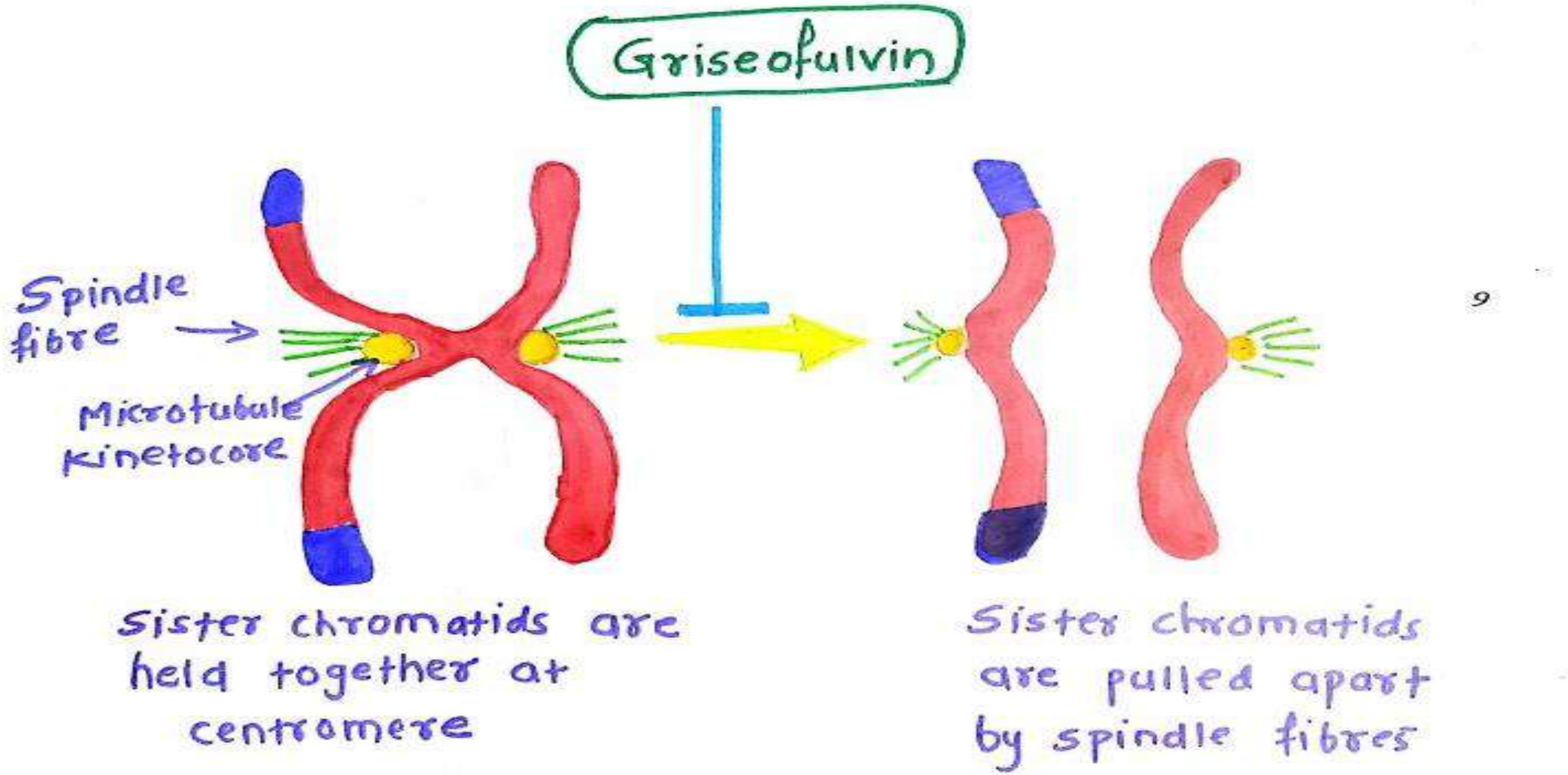
- Antifungal drug used to treat number of dermatophytos
- It is a spiro compound.
- Fungistatic for most dermatophytes including *Epidermo*

Trichophyton, Microsporum.

- Lacks antibacterial activity

- Produced by fungus *Penicillium griseofulvum*.
- Gets deposited in newly formed skin and binds to keratin and protects skin from getting infected
- It binds to microtubules and inhibits mitosis.
- Poor absorption in circulation.
- Poorly soluble in water
- Taken orally

Mechanism of action



Uses of Griseofulvin

- Used to treat dermatophytic infection including **infections of skin, nails and scalp.**

Side effects

- Allergic reactions
- Nausea
- Diarrhea
- Headache
- Trouble speaking
- Feeling tired

THANK YOU……

ISOLATION OF ANIMAL VIRUSES

Egg inoculation

Isolation of animal viruses

❖**Purposes:**

✓ To isolate and identify viruses in clinical samples.

✓ To study virus structure, replication, genetics and effects on host.

✓ To prepare viruses for vaccine production.

Egg inoculation

❖This method was first time used by **Good Pasture** in 1931.

❖Process of cultivation of viruses in embryonated egg depends on type of egg.

❖**For inoculation, eggs are first prepared for cultivation.**

✓5-12 day old fertile egg is selected.

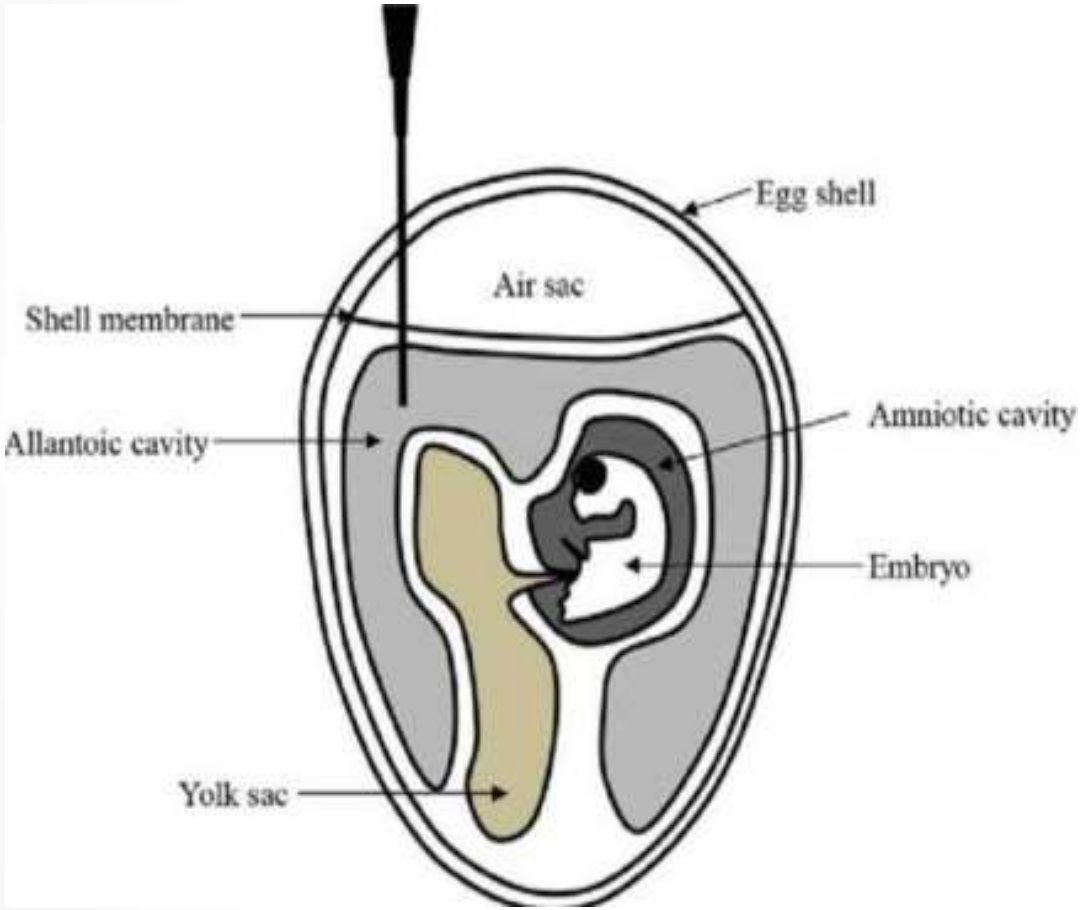
✓Egg is checked for cracks.

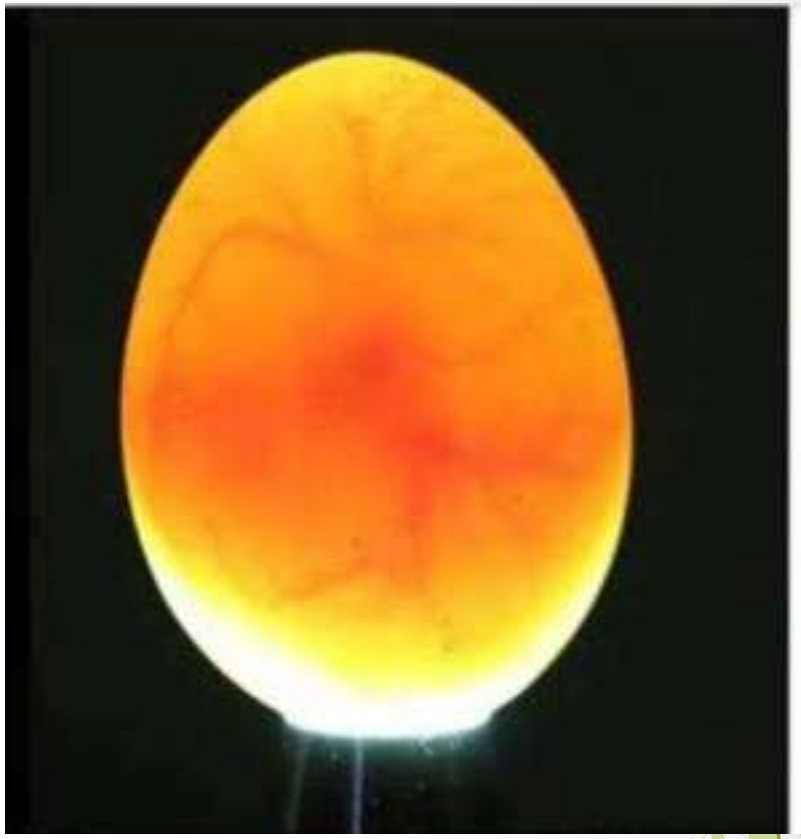
✓Shell surface is disinfected with iodine.

Procedure

1. Locate air sac in the egg by candling.
2. Drill small hole in the shell aseptically.
3. Inoculate material (virus) through the opening.
4. Close the opening with paraffin wax.
5. Incubate the egg at 37° C for 36 to 72 hours

Viruses can be cultivated in various parts of egg like chorioallantoic membrane, allantoic cavity, amniotic cavity and yolk sac.





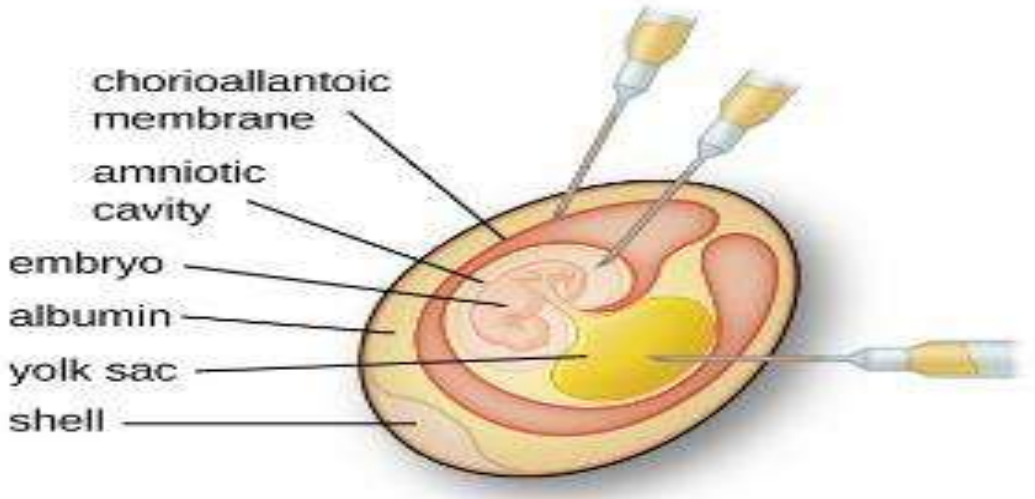
❖ Chorioallantoic membrane (Cam):

✓Mainly for growing pox viruses

✓After incubation pocks can be observed, which is grey white area on transperent CAM.

✓Herpes simplex viruse can also be grown

✓Suitable for virus enumeration



❖ Allantoic cavity

✓Inoculation for production of vaccine against influenza virus, rabies and yellow fever.

✓Commonly used for isolation of avian viruses

❖ Amniotic sac

✓Inoculation for isolation of influenza and mumps viruses.

✓To study growth and replication of viruses

❖ **Yolk sac:**

✓Simplest method for growth and multiplication of virus.

✓ For cultivation of Rickettsiae and Chlamydia.

Advantages of egg inoculation method

1. Widely used method for isolation and cultivation of viruses.
2. Ideal substrate for viral growth and replication.
3. Isolation and cultivation of many avian and few mammalian viruses.
4. Cost effective
5. Less labor needed.
6. Embryonated egg readily available.
7. Sterile and wide range of tissues.
8. Specific and non-specific defense not involved.

Disdvantages

✓Inoculation site varies with virus.

Sr. No.	Inoculation site	Cultivation
1.	Chorioallantoic membrane	Vaccinia virus, Herpes simplex virus, Pox virus, Rous sarcoma virus
2.	Allantoic cavity	Influenza virus, Mumps virus, Avian adenovirus
3.	Amniotic sac	Influenza and mumps virus
4.	Yolk sac	Herpes simplex virus

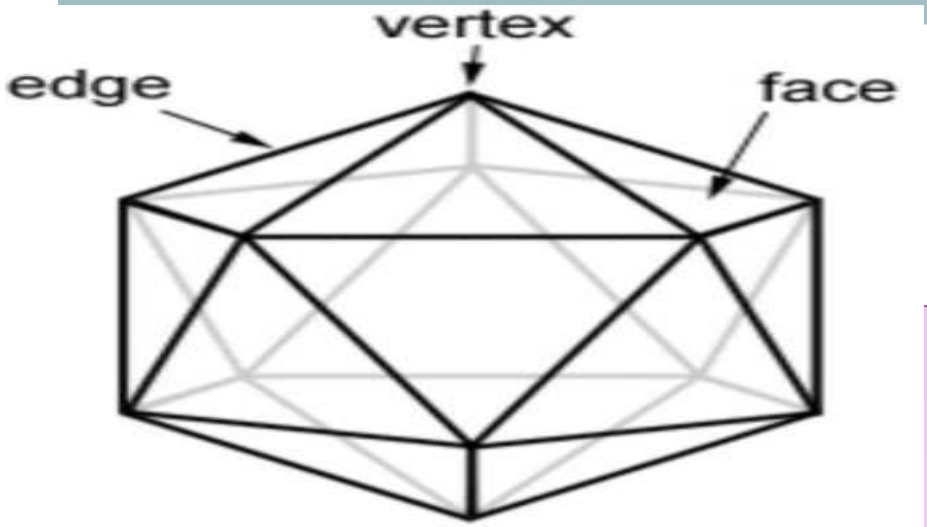
Structure of Viruses

Viral morphology

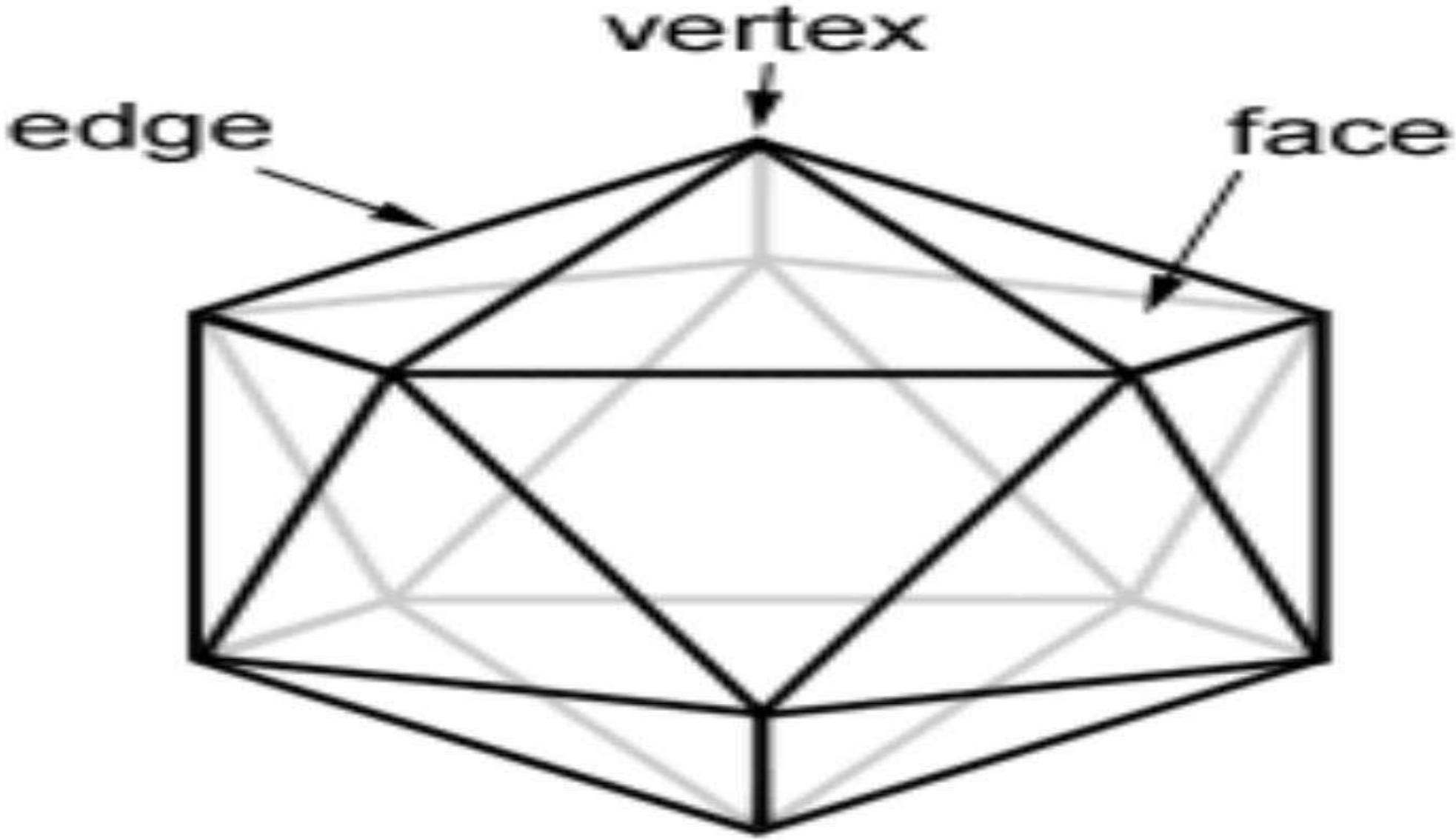
- The basic structural unit of virus is ‘capsid’.
- Capsids have highly ordered architecture.
- As viruses contains limited amount of genetic information, one or few proteins are used any times.
- Thus capsid subunits are arranged in symmetrical manner.

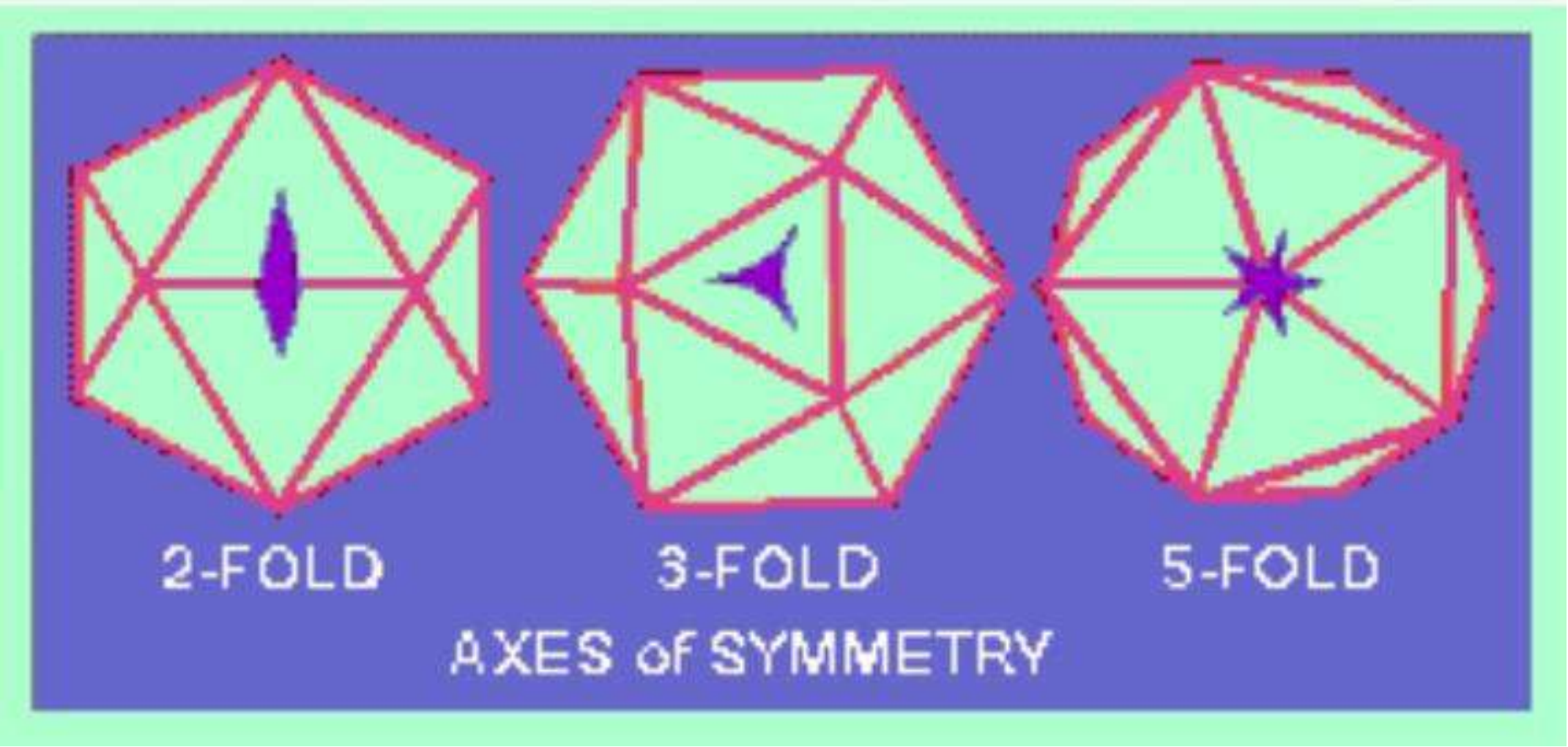
- Three types of symmetry:
  1. Icosahedral (Cubic symmetry)
  2. Helical symmetry
  3. Complex symmetry

Icosahedral capsid



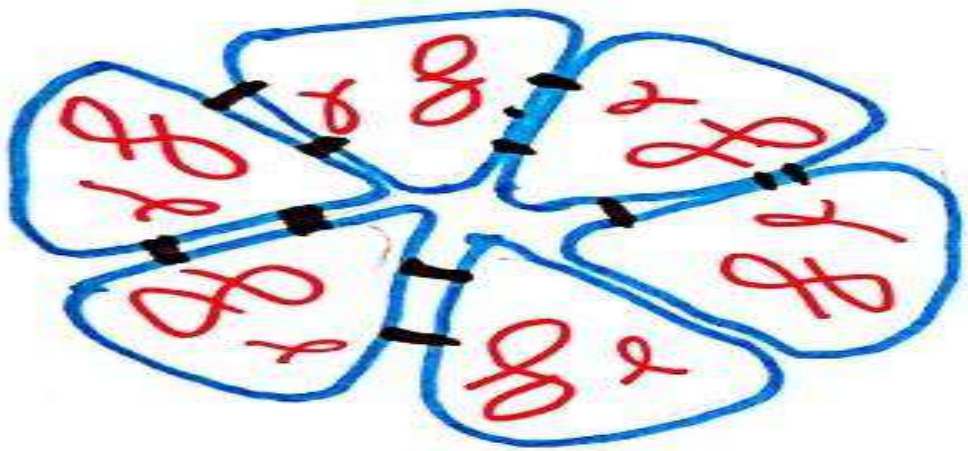
- Regular polyhedrons
- Contains 20 triangular faces, 12 corners and 30 edges.
- Protein appendages at faces are present in multiples of 20, whereas at vertices in the multiples of 12.
- Capsid size determines genome size.
- Icosahedron surface is mostly rough
- Capsid of many spherical viruses is icosahedral.


vertex
edge
face

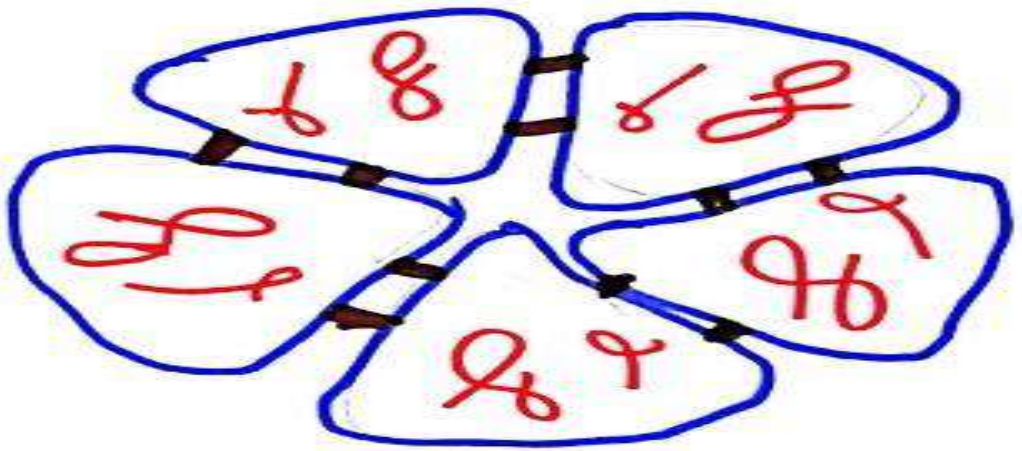

2-FOLD
3-FOLD
5-FOLD
AXES of SYMMETRY

Polypeptides

Monomer



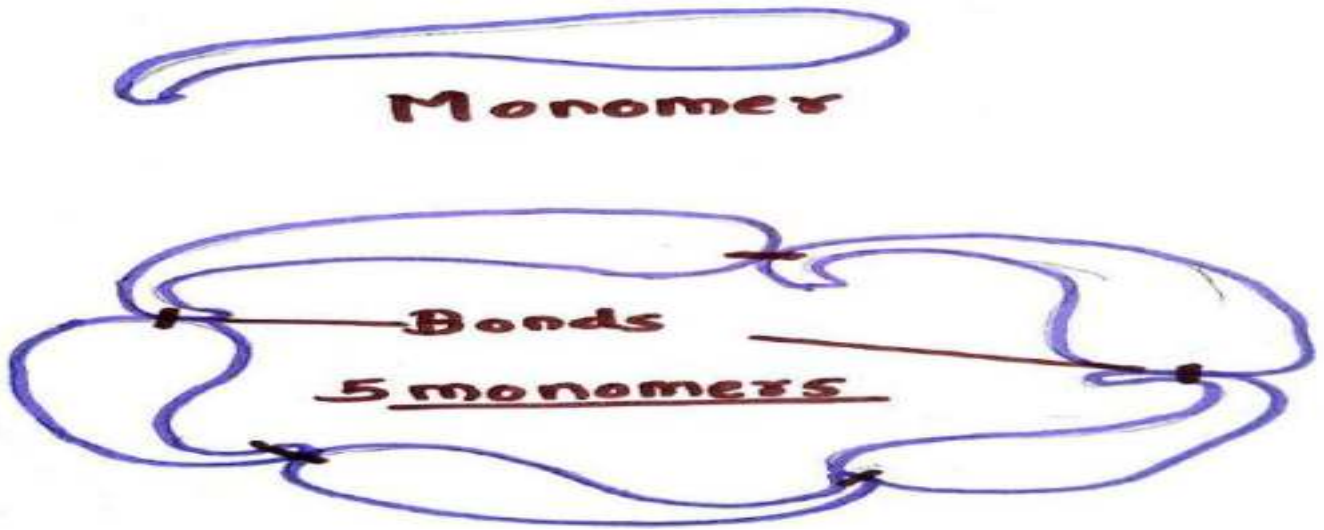
Hexamer
[6 subunits]



Pentamer
[5 subunits]

Helical capsids

- Capsomers arranged in helix around a single rotational axis.
- Mononers are thicker at one end than the other.

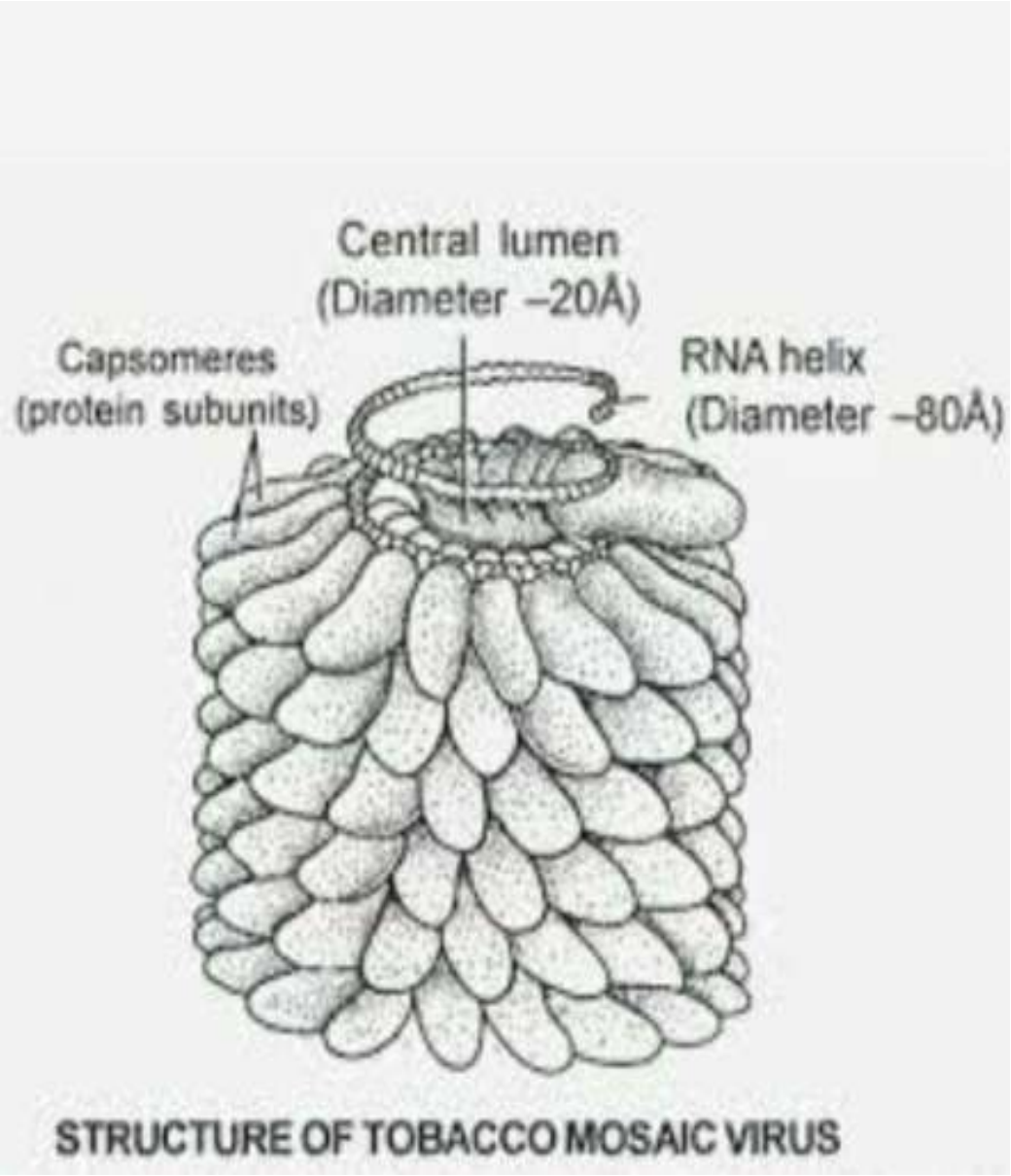


- **Genome size determines the size of capsid.**
- Helical capsid may be naked (e.g. TMV, M13) or enveloped (e.g. Influenza, mumps, measles virus)

Tobacco mosaic virus (TMV)

- Plant virus
- Infects tobacco plant and causes mosaic pattern (mottling and discolouration)
- Also infects other members of family ‘Solanaceae’.
- It was first virus to be discovered

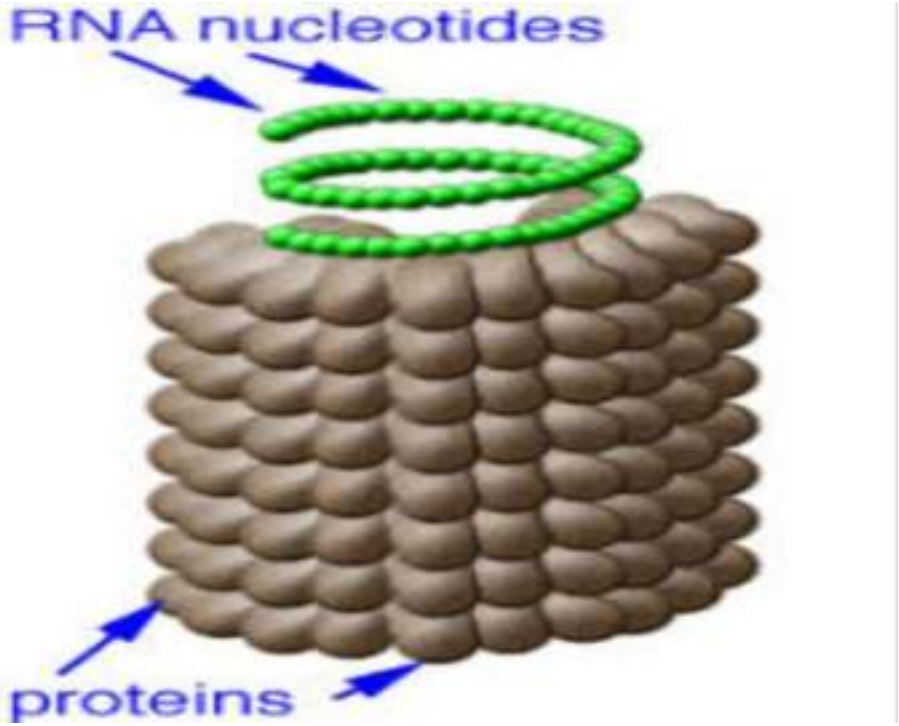
- Helical and naked capsid
- Size: 300nm x 18nm
- Molecular weight of TMV particle is $40x10^6$ d.
- Capsid contains **2130 casomers.**
- Each capsomer made up of 158 amino acids.



STRUCTURE OF TOBACCO MOSAIC VIRUS

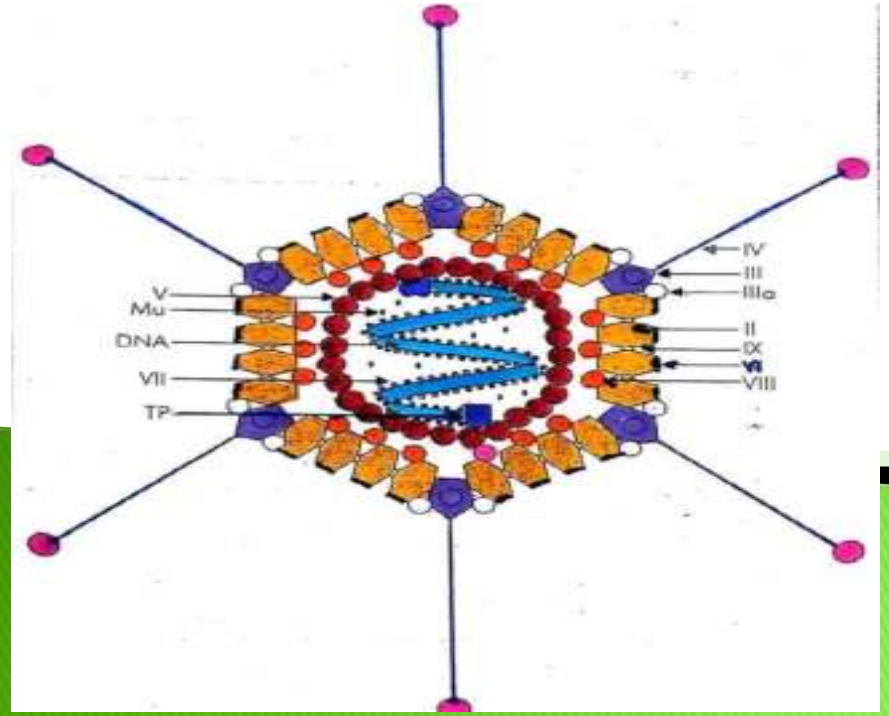
Genome:

- ✓**Single stranded positive sense RNA**.
- ✓6395 nucleotide
- ✓Molecular weight- 2.1x 10^6 d.



- ✓Each turn of RNA helix contains 49 nocleotides.
- ✓16.3 capsomers are attached to RNA per turn of helix.

Multiplication of Adenovirus



Mr. Suraj Dipak Gabale
Assistant Professor
Department of Microbiology,
Vivekanand College, Kolhapur (Autonomous)

ADENOVIRUS

- It is an animal virus of the family **Adenoviridae**.
- The name Adeno is derived from human **adenoids**.
- "**Adeno**" is a Latin word that means a '**gland**'.
- **Rowe et al.** First isolated adenoviruses from infected tonsils and adenoid glands of healthy children in 1953.

❖Widespread in nature.

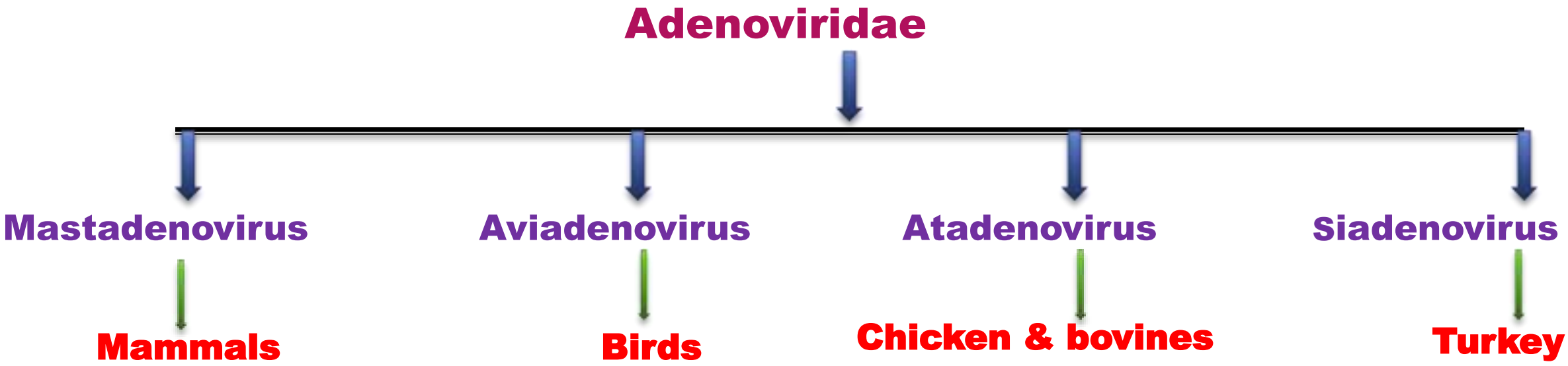
❖Mild human and animal pathogen.

❖Infections are common in children and immuno-compromised patients.

❖It causes respiratory and other infections as **common cold, pneumonia, bronchitis, gastroenteritis, conjuctivitis and cystitis**.

❖It can cause latent infection in healthy people which may convert later to productive infection.

❖**Causes malignancies in rodents.**



- Over 100 serotypes of adenovirus have been identified.
- 47 serotypes infects humans.

SUB GENERA	SEROTYPE	ONCOGENICITY
A	12,18,31	Oncogenic
B	3,7,11,14,16,21,34,35	3,7 are oncogenic
C	1,2,5,6	Non-oncogenic
D	8,9,10,13,15,17,19,20,22-30, 32,33,36,39,42-47	Non-oncogenic
E	4	Non-oncogenic
F	40,41	Non-oncogenic

- May cause 3 types of infections:

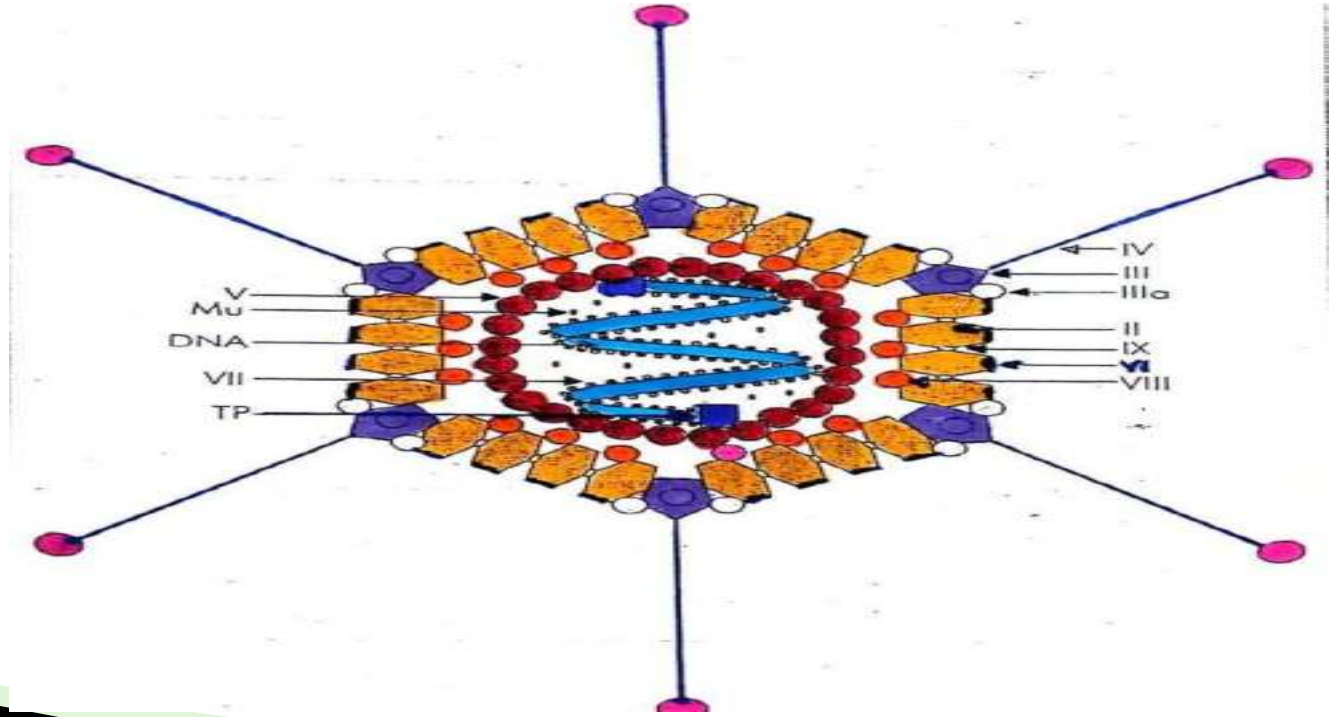
a. **Productive**: Complete replication of infectious virion

b. **Abortive**: Synthesis of viral gene products occurs but no formation of complete virion.

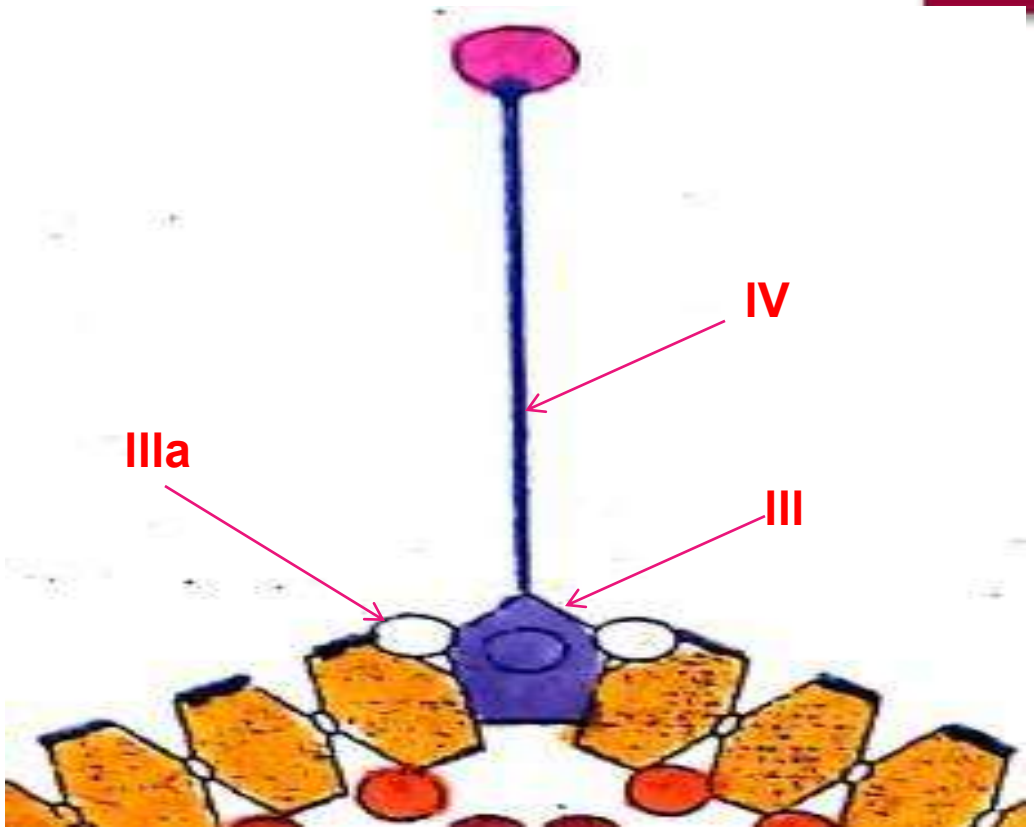
c. **Latent**: Persistence of viral genome on host cells.

Morphology of Adenovirus

- Non-enveloped
- Size: 70-100 nm in diameter.
- Icosahedral capsid

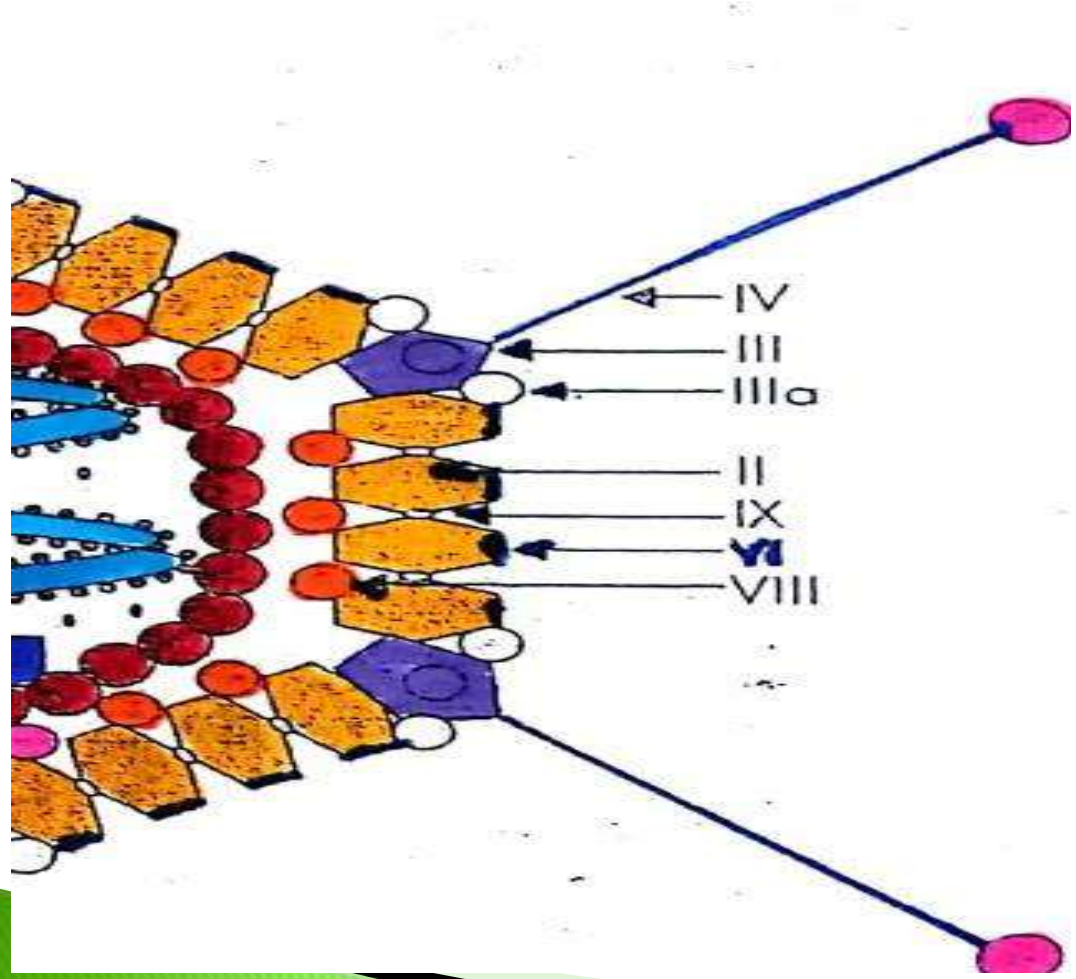


PENTON



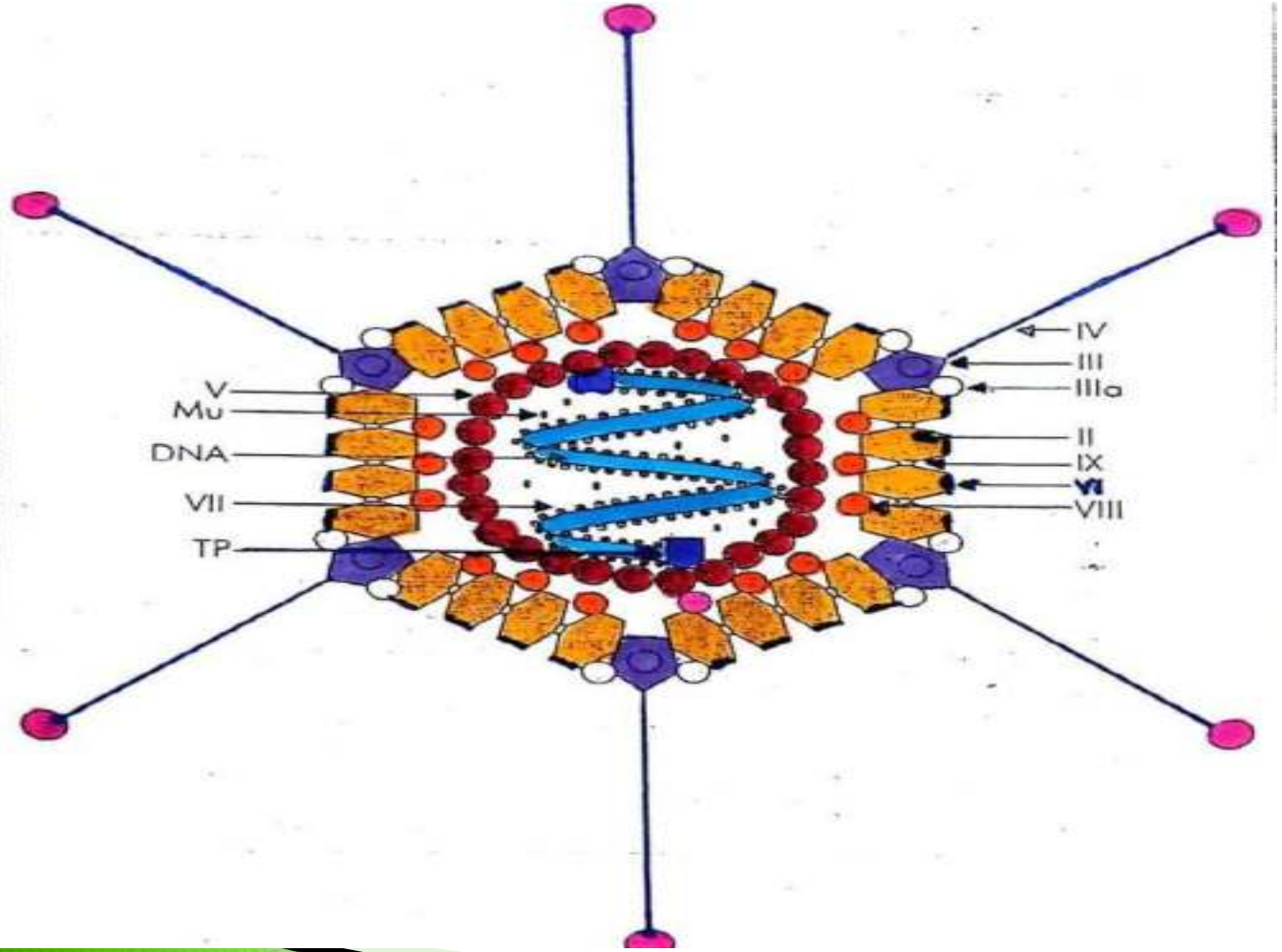
- Contains base and a fiber.
- Base consists of pentamer of protein III.
- 5 molecules protein IIIa are associated with penton base.
- Trimeric protein IV is attached to penton base.

HEXON



- Made of **protein II**.
- 3 minor proteins VI,VIII and IX are associated with protein II.

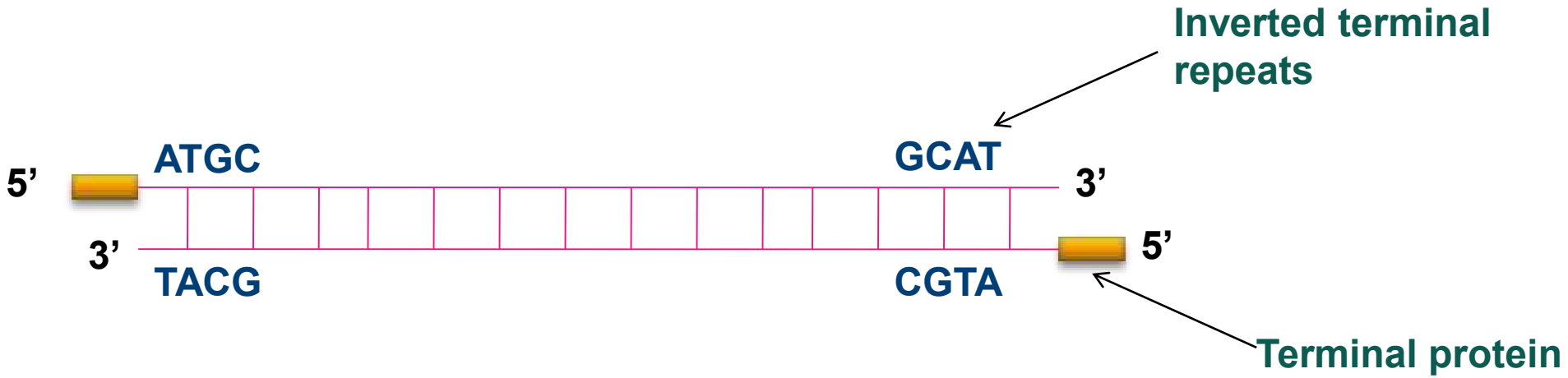
CORE

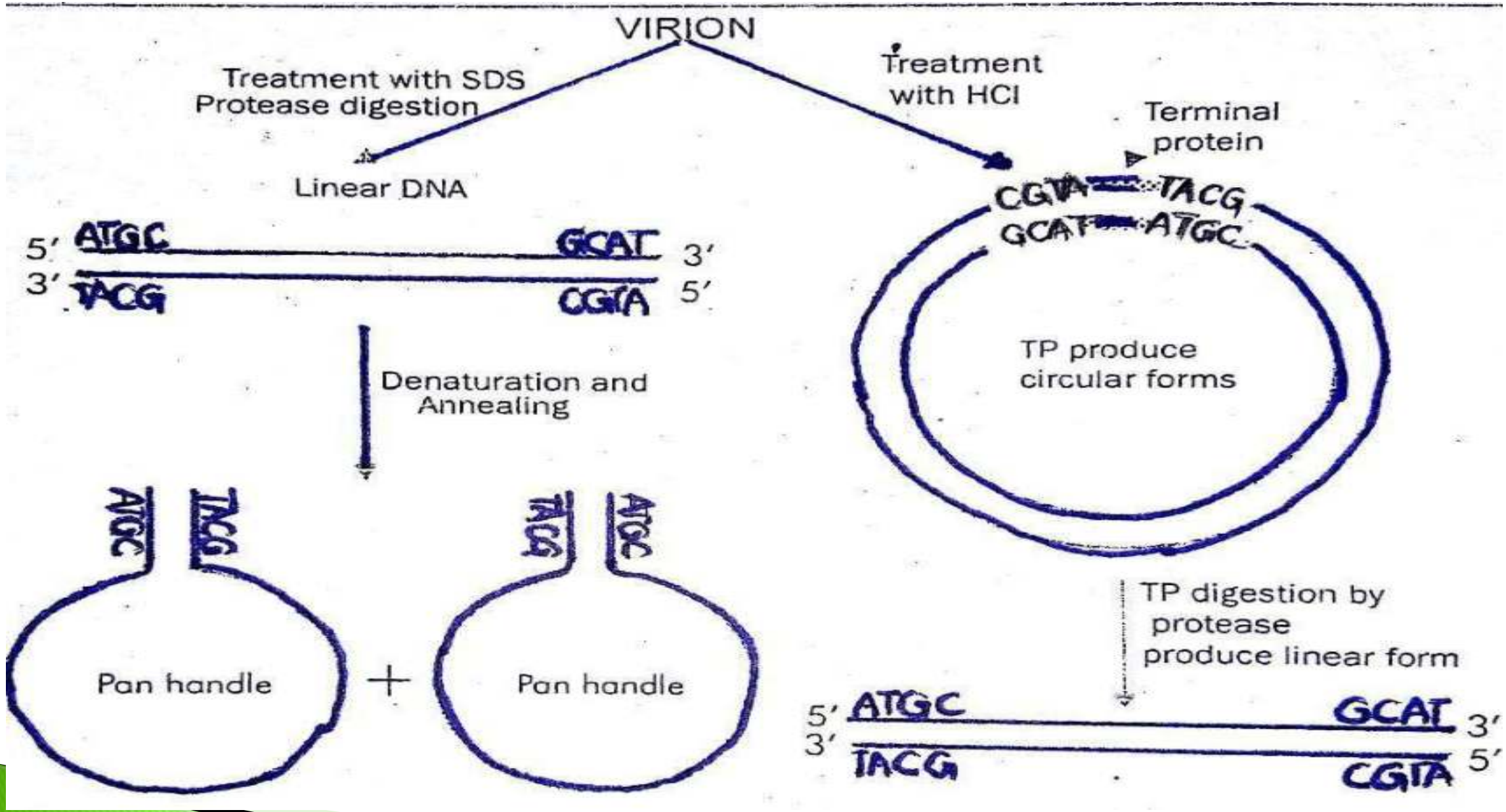


- TP: Covalently attached to 5’ end of genome.
- V: 180 copies per virion
- VII:1070 copies per virion
- Mu: 4kD.

GENOME

- ✓ Linear, non-segmented, dsDNA
- ✓ 36-38 kb long.
- ✓ Molecular wt.: 20-25x10 ^{6} d.
- ✓ Genome contains over 30 genes.




VIRION
Treatment with SDS
Protease digestion
Linear DNA
5′ ATGC GCAT 3′
3′ TACG CGTA 5′
Denaturation and
Annealing
Pan handle
+
Pan handle
Treatment
with HCl
Terminal
protein
CGTA TACG
GCAT ATGC
TP produce
circular forms
TP digestion by
protease
produce linear form
5′ ATGC GCAT 3′
3′ TACG CGTA 5′

Reproductive cycle

- Burst size: 10,000 virions per cell.

- Multiplication cycle involves following steps:

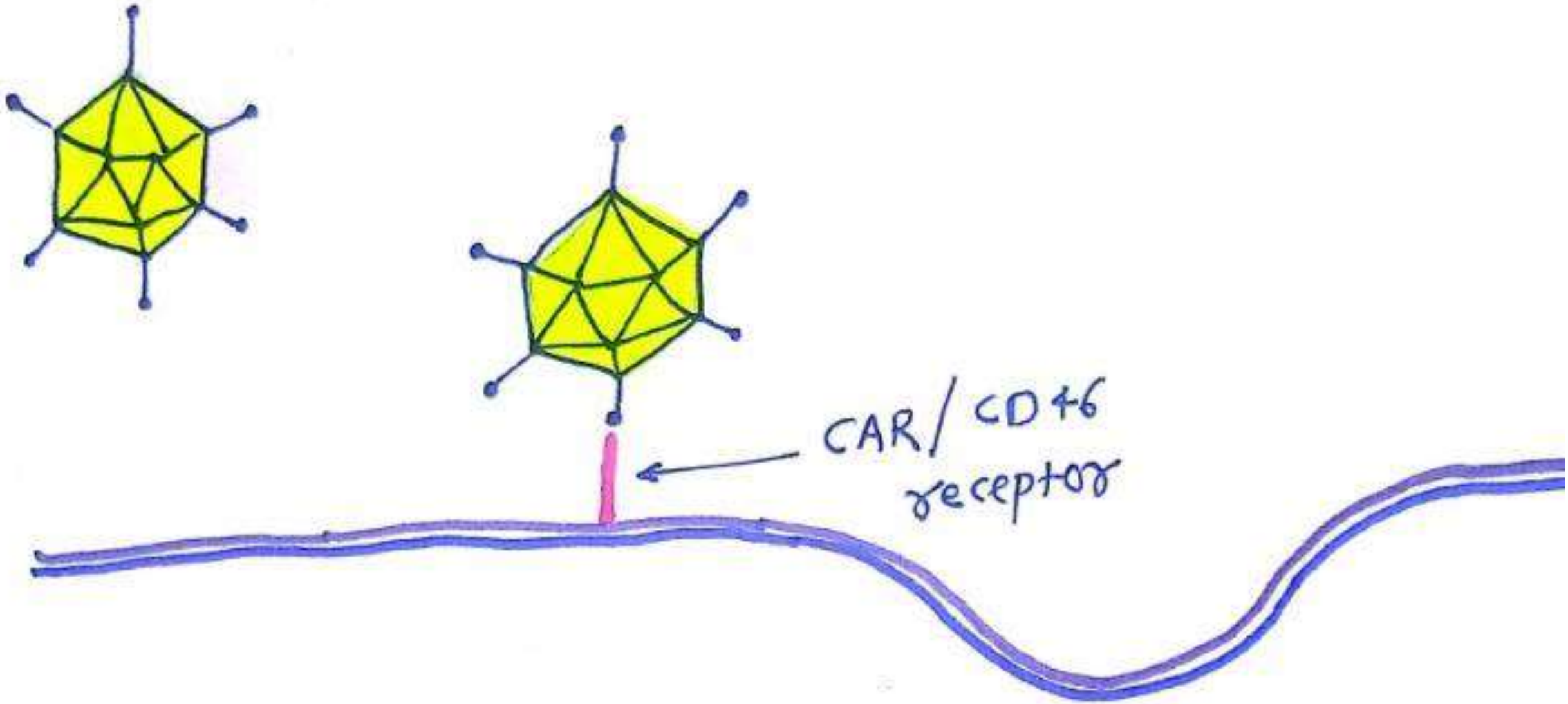
1. **Attachment**
2. **Penetration**
3. **Uncoating**
4. **Biosynthesis**
5. **Assembly**
6. **Release**

1. Attachment

❖**Adenovirus attachment organ: Penton fibres**

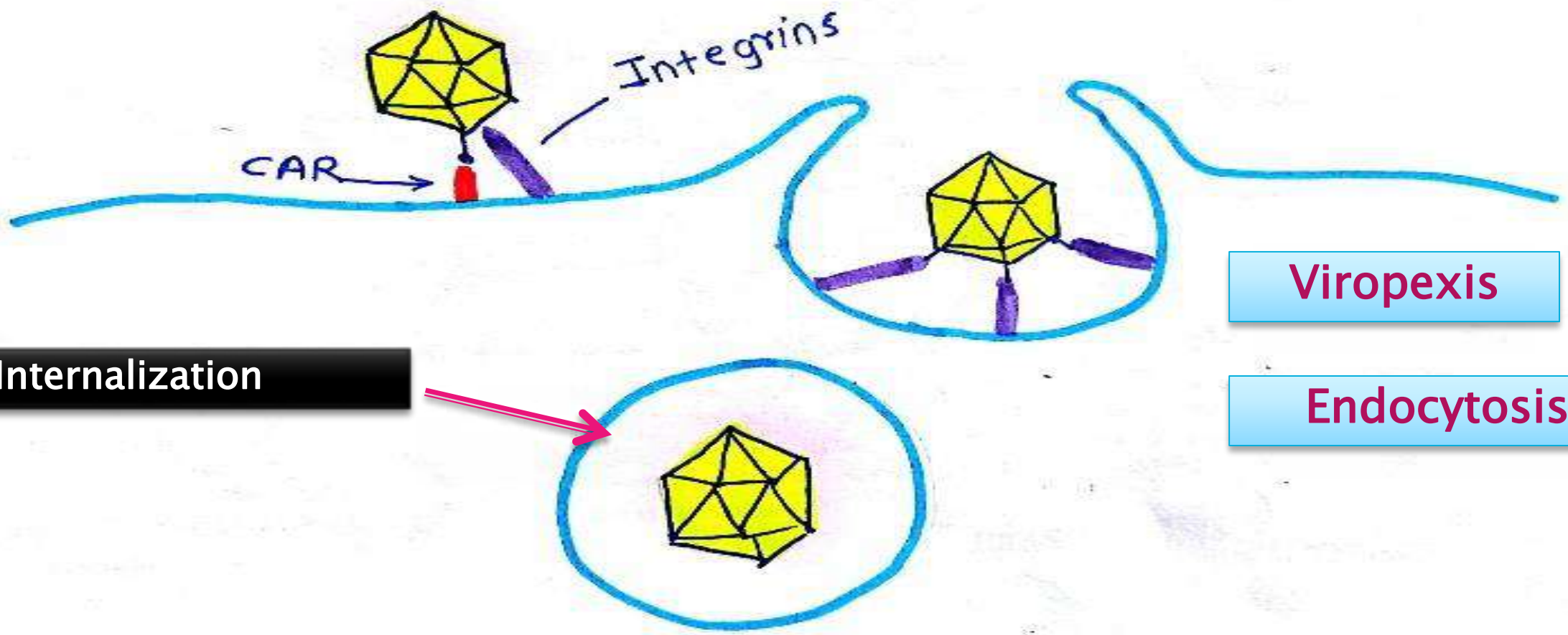
❖**Receptor sites:**

- **CD46** for group B adenovirus serotype.
- **Coxsackievirus Adenovirus Receptor** (CAR) for all other serotypes.
- **MHC class I** and **sialic acid** can also serves as receptor site.


CAR / CD46
receptor

2. Penetration

- Energy dependent process.

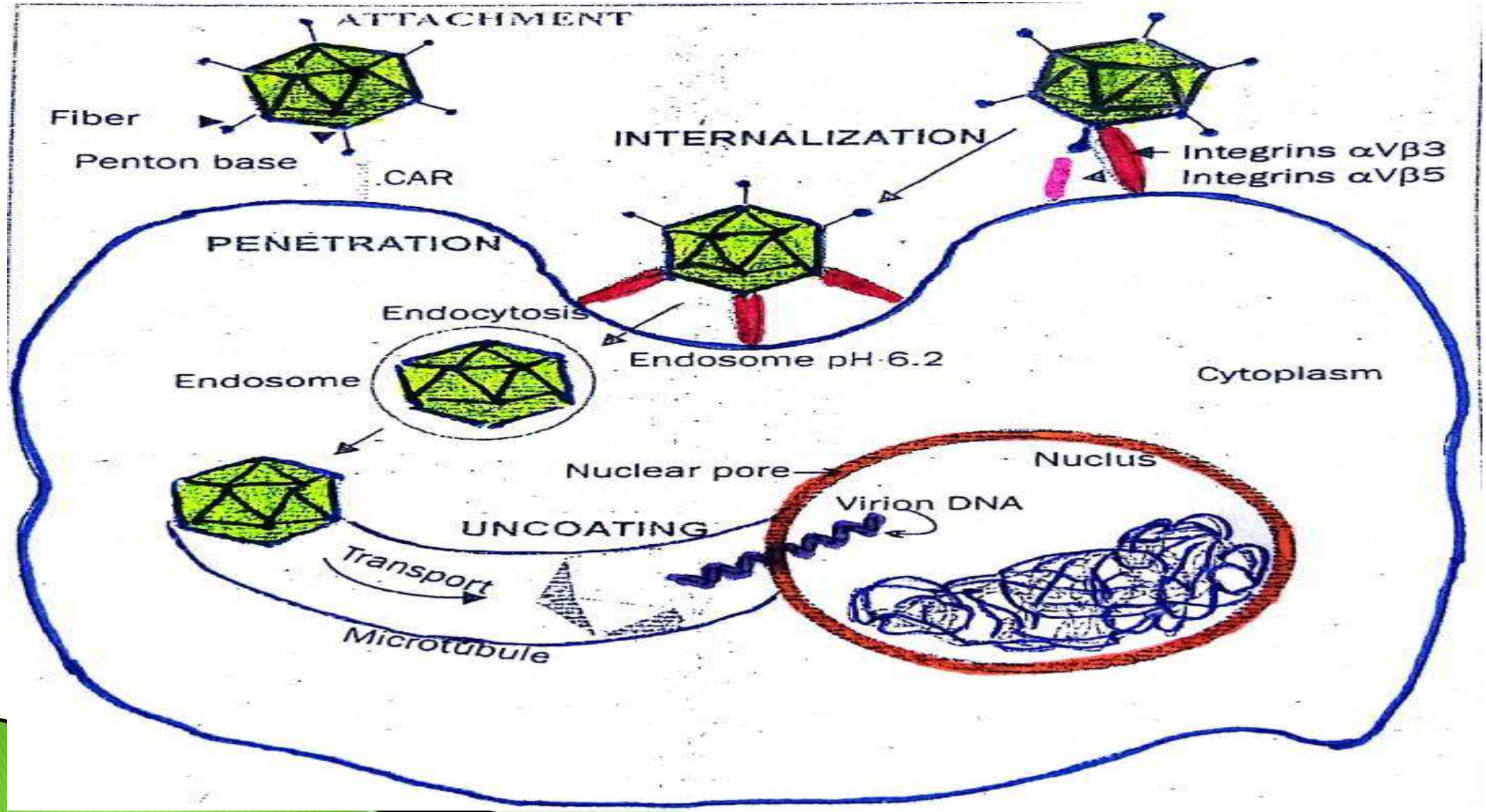


Viropexis

Internalization

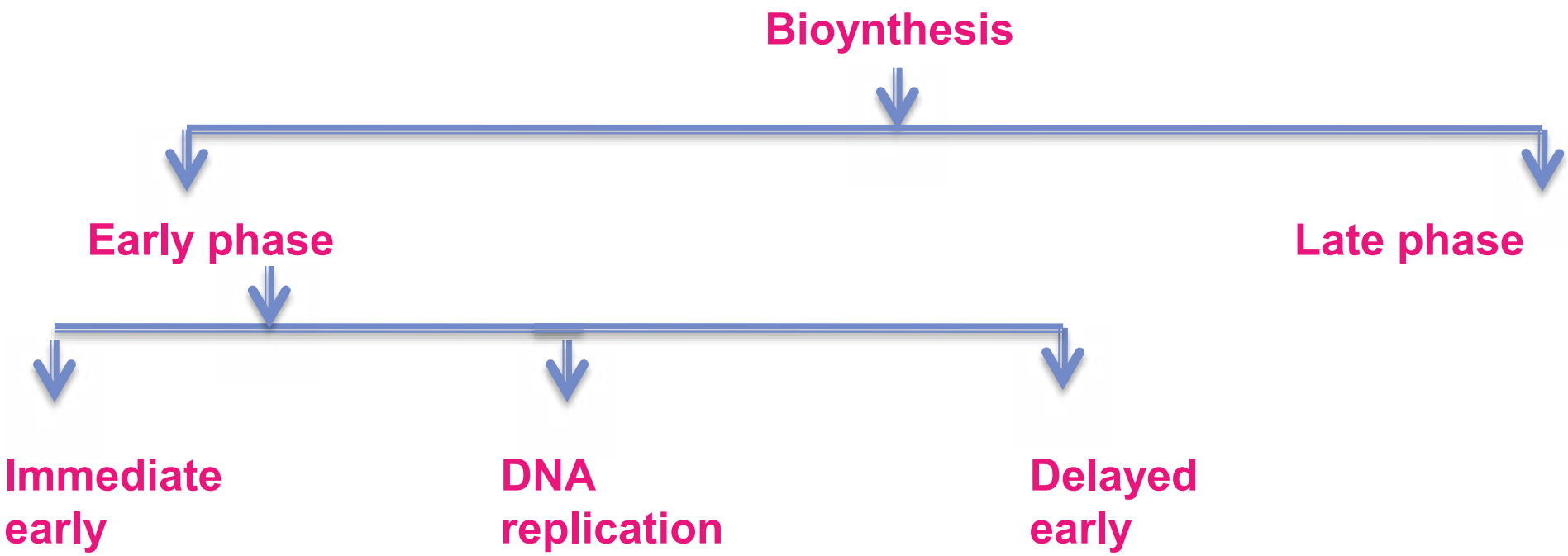
Endocytosis

3. Uncoating


ATTACHMENT
Fiber
Penton base
CAR
INTERNALIZATION
Integrins αVβ3
Integrins αVβ5
PENETRATION
Endocytosis
Endosome
Endosome pH 6.2
Cytoplasm
Nuclear pore
Nuclus
Virion DNA
UNCOATING
Transport
Microtubule

4. Biosyntheis

Upon penetration, rapid shut-off of host protein synthesis occurs.



1. Immediate early phase:

- **E1A gene transcript-** E1A protein activates transcription of delayed early genes.

2. Delayed early phase:

- Five regions are transcribed as E1A, E1B, E2, E3 and E4.

- **E1A**: Activates transcription of other genes.

  Induces host cell to enter ‘S phase’ of growth.

- **E1B**: Induces cell growth in co-operation with E1A

- **E2** (E2A and E 2B): Encodes 3 proteins.

  a. DNA polymerase

  b. Terminal protein

  c. DNA binding protein (DBP)

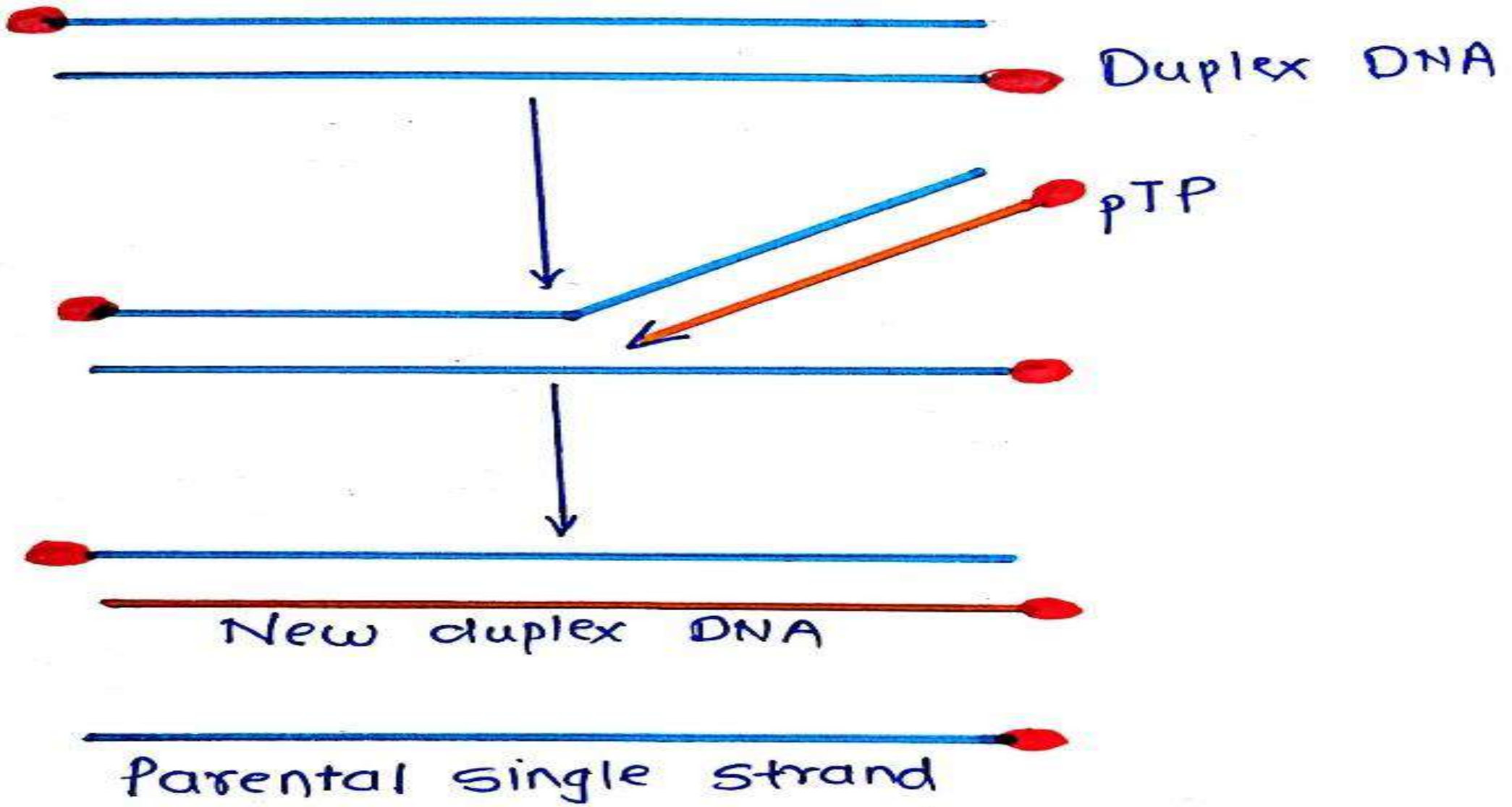
- **E3**: Regulates host defence mechanism.

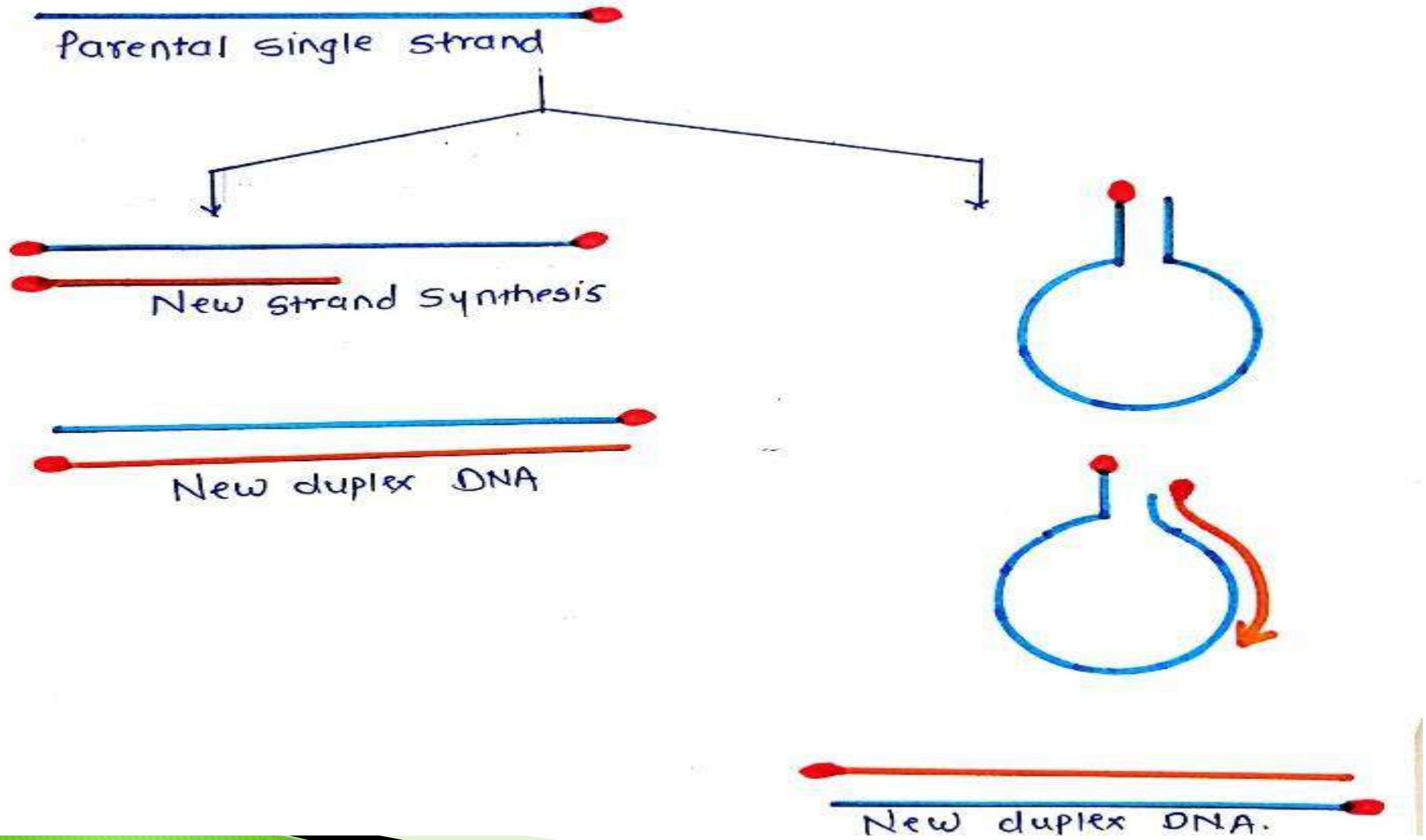
  Thought to involved in virus release.

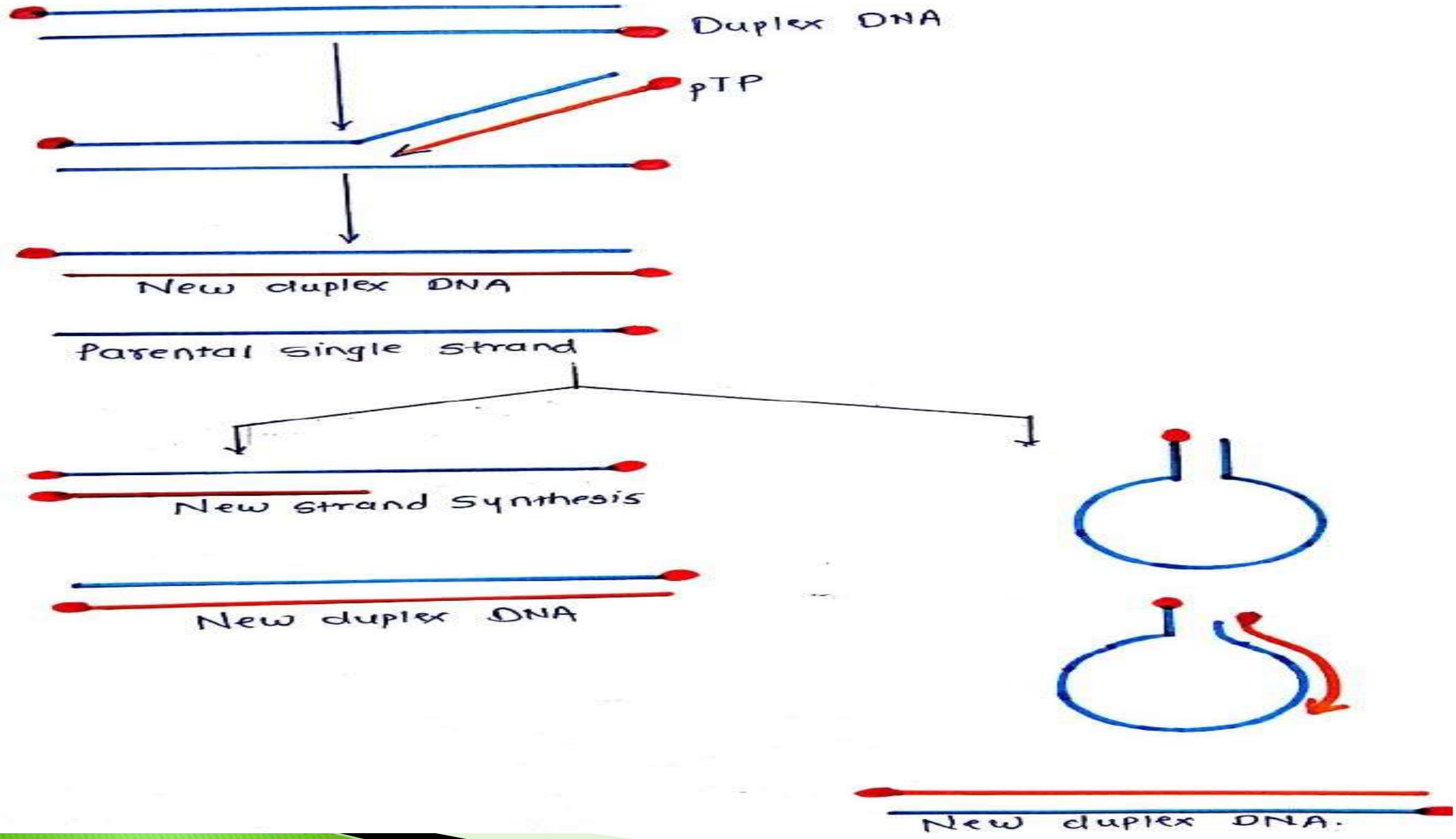
E4: Involved in transition from early to late phase.

- ✓ mRNA transport.
- ✓ Shut off of host gene expression.
- ✓ Viral DNA replication
- ✓ Assembly of virion

DNA replication




Parental single strand
New strand Synthesis
New duplex DNA
New duplex DNA.


Duplex DNA
pTP
New duplex DNA
Parental single strand
New strand synthesis
New duplex DNA
New duplex DNA.

Late phase

- Capsid proteins and packing proteins are synthesized.
- 2 genes are expressed-
  - **IX and IVa2**: Plays role in packing of phage DNA into capsid.
- **L1 to L5**: Assembly

5. Assembly

- **Assembly begins in cytoplasm and completes in nucleus**.
- Pentons and hexons are synthesized in cytoplasm and transported to nucleus.
- Immature empty capsid is assembled in nucleus.
- DNA enters nucleus and mature virions are produced.

6. Release

- Specific mechanism of release is unknown.
- E3 gene product (11.5kD) induces apoptosis.
- E3 protein also facilitates cell lysis that leads to release of virions from host cell.