AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 3.

Ultrastructure of bacteria. Acid-fast bacteria and Ziehl-Neelsen stain.

Spores and Ojeshko stain. Intracellular additives and Neisser stain

FACULTY: General Medicine
SUBJECT: Medical microbiology - 1

Discussed questions:

- The structure of the bacterial cell (structural features of acid-fast bacteria).
- Sil-Nielsen staining technique of acid-fast bacteria.
- The role of the Ziehl-Neelsen method in the diagnosis of tuberculosis.
- Spores, the stages of spore formation and stages.
- Technique of spores staining.
- Volutin granules and their detection by Neisser method.

Purpose of the lesson:

- Explain to students acid-fast bacteria, their Sil-Nielsen staining technique, the role of Sil-Nielsen method in diagnosis. To teach them spores, spore-forming bacteria, the form, location, importance of spores, the process of formation and the technique of staining by the Ojeshko method. Explain to students the capsule, its chemical composition and function, its detection by the Gins-Burry method and its role in diagnostics. Explain to students the structure, location, chemical composition and function of flagella, the organ of movement of bacteria, the methods used to study the movement of bacteria and their role in diagnosis.

Acid resistant bacteria

Due to the weak permeability of the cell wall, they are resistant to decolorization with acid, alcohol and alkalis. This specificity is due to the presence of the following substances in their cell wall:

Lipids

Similar substances

Oxytocin

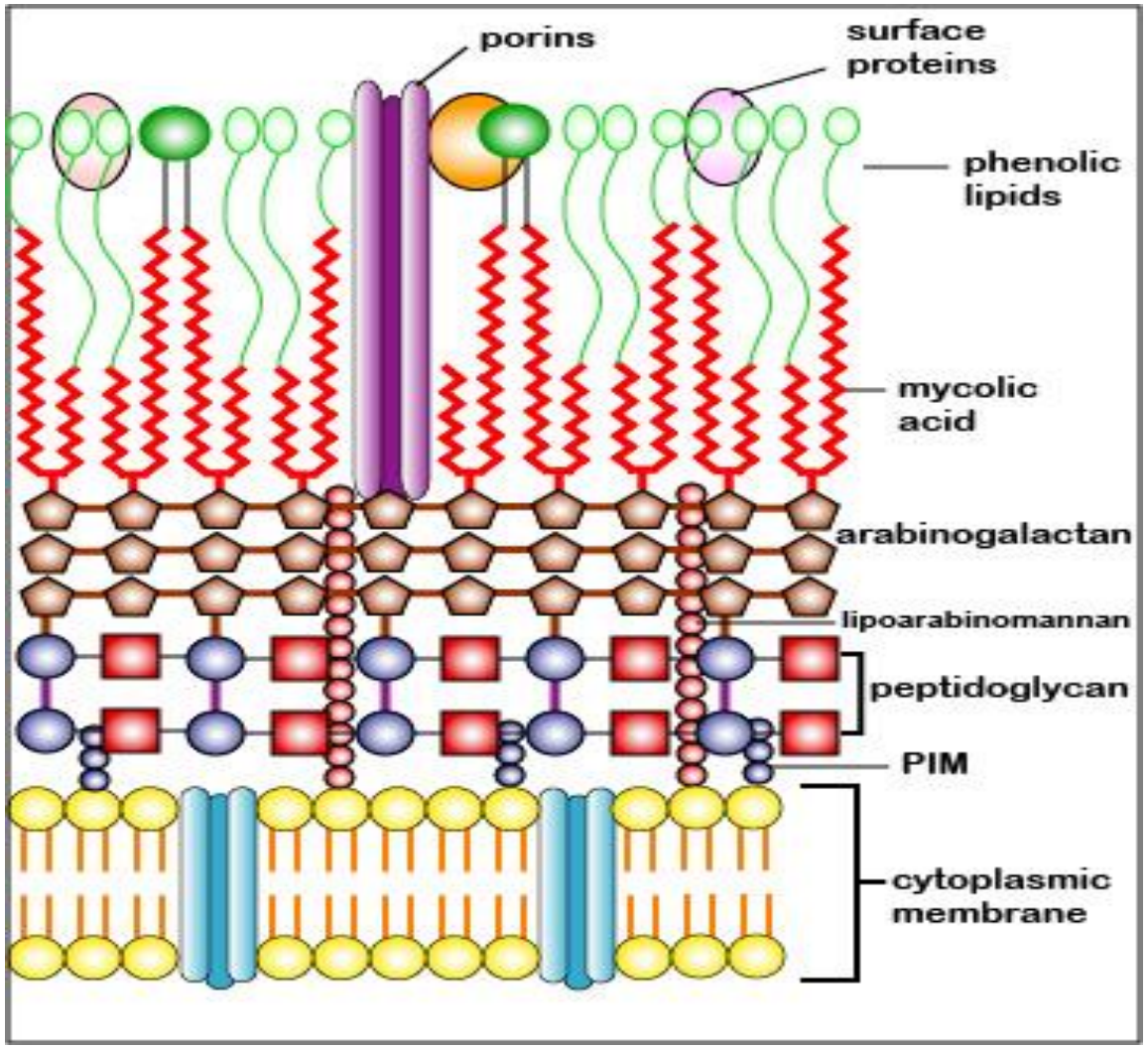
Mycolic acid, etc.

Mycobacterium tuberculosis (TB)

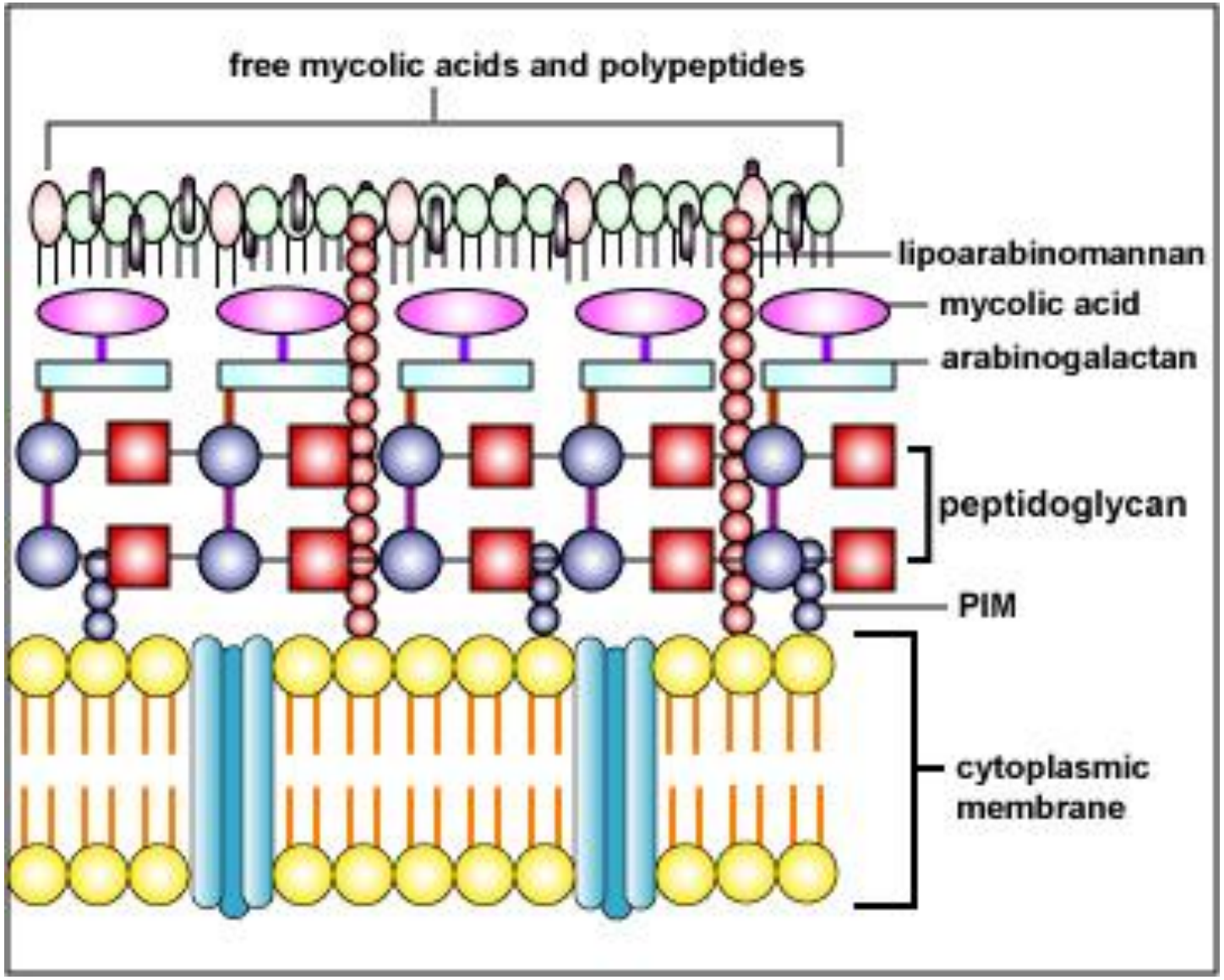
M.leprae (leprosy)

Some species of Actinomyces

Cell wall of acid fast bacteria



Cell wall of acid fast bacteria



Ziehl-Neelsen technique (AFB Staining)

Place slide with heat fixed smear on staining tray. Put a filter paper on the slide and add carbol fuchsin, heat the smear until vapour just begins to rise. Do not overheat (boil or dry). Add additional stain if necessary. Allow the heated stain to remain on the slide for 5 minutes.

Tilt the slide slightly and filter paper are discarded. Add 5% sulfuric acid or 3% acid alcohol solution for 10-15 seconds until the smear is sufficiently decolorized.

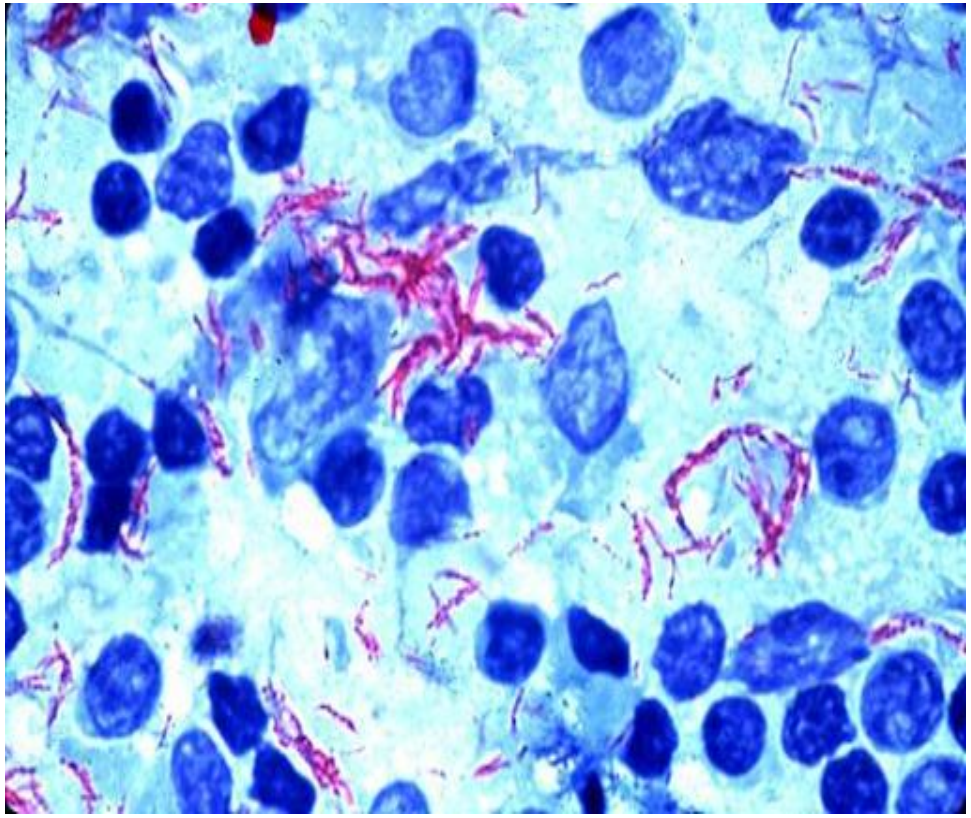
Tilt the slide slightly and gently rinse with tap water. Add methylene blue for 1 min. Tilt the slide slightly and gently rinse with tap water. Allow the slide to dry with bibulous paper. View the smear using a light-microscope under oil-immersion. Acid fast bacteria is stained red-pink, non-acid fast bacteria is blue

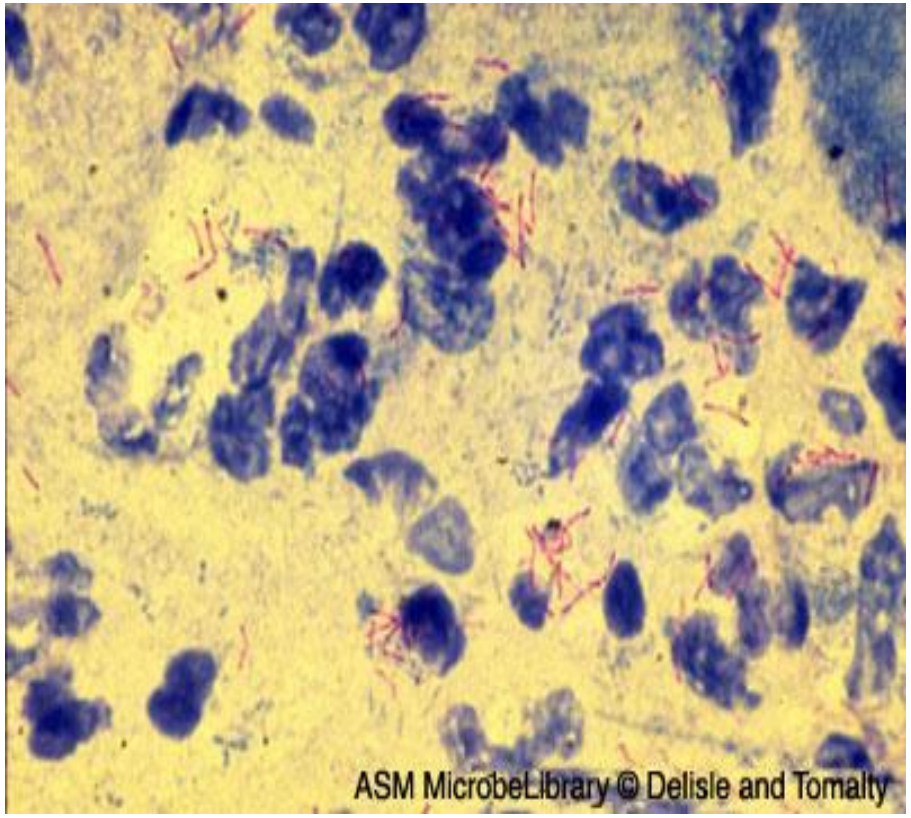
Results of Acid Fast Staining:

Reagent	Acid Fast	Non-Acid Fast
Carbol Fuchsin with heat	Red (Hot Pink)	Red (Hot Pink)
Acid Alcohol	Red	Colorless
Methylene Blue/Malachite Green	Red	Blue/Green

1. **AFB:** Red, straight or slightly curved rods, occurring singly or in small groups, may appear beaded.
2. **Cells:** Green
3. Background material: Green

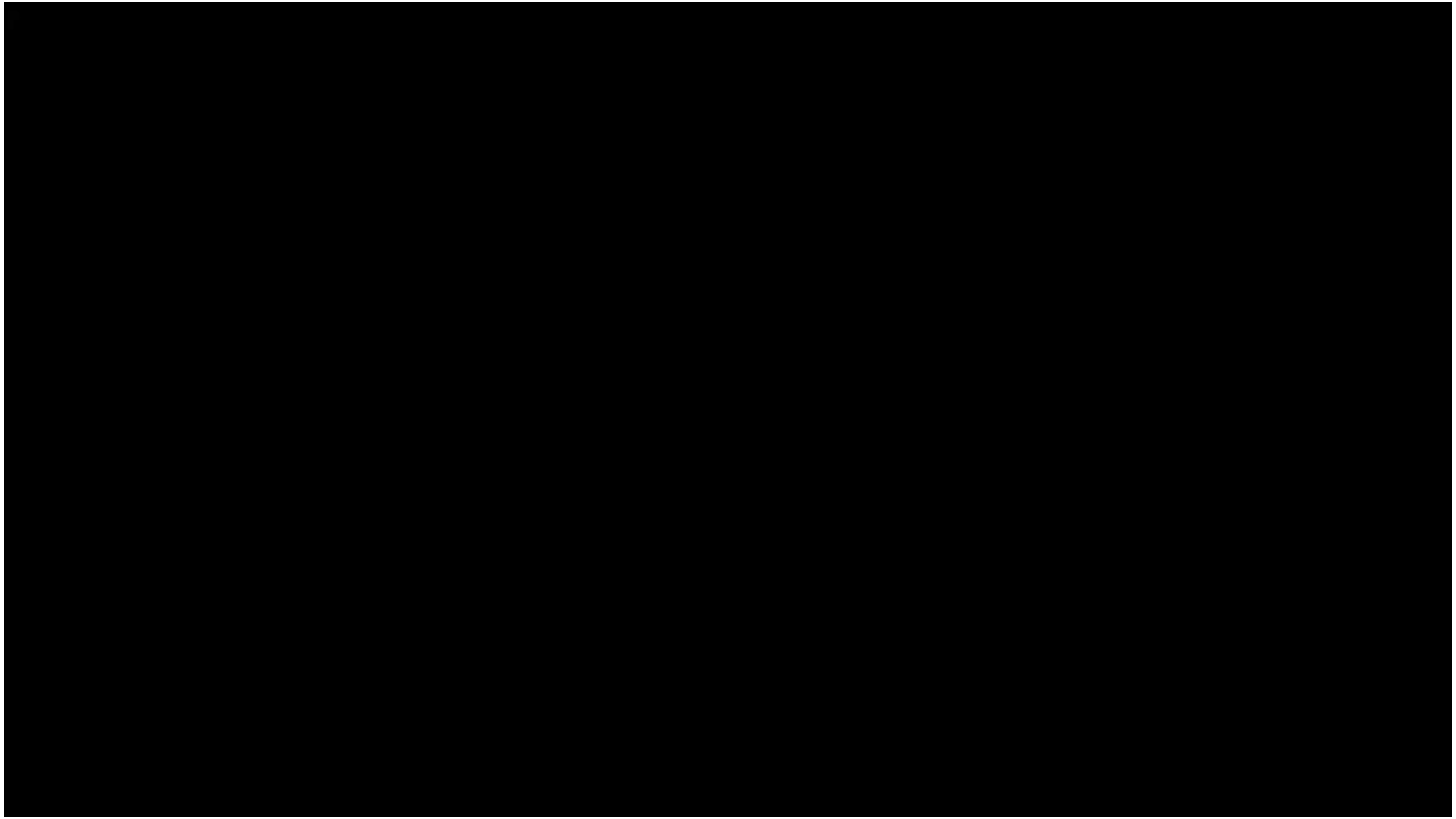
Acid fast bacteria





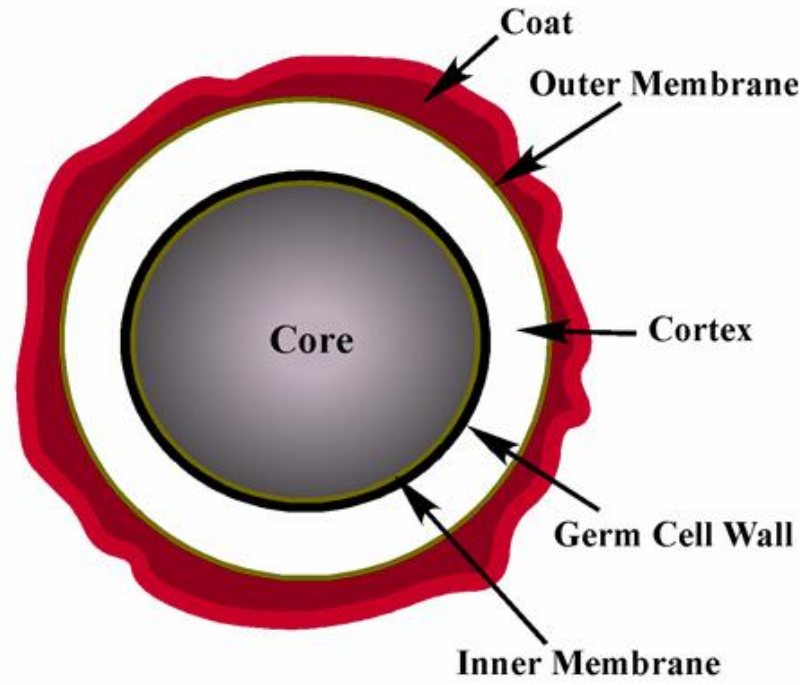
Acid fast bacteria are red,
Non acid fast bacteria appear in blue

Ziehl-Neelsen technique (AFB Staining)

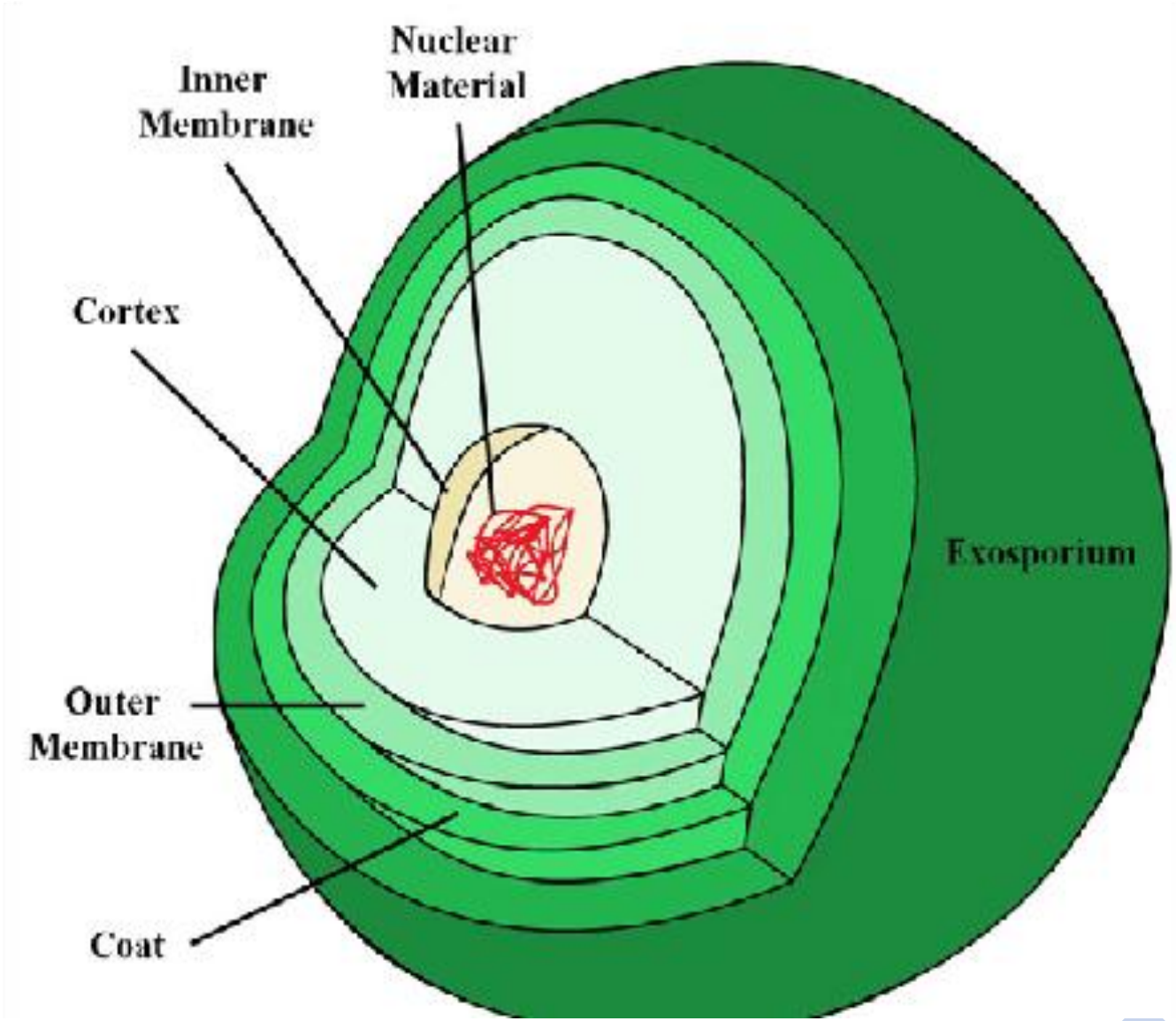


Bacterial spores

- *Form of conservation of the species in adverse conditions*
- *Sporulation lasts 20-24 hours*
- *Metabolic activity is very poor*
- *The conductivity is very weak, they are resistant to acid, alkaline, alcoholic acid*
- *It is mainly found in Gram positive bacteria (clostridia and bacilli)*

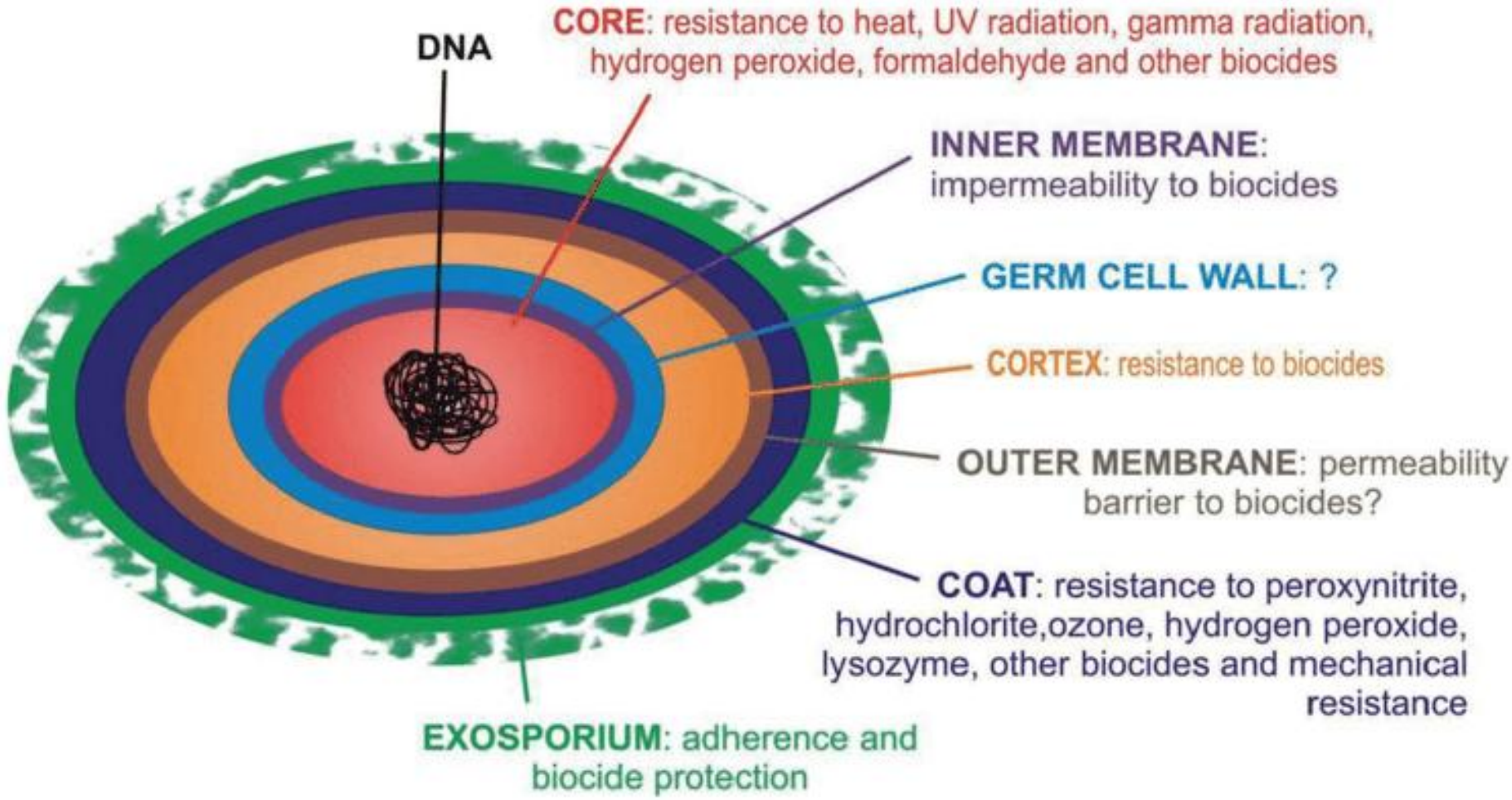


- Highly resistant resting stages formed during adverse environment (depletion of nutrients)
- Formed inside the parent cell, hence called Endospores
- Very resistant to heat, radiation and drying and can remain dormant for hundreds of years.
- Formed by bacteria like *Clostridia*, *Bacillus*

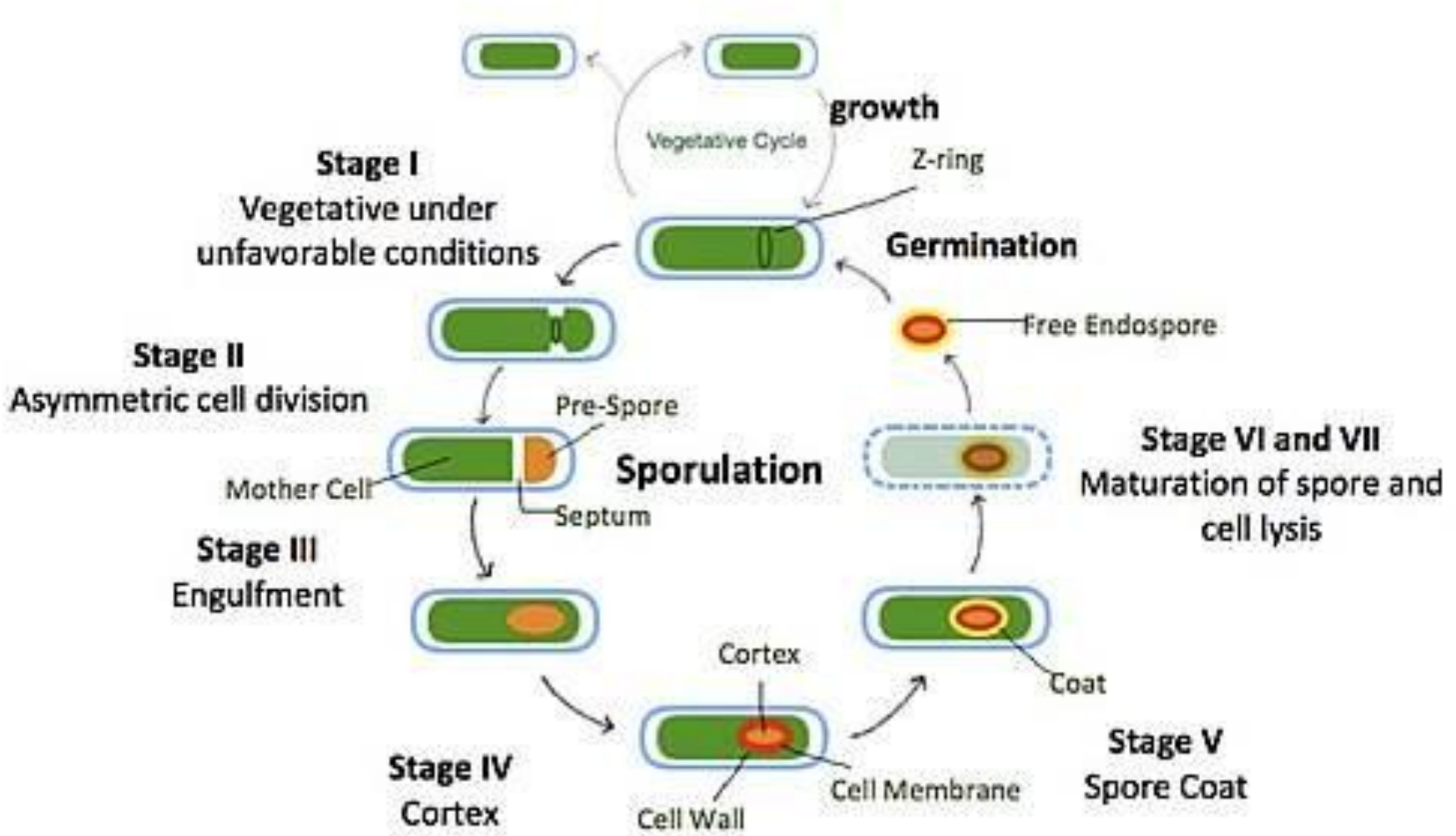

Nuclear
Material
Inner
Membrane
Cortex
Exosporium
Outer
Membrane
Coat

Sporulation

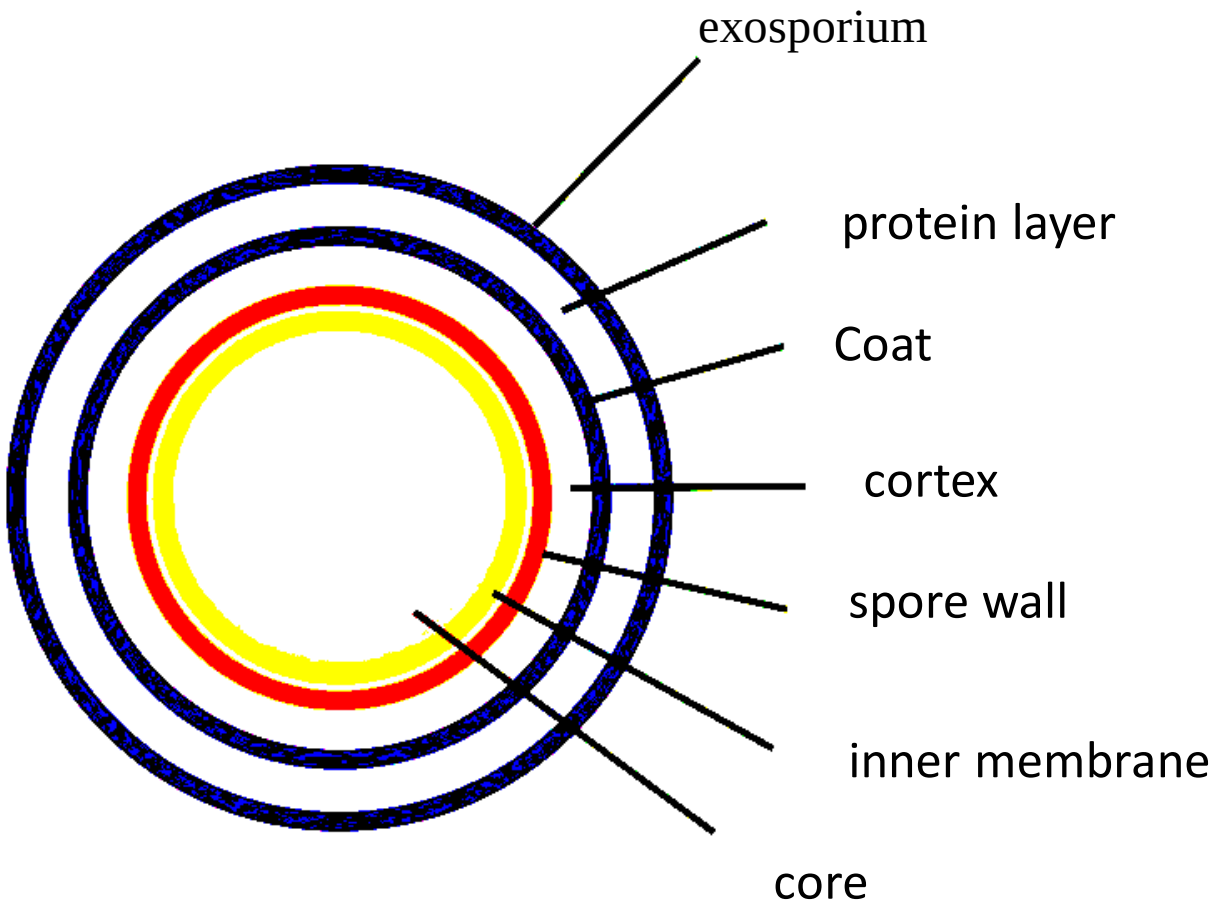
- *Form of conservation of the species in unfavorable conditions*
- *Sporulation lasts 20-24 hours*
- *The protoplasm is compacted to the nucleoid. This part is called the spore core.*
- *Enzyme activity increases.*
- *Unique enzymes are dipicolinsintetase (5-10%).*
- *The core contains calcium salt of dipicolinic acid*
- *The core is surrounded by a peptidoglycan wall is called the prospore*
- *Between these layers, the peptidoglycan is formed by the cortex.*
- *A layer of keratin-like protein is formed over the cortex layer*
- *The outer membrane of the spore contains lipoprotein and a small amount of carbohydrates.*


DNA
CORE: resistance to heat, UV radiation, gamma radiation, hydrogen peroxide, formaldehyde and other biocides
INNER MEMBRANE: impermeability to biocides
GERM CELL WALL: ?
CORTEX: resistance to biocides
OUTER MEMBRANE: permeability barrier to biocides?
COAT: resistance to peroxynitrite, hydrochlorite,ozone, hydrogen peroxide, lysozyme, other biocides and mechanical resistance
EXOSPORIUM: adherence and biocide protection

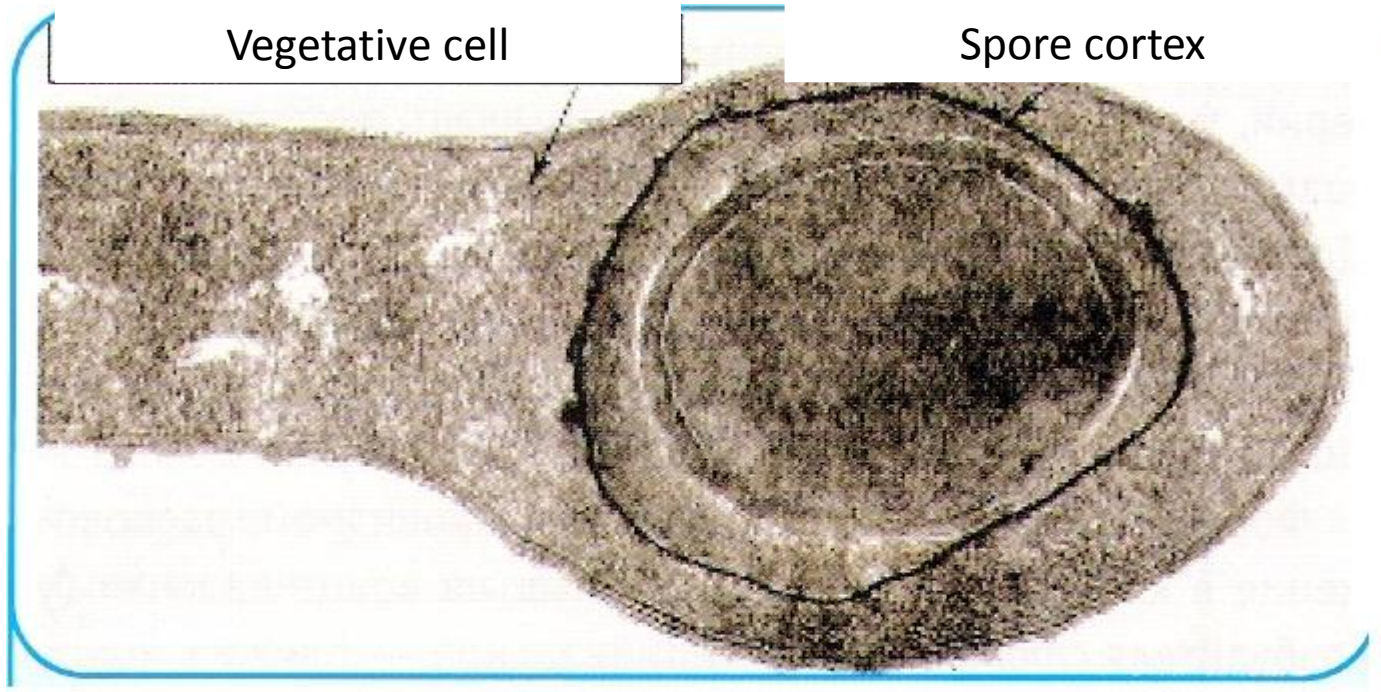
Sporulation



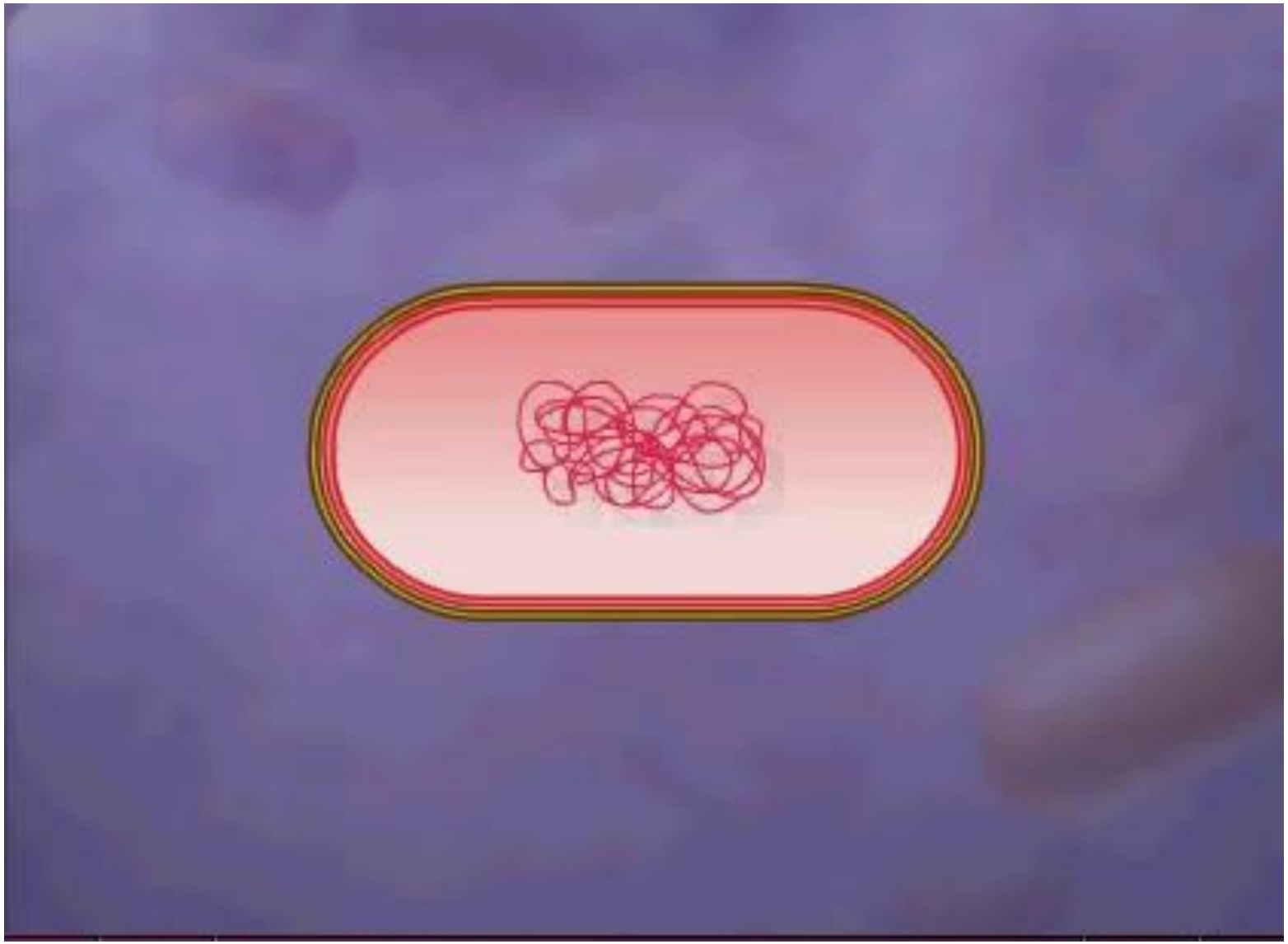
Structure of spore



Bacterial spore

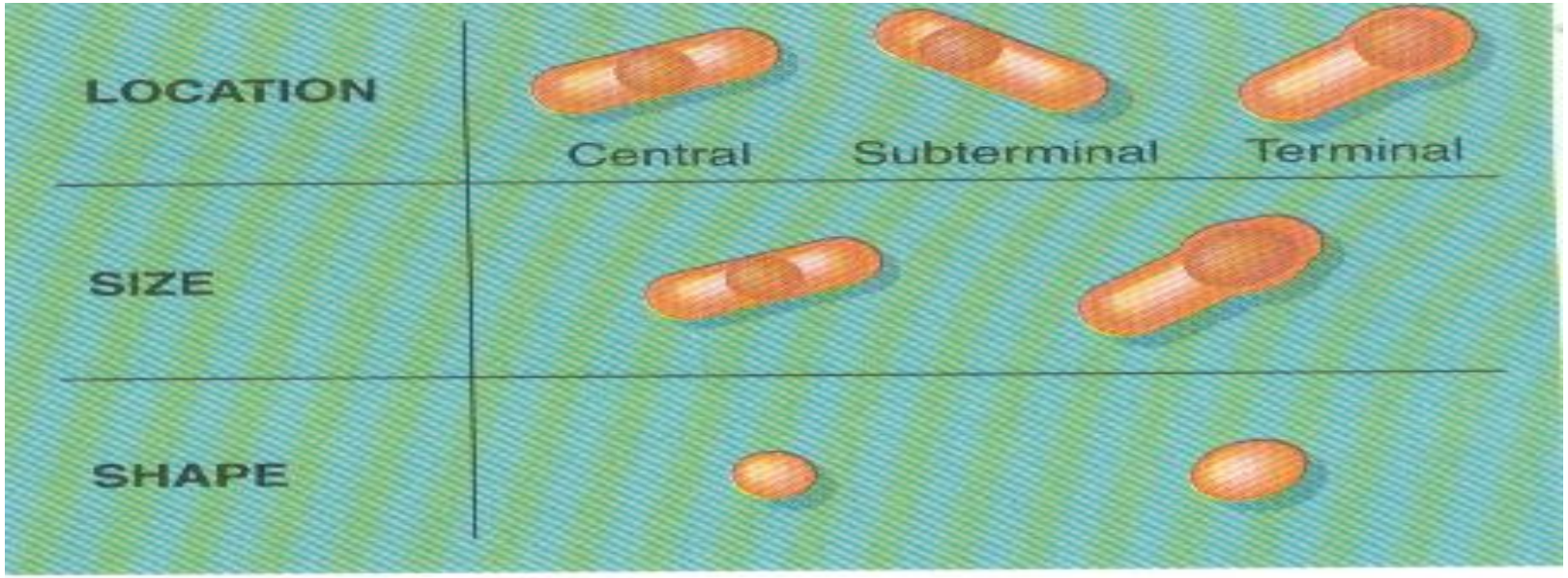


Process of sporulation

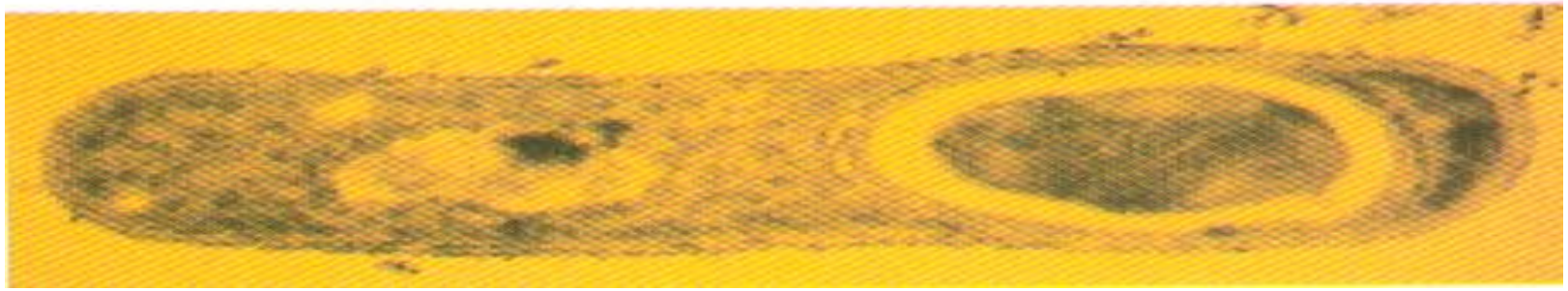


Types of spores in bacteria

***central** – causative agent of anthrax (B.antracis)*
***terminal** – causative agent of tetanus (C.tetani)*
***subterminal** – causative agent of botulism (C.botulinum) and gas gangrene (C.perfringens)*



(a)



Sporulation and germination

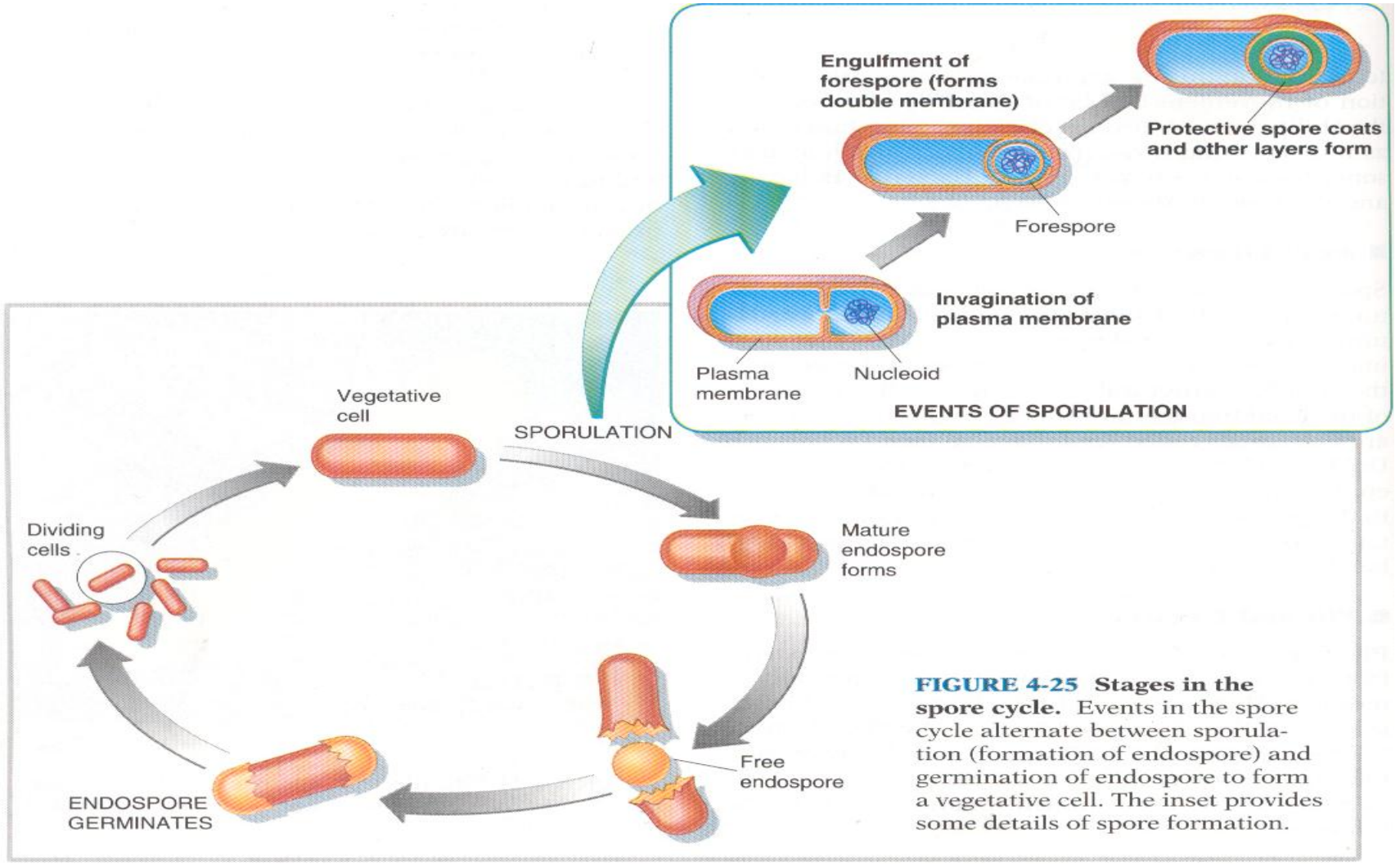
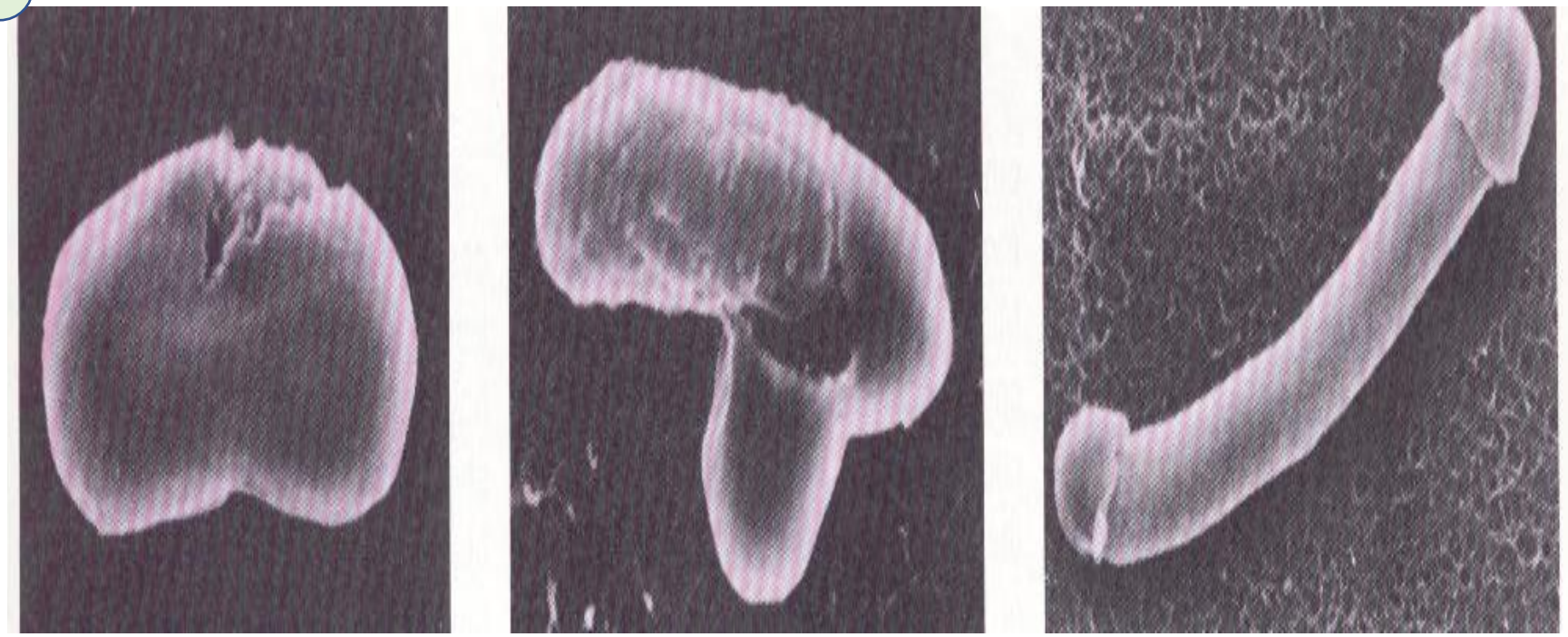


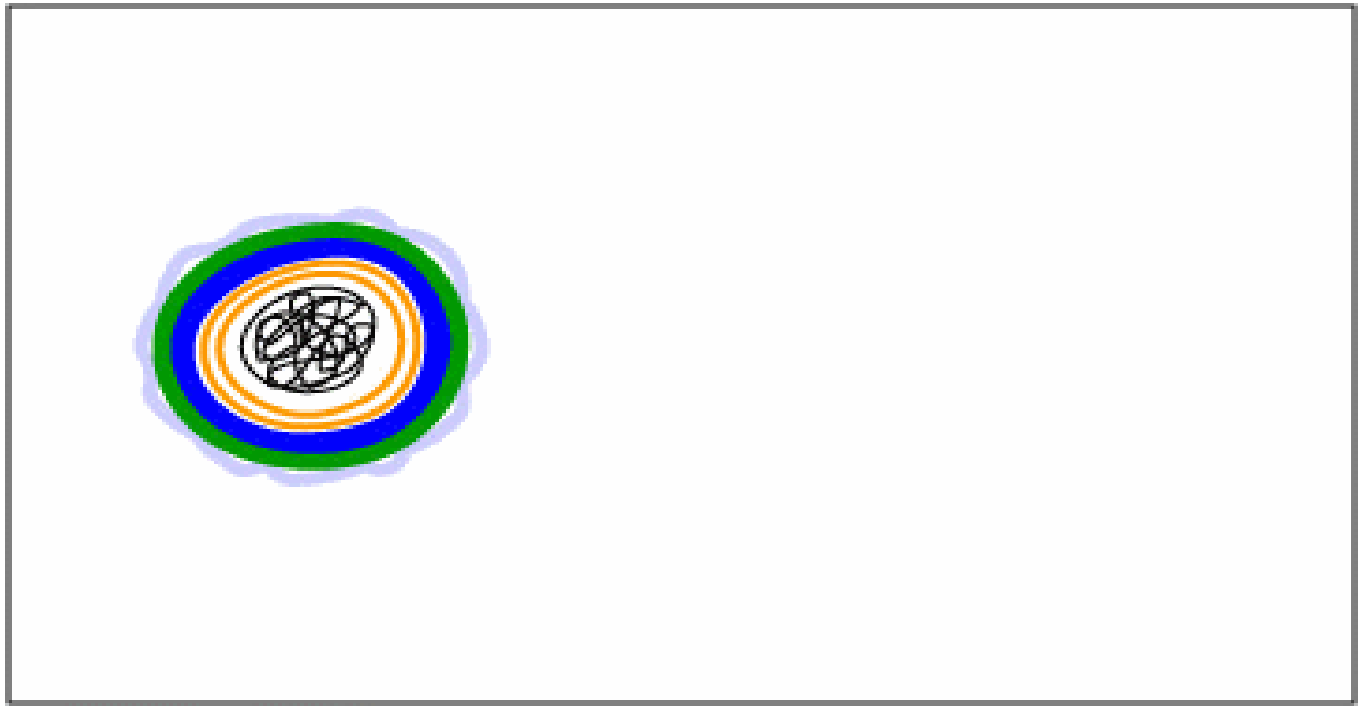
FIGURE 4-25 Stages in the spore cycle. Events in the spore cycle alternate between sporulation (formation of endospore) and germination of endospore to form a vegetative cell. The inset provides some details of spore formation.

Germination

Under favorable conditions (in the human body) spores are converted into vegetative forms. This process is called germination and lasts 3-5 hours. First of all, the cortex is decomposed by the lysosome, and the vegetative form is removed. Then the process of cell growth and division is underway.



Process of germination



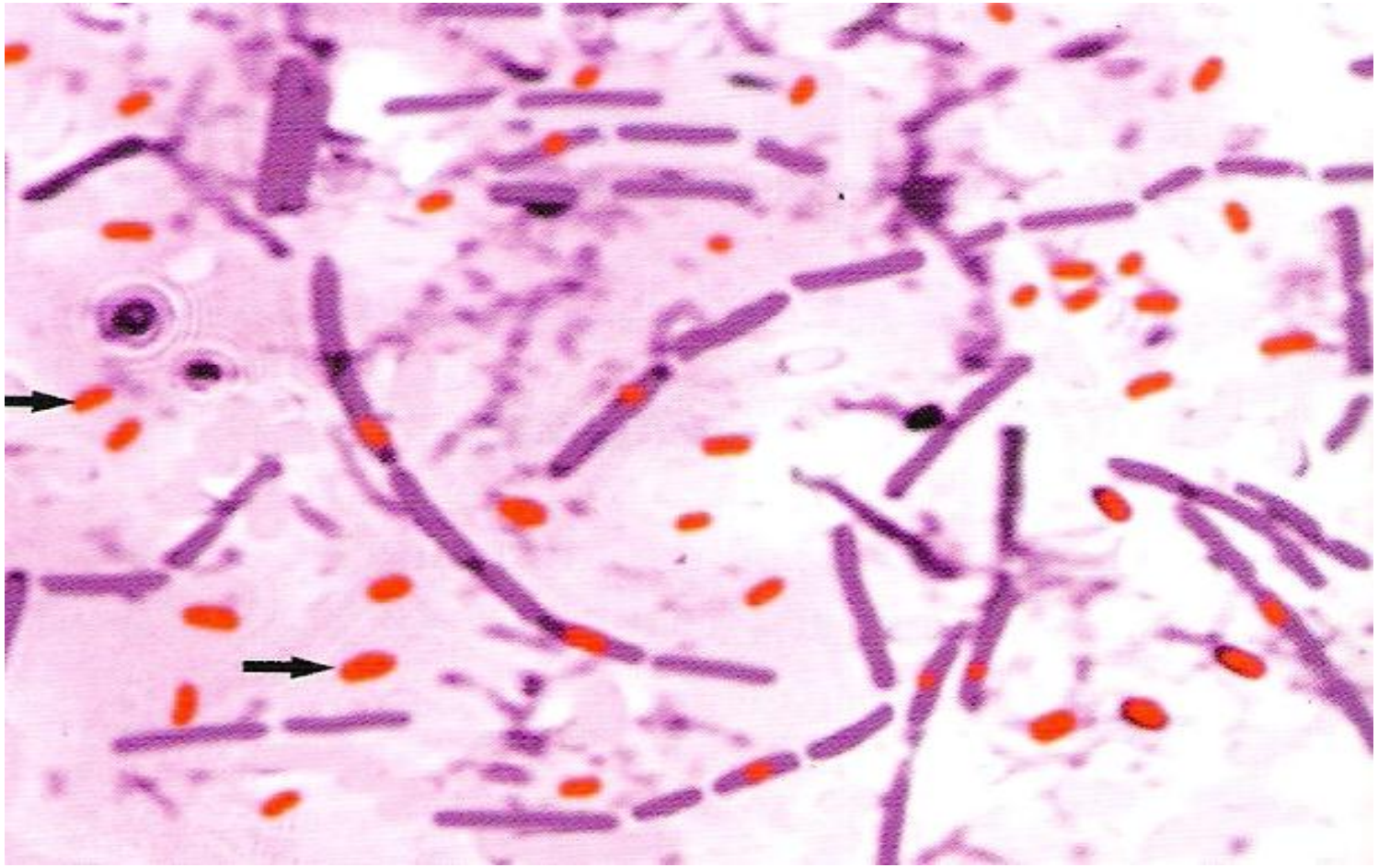
Technique of stainin of spores by Ojeshko method

Place slide with heat fixed smear on staining tray. Add 0,5% salt acid (HCl) heat the smear keep the slide steaming. Do not overheat (1-2 min.)

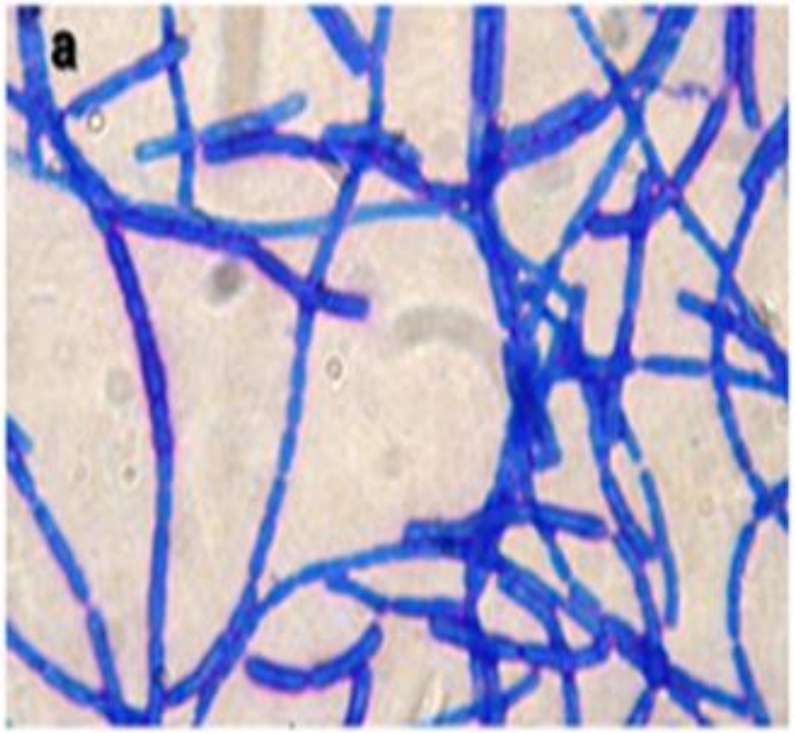
Tilt the slide slightly and gently rinse with tap water. Allow the slide to dry with bibulous paper or heat fix the smear.

After stain by the Ziehl-Neelsen method. The spores are stained red and vegetative forms are stained blue.

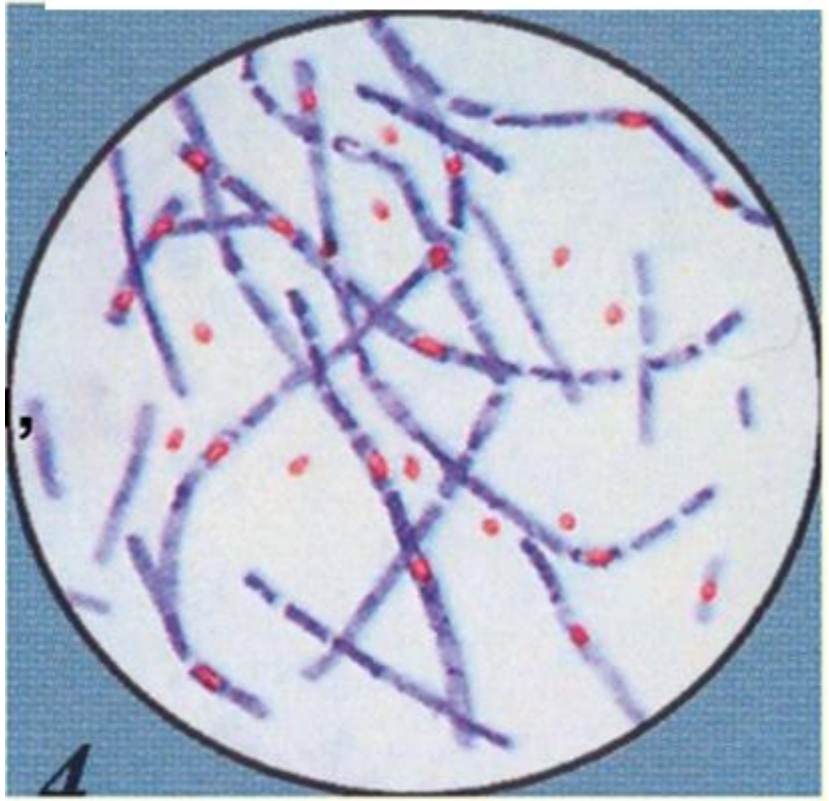
Spore staining (Ojeshko)



Bacillus antracis (vegetative and spore forms)



B.antracis vegetative form
(staining with methylene blue)

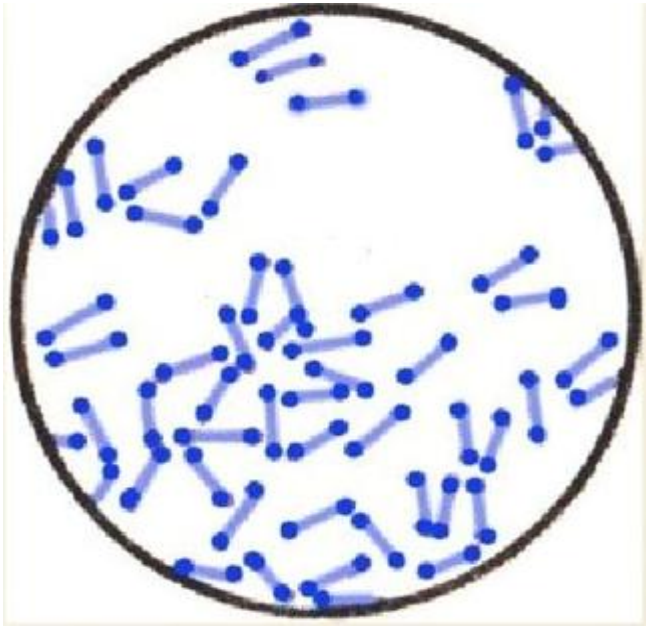


B.antracis spore– Ojeshko method

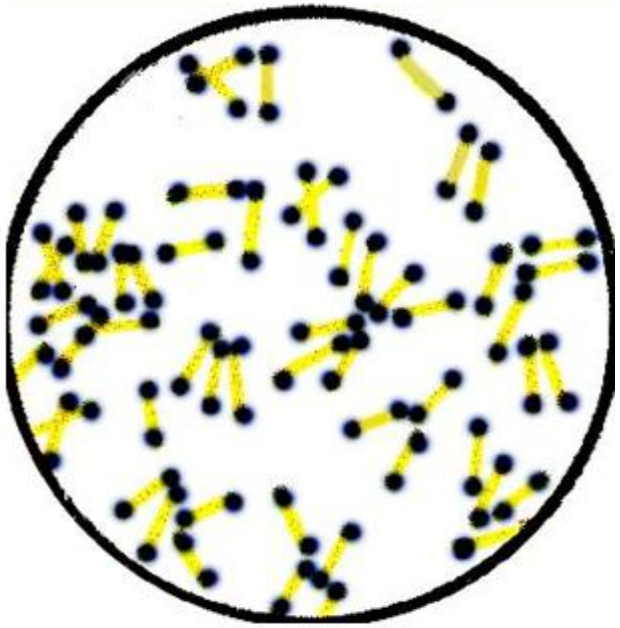
Volutin granules

- In microorganisms, polyphosphate is a cytoplasmic additive in the form of granules. It was first described in the bacterium Spirillum volutans (hence the name)
- Volutin is an internal reserve of phosphates, and when there is a lack of phosphorus in the environment, the cell divides several times due to it.
- Many bacteria accumulate volutein when some nutrients are deficient. Yeasts, corynebacteria, and mycobacteria usually form appendages in the later stages of growth

Corynobacterium diphteria – volutin granules



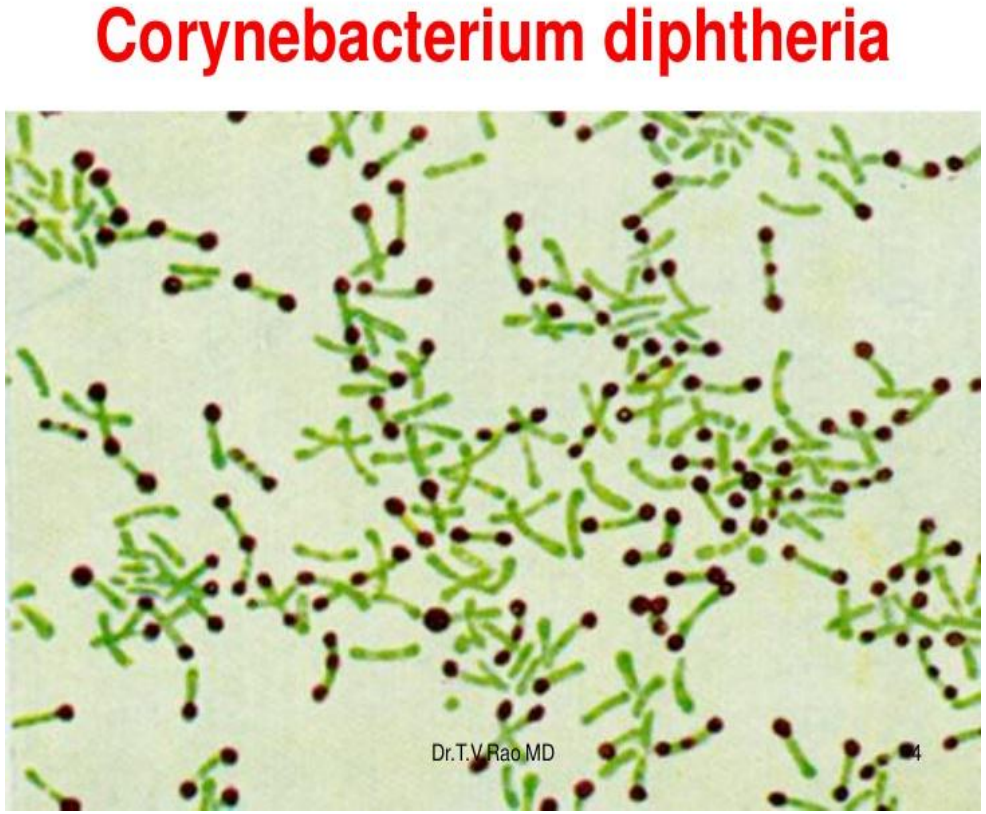
Leffler method



Neisser method

Volutin granules

- *Polyphosphate granules-metachromatic grains (Babes-Ernest bodies) are found in cornebacteria (Corynebacterium diphteria, etc.), a sign of recognition of these bacteria*
- *It is determined by Neisser method.*



Neisser staining method

Neisser staining method

Place slide with heat fixed smear on staining tray. Add Neisser Glacial acetic acid and methylene blue for 2-3min. Tilt the slide slightly and gently rinse with tap water.

Add iodine for 30 sec-1 minute. Tilt the slide slightly and gently rinse with tap water.

Add Chrysoidin Y for5-7 min. Tilt the slide slightly and gently rinse with tap water Allow the slide to dry and then view with a 100x bright field objective.

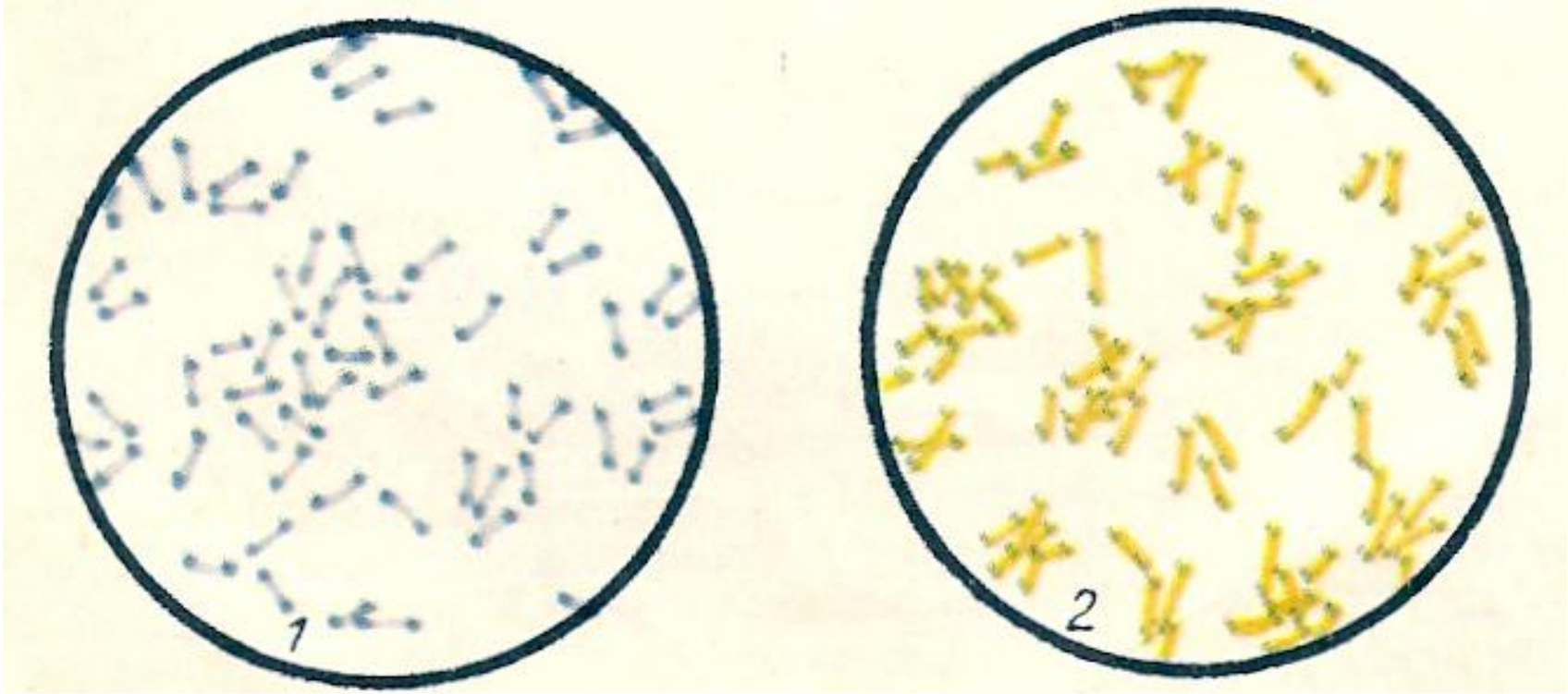
The result of Neisser's method

A.L.Neisser
(1855-1916)

Because the voluetine grains are alkaline, they turn a dark blue color, taking on the color of acetate.

Because the cytoplasm is acidic, it takes on the color of vesuvin and turns yellow

Corynobacterium diphteria – volutin granules



Methylene blue **Neisser method**