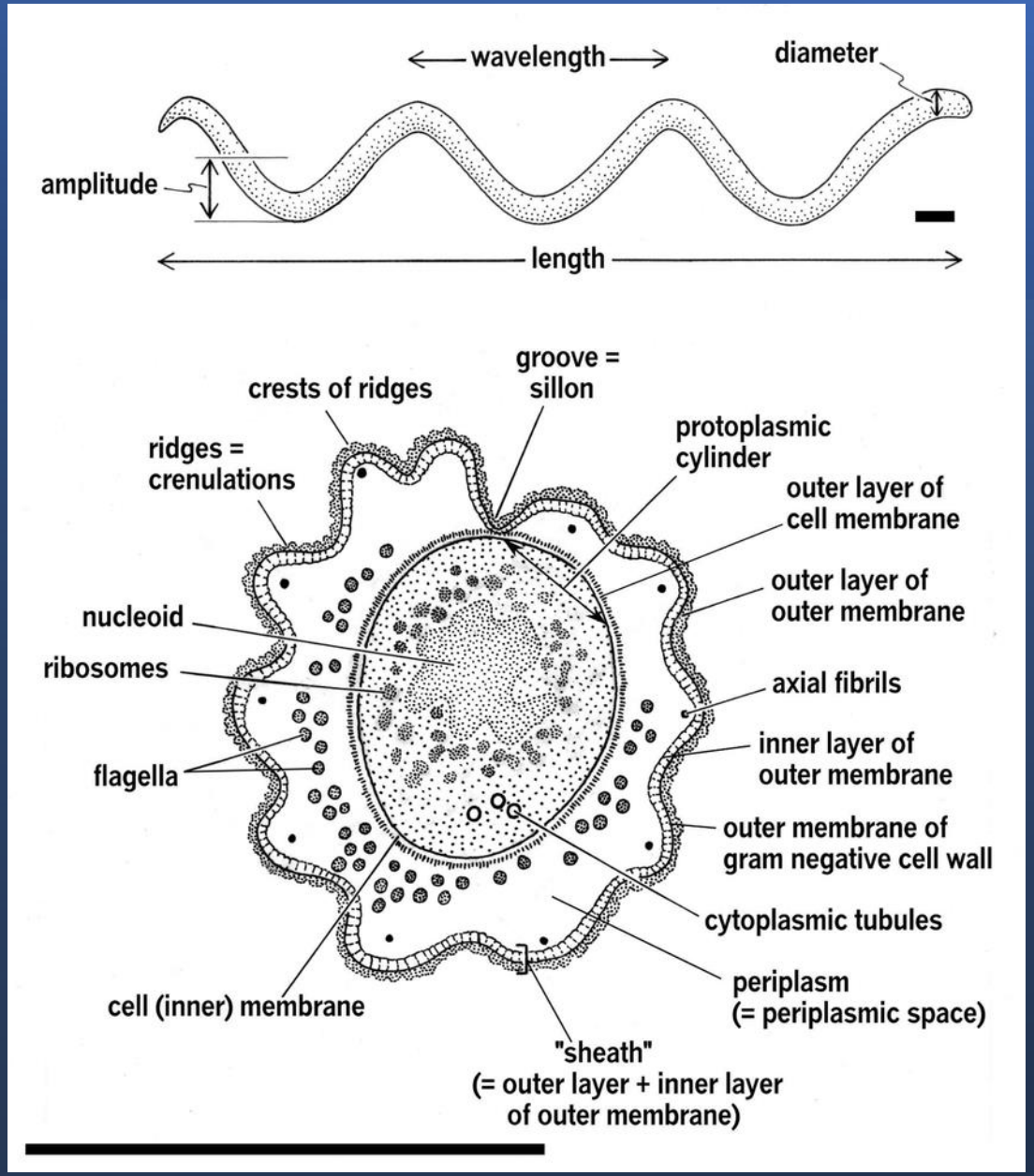
Practical lesson 3
Morphology and classification of Spirochetes, Rickettsia, Chlamydia, Mycoplasma and Actinomycetes. Giemsa staining.

- ***Morphology and Ultrastructure of Spirochaetes.***
- Genetically spirochaetes (L. *spira* curve, Gk. *chaite* cock, mane) differ from bacteria and fungi in structure with a corkscrew spiral shape. The body of the spirochaete consists of an axial filament and cytoplasm wound spirally around the filament. No special membrane separates the nucleoid from the cytoplasm. Spirochaetes have a three-layer outer membrane. As demonstrated by electron microscopy, they possess a fine cytoplasmic membrane enclosing the cytoplasm. The spirochaetes do not possess the cell wall characteristic of bacteria, but electron microscopy has revealed that they have a thin cell wall (periplast) which encloses the cytoplasm (Fig.42). Spirochaetes do not produce spores, capsules, or flagella. Very delicate terminal filaments resembling flagella have been revealed in some species under the electron microscope.
- *Classification of spirochaetes.* The order *Spirochaetales*, family Spiro-chaetaceae includes the sprophytes *(Spirochaeta, Cristispira)* representing large cells, 200-500 mcm long, some of which have crypts (undulating crests); the ends are sharp or blunt. They live on dead substrates, in foul waters and in the guts of cold-blooded animals. They stain blue with the Giemsa stain.
- Three pathogenic genera belong to the family Spirochaetaceae:
- *Borrelia,*
- *Treponema,*
- *Leptospira.*

spirochetes


wavelength
diameter
amplitude
length
groove =
sillon
crests of ridges
ridges =
crenulations
protoplasmic
cylinder
outer layer of
cell membrane
outer layer of
outer membrane
nucleoid
ribosomes
axial fibrils
inner layer of
outer membrane
flagella
outer membrane of
gram negative cell wall
cytoplasmic tubules
periplasm
(= periplasmic space)
cell (inner) membrane
"sheath"
(= outer layer + inner layer
of outer membrane)

Giemsa staining procedure:

- ***Thin blood films*** (only)
- 1. Fix air-dried film in absolute methanol by dipping the film briefly (two dips) in a Coplin jar containing absolute methanol.
- 2. Remove and let air dry.
- 3. Stain with diluted Giemsa stain (1:20, vol/vol) for 20 min. For a 1:20 dilution, add 2 ml of stock Giemsa to 40 ml of buffered water in a Coplin jar. 4. Wash by briefly dipping the slide in and out of a Coplin jar of buffered water (one or two dips). Note: Excessive washing will decolorize the film.
- 5. Let air dry in a vertical position.
- ***Thick blood films*** (only)
- 1. Allow film to air dry thoroughly for several hours or overnight. Do not dry films in an incubator or by heat, because this will fix the blood and interfere with the lysing of the RBCs. 2. DO NOT FIX.
- 3. Stain with diluted Giemsa stain (1:50, vol/vol) for 50 min. For a 1:50 dilution, add 1 ml of stock Giemsa to 50 ml of buffered water in a Coplin jar.
- 4. Wash by placing film in buffered water for 3 to 5 min.
- 5. Let air dry in a vertical position

- ***Morphology and Ultrastructure of Rickettsiae***

- Rickettsiae are included in the order *Rickettsiales* of obligate intracellular bacteria containing DNA and RNA and are pleomorphic organisms. They live and multiply only within the cells (in the cytoplasm and nucleus) of the tissues of humans, animals, and vectors.

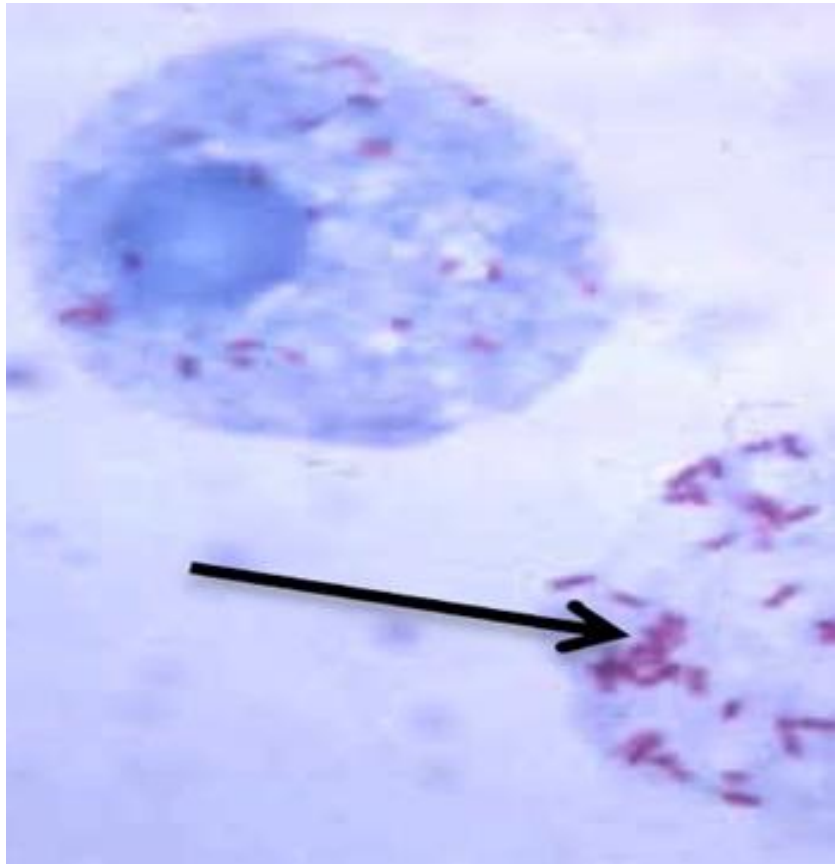
- Coccoid forms resemble very fine, homogeneous, or single-grain ovoids about 0,5 mcm in diameter, quite often they occur as the diploforms. Rod-shaped rickettsiae are short organisms from 1 to 1,5 mcm in diameter with granules on the ends; or long and usually curved thin rods from 3 to 4 mcm in length. Filamentous forms are from 10 to 40 mcm and more in length; sometimes they are curved and multigranular filaments.

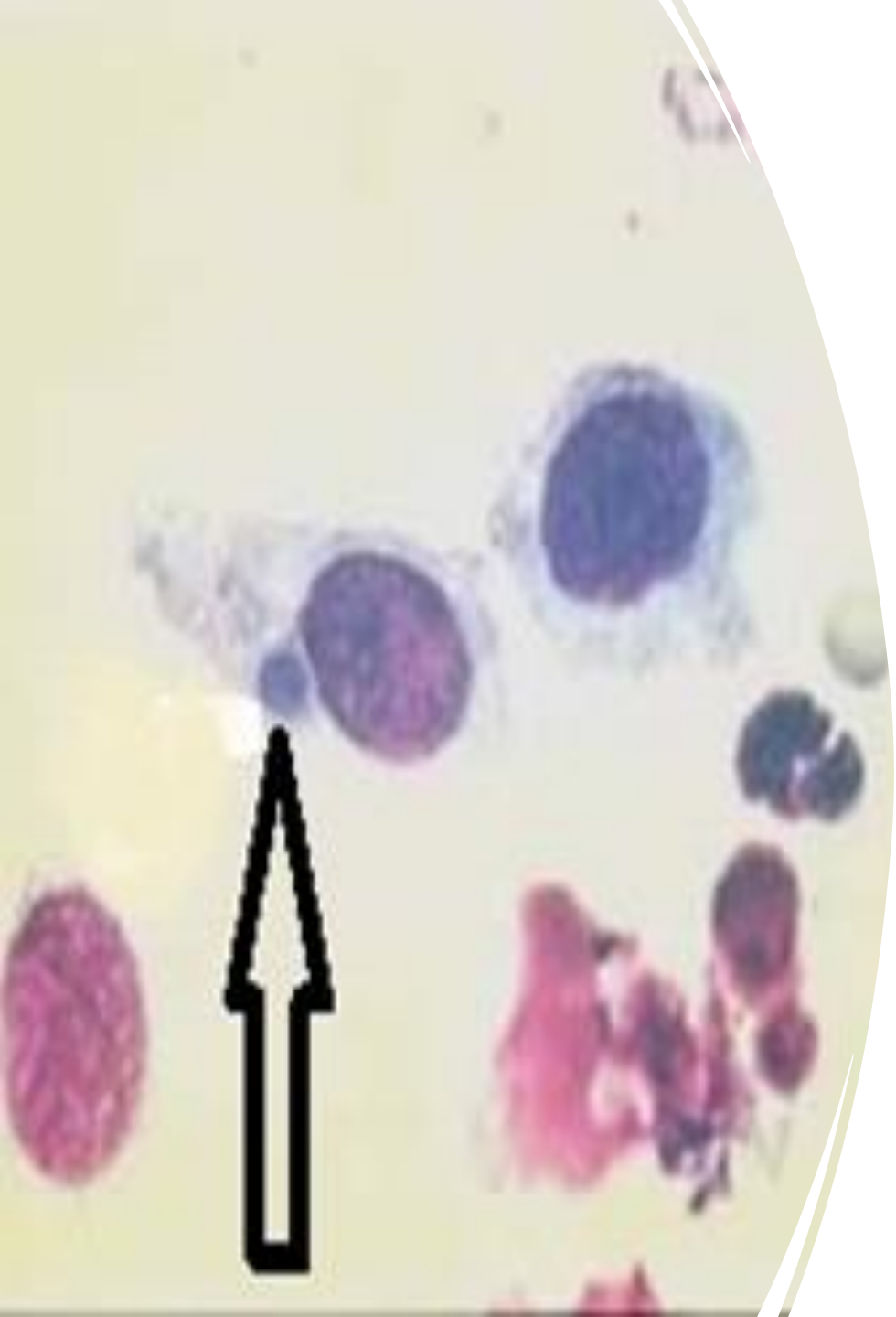
- Rickettsiae are non-motile, do not produce spores and capsules and stain well by the Giemsa stain and the Ziehl-Neelsen stain.

- Electron microscopy and cytochemical study have shown that the rickettsiae have an inner (0,06 mcm) and an outer membrane acting as a wall and consisting of three layers. Granules of the ribosome type measuring 2-7 mcm and vacuole-like structures 0,06-0,08 mcm in diameter have been found in the cytoplasm or rickettsiae.

- Ricketsiae multiply by division of the coccoid and rod-shaped forms wich give rise to homogeneous populations of the corresponding type, and also by the breaking down of the filamentous forms giving rise to coccoid and rod-shaped entities.

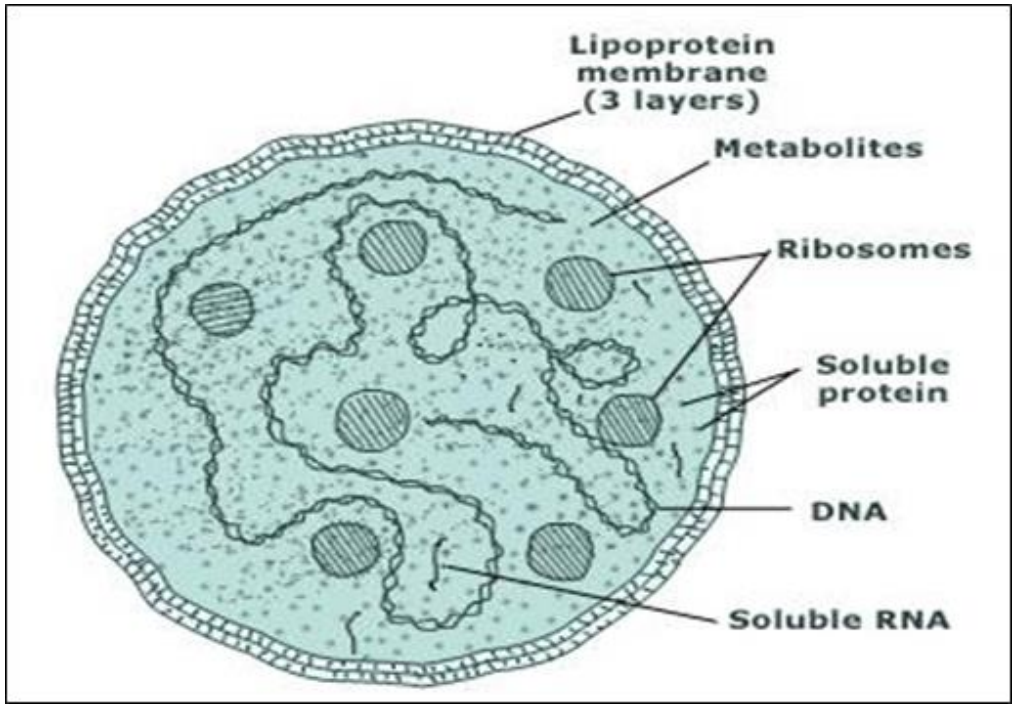
- Pathogenic rickettsiae invade various species of animals and man. The diseases caused by rickettsiae are known as rickettsioses. A typical representative is *Ricckettsia prowazekii* (the name was given in honour of the scientists, the American Howard Ricketts and Czech Stanislaus Prowazek), the causative agent of typhus fever.





- The order *Chlamydiales* includes the family Chlamydiaceae, genus *Chlamydia*; these are the causative agents of ornithosis, trachoma, venereal lymphogranuloma and other diseases.
- The chlamydial cell is roughly spherical and measures between 0.3 and 1.0 u in diameter, according to the stage of development. Both the small and the large cell types contain complete cell walls which are similar to the cell walls of gram-negative bacteria. Chlamydia are non-motile, do not produce spores and capsules and multiply in the cytoplasm of the host cell. The chlamydia, are obligate intracellular parasites of higher animals (mammals and birds). These organisms are termed basophilic because they take up the Giemsa stain.
- Under the cell wall lies a separate cytoplasmic membrane made up of large amounts of lipid. The DNA occurs as an irregular mass in the cytoplasm. There is no nuclear membrane. Ribosomes can be seen throughout the cytoplasm.
- The chlamydia fall into two main ecological groups. In the first group, are the agents causing trachoma, inclusion conjunctivitis and lymphogranuloma venereum (*Chlamydia trachomatis*), atipical pneumonia (*Chlamydia pneumoniae*) which seem to infect man only. In the second group, are those agents transmitted to man as zoonotic infections. About 100 species of birds are naturally infected with chlamydia. This includes 71 species of parrots as well as finches, pigeons, chickens, ducks, turkeys and seabirds (*Chlamydia psittaci*).

- The mycoplasmas belong to the class *Mollicutes*, order *Mycoplasmatales*. These bacteria measure 100-150 nm, sometimes 200-700 nm, are non-motile and do not produce spores.
- Mycoplasmas are the smallest microorganisms. They were first noticed by Pasteur when he studied the causative agent of pleuropneumonia in cattle. However, at the time he was unable to isolate them in pure culture on standard nutrient media, or to see them under a light microscope. Because of this, these micro-organisms were regarded as viruses. In 1898 Nocard and Roux established that the causative agent of pleuropneumonia can grow on complex nutrient media which do not contain cells from tissue cultures. Elford using special filters determined the size of the microbe to be within the range of 124-150 nm. Thus, in size mycoplasmas appeared to be even smaller then some viruses.
- Since they do not possess a true cell wall, mycoplasmas are characterized by a marked pleomorphism. They give rise to coccoid, granular, filamentous, cluster-like, ring-shaped, filterable forms, etc (Fig.48). Pleomorphism is observed in cultures and in the bodies of animals and man.



- Actinomycetes (Gk. mykes fungus, actis ray) are unicellular microorganisms which belong to the class Bacteria, the order Actinomycetales. The body of actinomycetes consists of a mycelium which resembles a mass of branched, thin (0.2-1.2 mcm in thickness), non-septate, filaments — hyphae.

- In some species the mycelium breaks up into poorly branching forms. In young cultures the cytoplasm in the cells of actinomycetes is homogeneous, it refracts light to a certain extent, and contains separate chromatin grains. When the culture ages, vacuoles appear in the mycelial cells, and granules, droplets of fat and rod-shaped bodies also occur. The cell wall becomes fragile, breaks easily and a partial lysis of the cells occurs. In actinomycetes, as in bacteria, differentiated cell nuclei have not been found, but the mycelial filaments contain chromatin granules. The actinomycetes multiply by means of germinating spores attached to sporophores, and by means of fragmentation where they break up into hyphae (Fig.49).

- The order *Actinomycetales* consists of 4 families: Mycobacteriaceae, Actinomycetaceae, Streptomycetaceae, Actinoplanaceae. The family Mycobacteriaceae includes the causative agents of tuberculosis, leprosy, and the family Actinomycetaceae, the causative agents of actinomycosis
  and acid-fast species nonpathogenic for man.

- Among the actinomycetes of the family Streptomycetaceae are representatives which are capable of synthesizing antibiotic substances.These include producers of streptomycin, chloramphenicol, chlortetracycline, oxytetracycline, neomycin, nystatin, etc. No species pathogenic for animals and man are present in the family Actinoplanaceae.