Lesson 10

Microbiological diagnosis of gram negative (Neisseria) coccoid form bacterial infections

Gram-negative cocci classification:

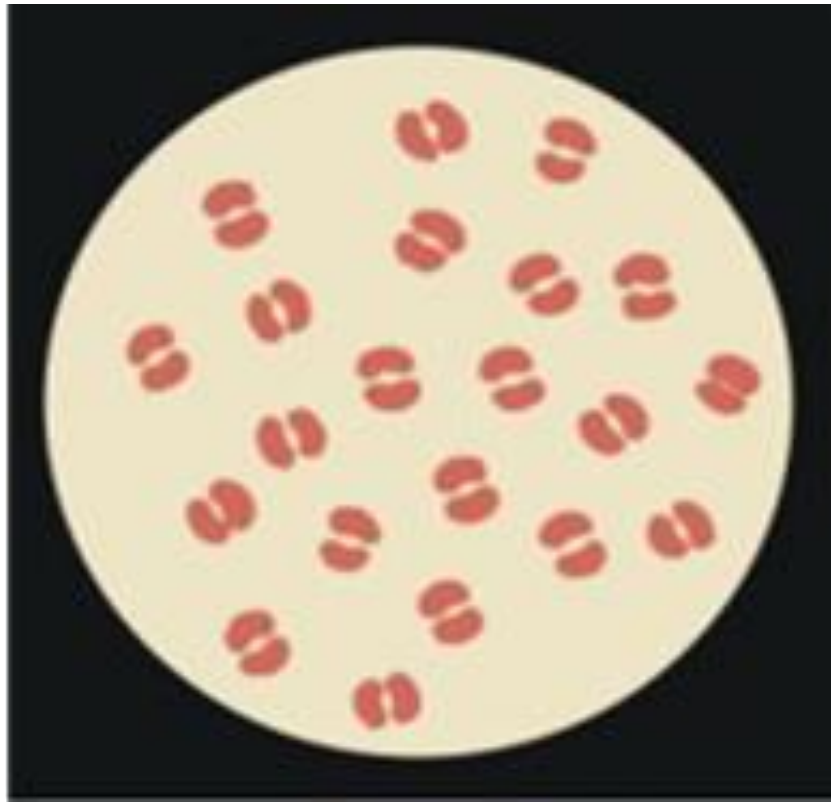
Family: Neisseriaceae

Genus: Neisseria S

pecies: N.meningitidis, N.gonorrhoeae (pathogenic) N.lactamica, N.sicca, N.subflava, N.cinerea, N.mucoza, (representatives of the normoflora of the upper respiratory tract, opportunistic)

Morphobiological features:

Neisseria meningitidis are Gram-negative, bean-shaped diplococci, 0.6-1.0 µm in size, whose concave surfaces are in contact with each other. They are immobile and do not form spores. Clinical isolates form a capsule, which is lost during growth on nutrient media.



Genus Neisseria (in a smear of bean-shaped diplococcus)

Antigenic structure of Neisseria meningitidis

- ✓ According to capsular antigens, 13 serogroups of meningococci are distinguished. The most important in human pathology are serogroups A, B, C, Y and W135.
- ✓ Serogroup A meningococci are characterized by high virulence, which is associated with their high invasive activity.

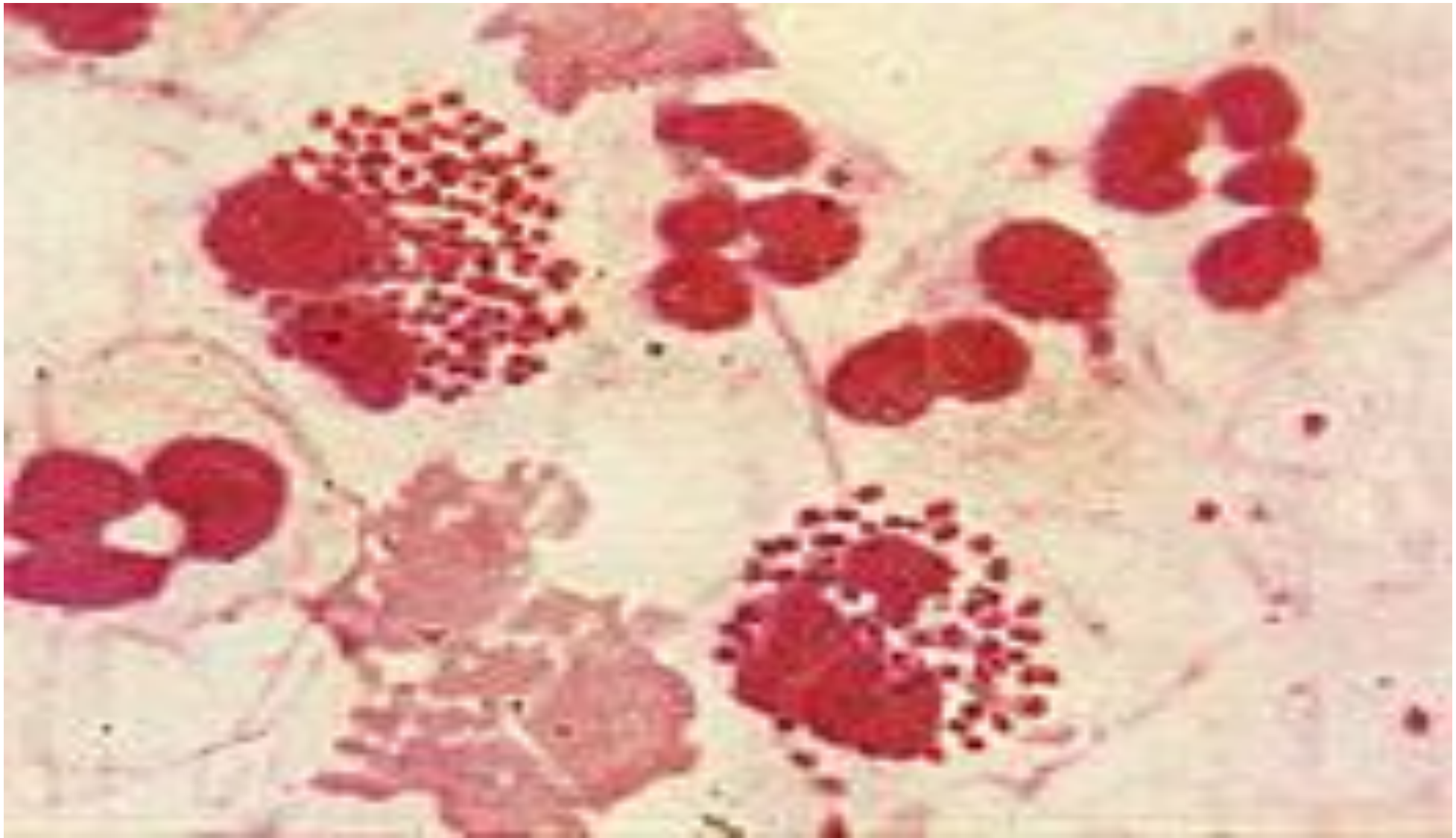
Cultural properties of Neisseria meningitidis

- They grow on dense nutrient media with the addition of native proteins (serum, blood, egg white), form delicate translucent rounded small colonies 2-3 mm in diameter. There is no hemolysis on blood agar.
- Capnophiles.
- On liquid nutrient media (whey broth) form turbidity and a slight sediment

N.meningitidis (non-hemolytic colonies on blood agar)

Neisseria meningitidis

in a smear prepared from the cerebrospinal fluid of patients with meningitis



N. meningitidis Biochemical activity

Properties	*N.meningitidis*
Glucose	+ (to acid)
Maltose	+ (to acid)
Gelatin	-
Indole	-
hydrogen sulfide	-
Nitrates	-
Oxidase	+
Catalase	+

Neisseria meningitidis

Pathogenic factors:

- capsule
- pilus
- endotoxin
- İgA protease

Epidemiology:

The source of infection is a sick person or a carrier

The transmission mechanism is aerogenic, the way is airborne

Meningococcal infections :

- meningococcal carriage
- acute nasopharyngitis
- meningococcemia
- epidemic cerebrospinal meningitis

Clinical symptoms of epidemic cerebrospinal meningitis:

- high fever
- headache
- neck muscle stiffness
- vomit
- rashes

Meningococcemia

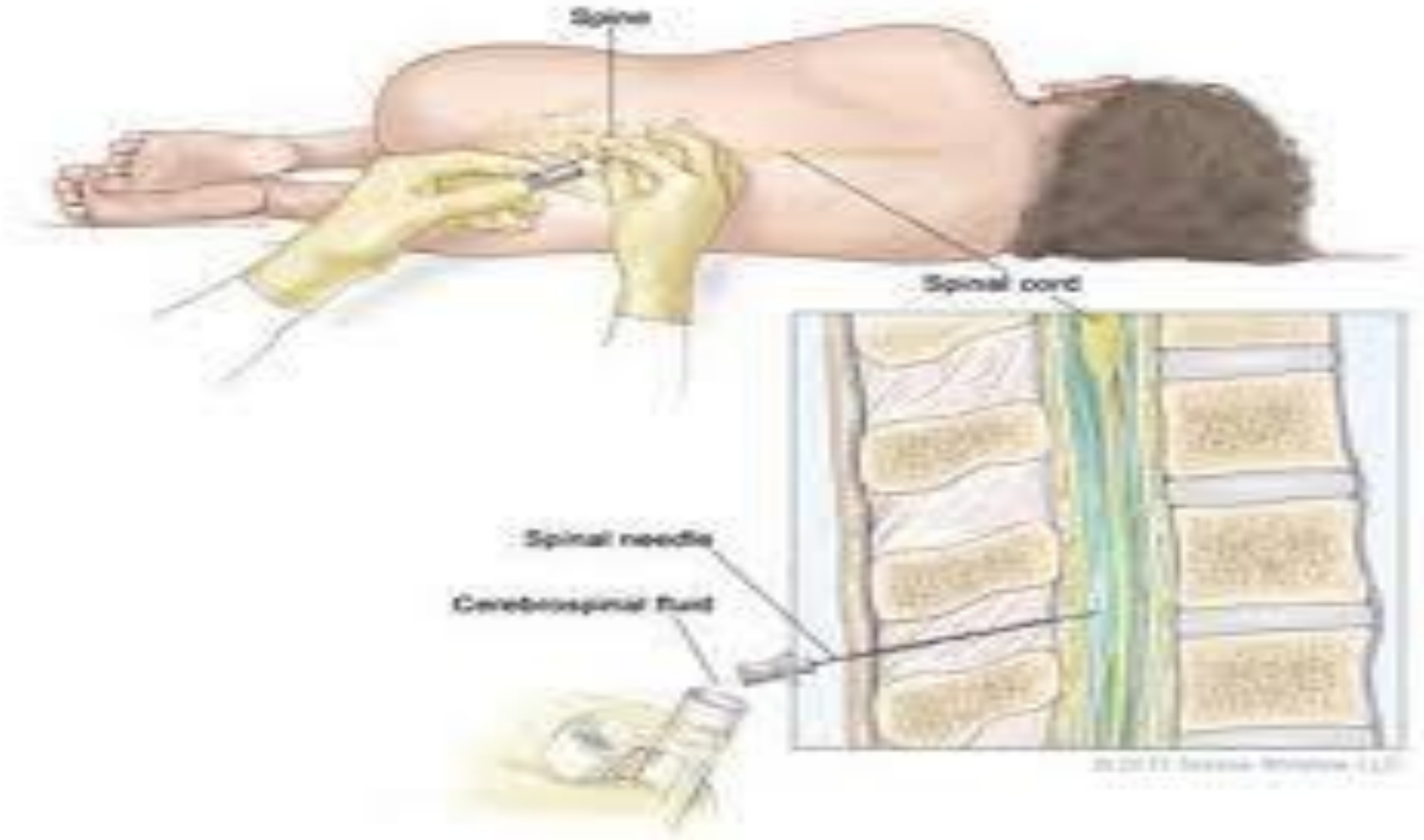
(hemorrhagic rash-exanthema)

Methods of microbiological diagnostics of meningococcal infections

Materials for research:

- CSF - cerebrospinal fluid
- blood
- nasopharyngeal mucus
- punctate from the elements of the rash

Collection of cerebrospinal fluid (lumbar puncture)



Methods of microbiological diagnostics:

➢ *Microscopic*

- Microscopy of a Gram-stained CSF smear (Gram-negative bean-shaped diplococci are visible inside the leukocytes)

➢*Bacteriological (cultural)*

- Inoculation of the test material on nutrient media (blood and serum)
- Incubation at 37ºC with elevated carbon dioxide for 18-24 hours
- Identification of the isolated culture by morpho-biological properties
- Determination of sensitivity to antibiotics

➢*Serological*

- Precipitation reactions and enzyme immunoassay with cerebrospinal fluid
- Passive haemagglutination test (RPHA) and serum ELISA

Treatment and specific prevention of meningococcal infections:

Treatment –

Benzylpenicillin

Chloramphenicol

3rd generation cephalosporins

Specific prophylaxis - for active immunization, vaccines from purified capsular polysaccharides of meningococcal serogroups A and C are used.

Gonococci

Classification:

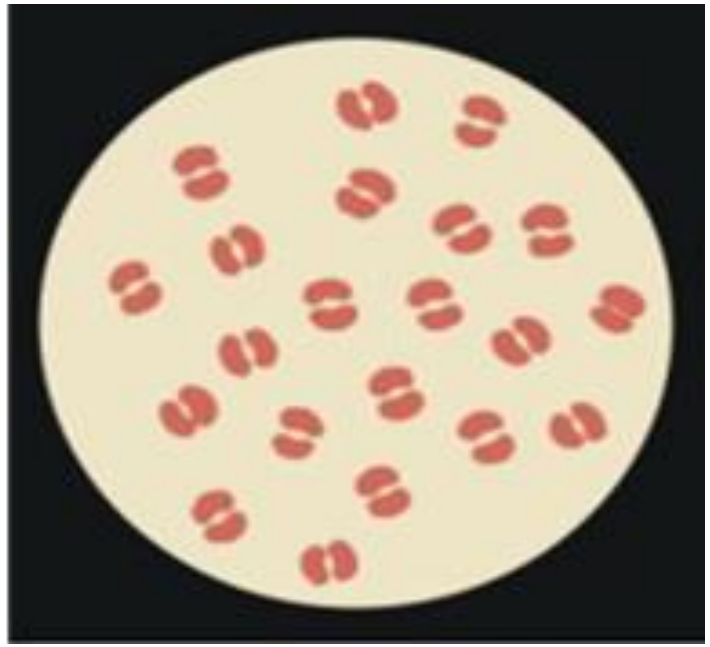
Family: Neisseriaceae

Genus: Neisseria

Species: N.gonorrhoeae (pathogenic)

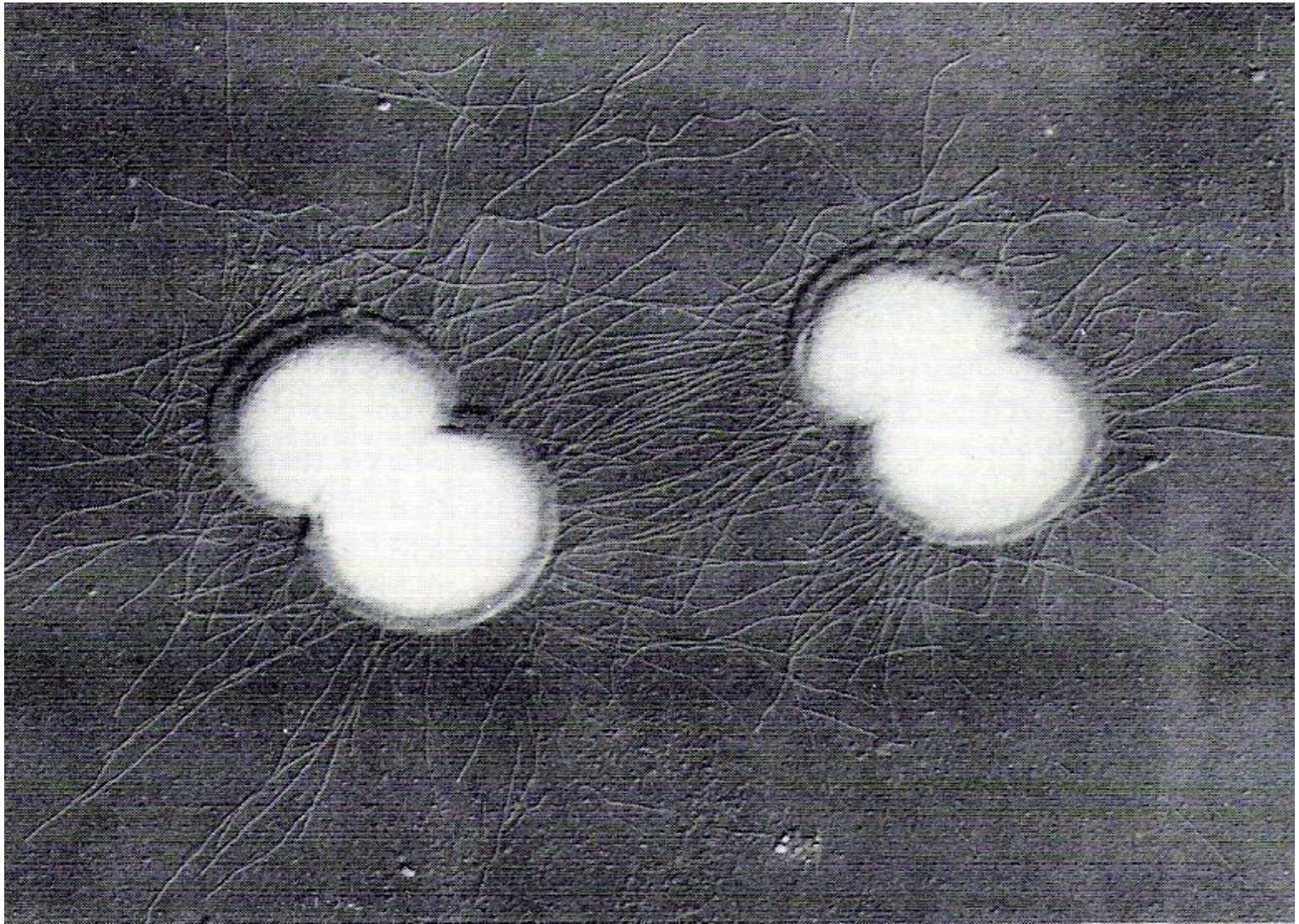
Morpho-biological properties:

Neisseria gonorrhoeae are gram-negative bean-shaped diplococci 1.25-1.0 x 0.7-0.8 µm in size. They are motionless, do not form spores, have a capsule.



Neisseria gonorrhoeae

(electron microscopy)



Antigenic structure of Neisseria gonorrhoeae

- drinking
- Surface proteins of the outer membrane:

- Por proteins - PorA and PorB
- Opa-proteins - turbidity proteins, enhance adhesion to cells macroorganism
- Opa (-) gonococci form transparent, Opa (+) form cloudy colonies

Cultural properties

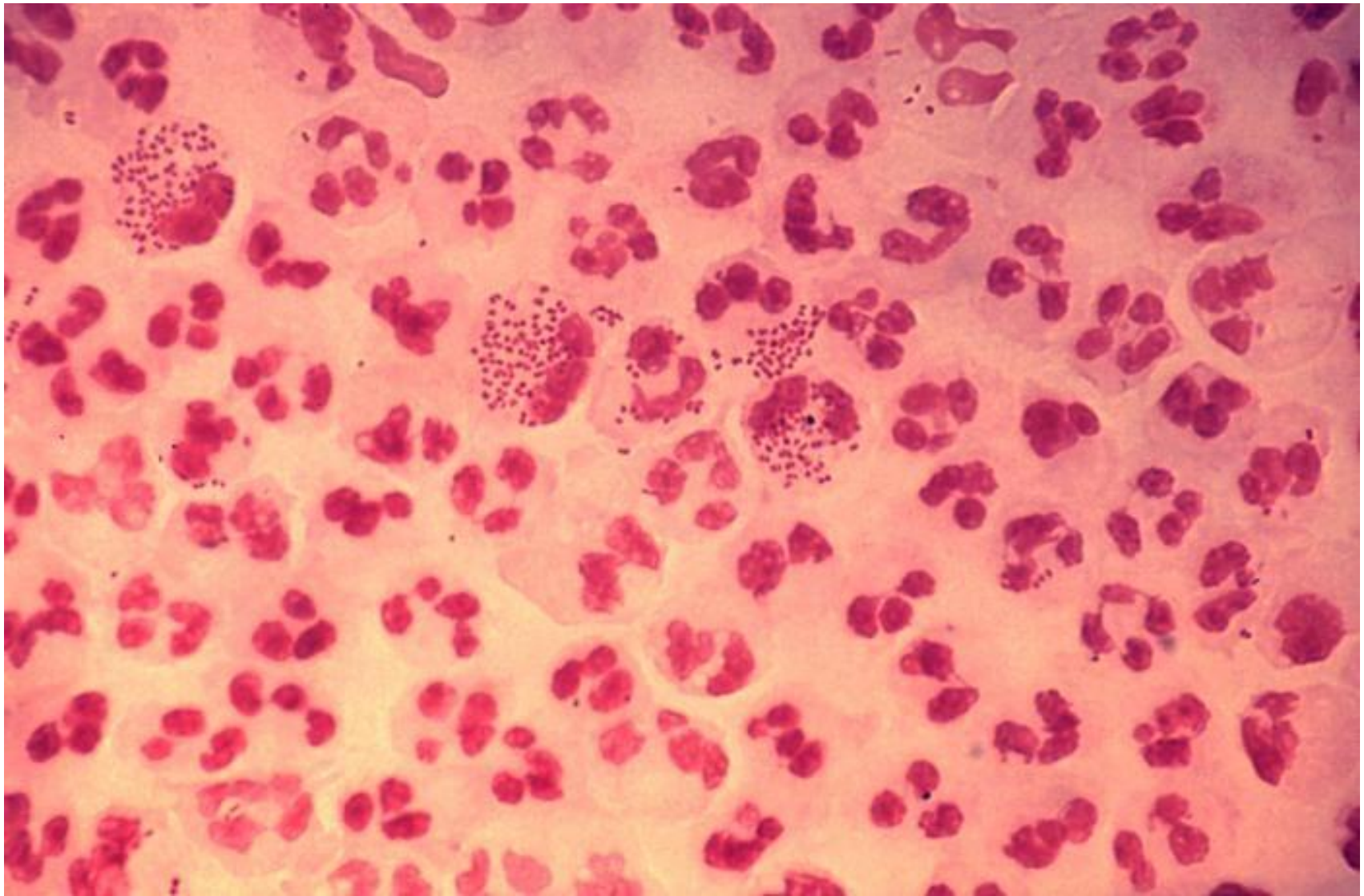
On dense nutrient media with the addition of whey, they form transparent or slightly cloudy shiny colonies resembling dew drops 1-2 mm in diameter.

On liquid nutrient media, they grow diffusely and form a surface film.

N.gonorrhoeae (non-hemolytic colonies on blood agar)

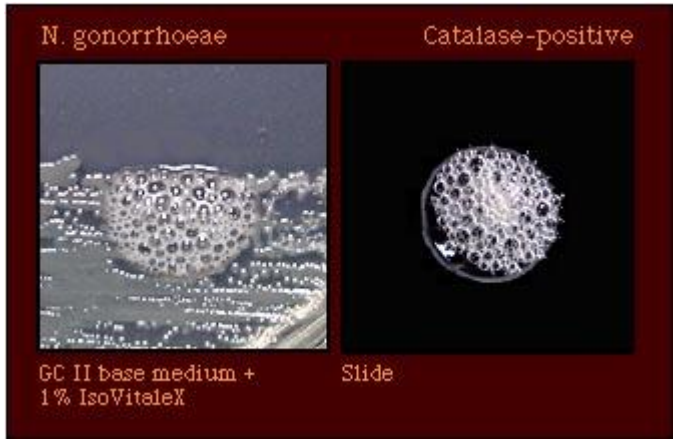
Neisseria gonorrhoeae

(in a smear from a purulent discharge of a patient with gonorrhea)



Biochemical properties

Sign	N.gonorrhoeae
Glucose	+ (to acid)
ammonia	-
indole	-
hydrogen sulfide	-
oxidase	+
Catalase	+
Nitrates	-



N.gonorrhoeae Catalase (+)

N.gonorrhoeae oxidase(+)

Neisseria gonorrhoeae

Pathogenic factors:

- capsule
- pilus
- lipooligosaccharides (LOS)
- outer membrane film (Por-, Opa-proteins)
- IgA protease

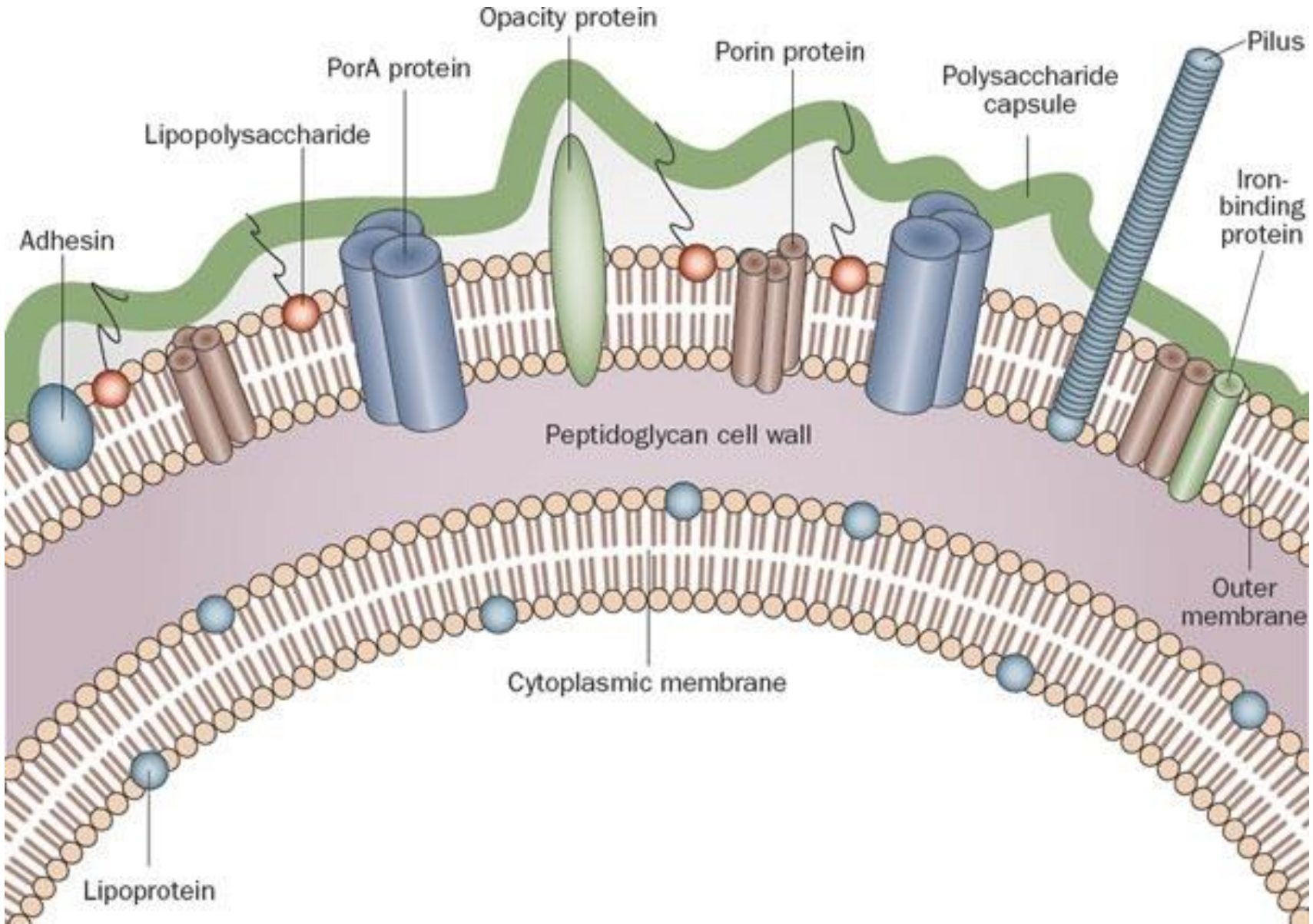
Epidemiology

The source of the infection is a sick person
Mechanism and route of transmission: contact (sexual)

Caused diseases:

- gonorrhea
- blennorrhea ("oftalmia neonatorum")
- Generalized infections and extragenital complications (bacteremia, arthritis, etc.)

Pathogenicity factors of gonococci



Microbiological diagnosis of acute and chronic gonorrhea:

The material for research is taken with a sterile cotton swab from:

- urethra
- vagina
- cervix
- conjunctiva
- rectum
- Pharynx

It is also possible to study the urine sediment

Microbiological diagnosis of acute and chronic gonorrhea:

➢*Microscopic (for acute gonorrhea)*

- Microscopy of a smear prepared from the test material (taken with a swab from the vagina and urethra) and stained by Gram (gram-negative intracellular bean-shaped diplococci are visible)

➢*Bacteriological (cultural)*

- Inoculation of the test material on nutrient media (with the addition of serum or ascitic fluid) Incubation at 37ºC with elevated carbon dioxide for 18-24 hours
- The selected culture is identified by morpho-biological properties
- Determination of sensitivity to antibiotics

➢*Serological (for chronic gonorrhea)*

- ELISA method (ELISA)

➢*Molecular genetic method*

- Polymerase chain reaction (PCR)

Treatment and prevention of gonorrhea:

Treatment:

cephalosporins

azithromycin

Immunotherapy - gonovaccine. pyrogenal

Prevention - for the prevention of blennorrhea, newborns are instilled in the eyes of 1% silver nitrate, or a 30% solution of albucid

Means of specific prophylaxis for gonorrhea are absent!