Ministry of Health, Republic of Belarus

Institution of Education

“Grodno State Medical University”

Department of Microbiology, Virology and Immunology named after S.I.Gelberg

MEDICAL BACTERIOLOGY WITH BASICS OF MYCOLOGY AND PROTOZOOLOGY

Training appliance for students of the Department for International Students

PIOGENIC COCCI

(piogenic cocci – pus producing cocci)

Theme N18

STAPHYLOCOCCI

Species of Staphylococci

- S.aureus
- S. epidermidis
- S. saprophyticus

- ✓ High resistance to unfavorable conditions of the environment (drying, heat, etc).
- ✓ Major components of the normal flora of skin and nose.

Staphylococci: characteristics

- Morphology:

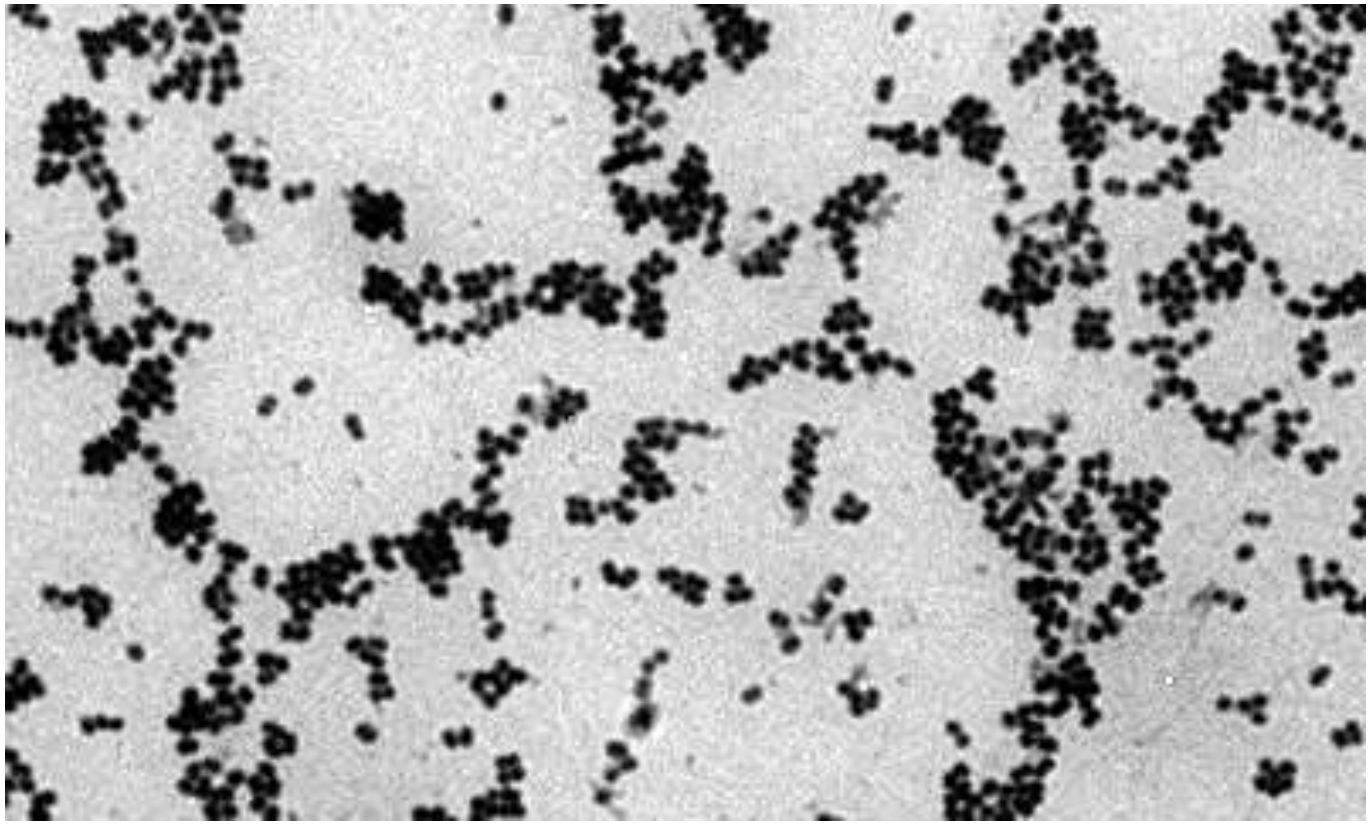
  spherical gram-positive cells, classically grow in irregular grape like-clusters, never produce spores and macrocapsules, nonmotile.

- Growth requirements:

  grow on simple nutritive media at 37^0C and produce pigmented S-shaped colonies after 24 hours of cultivation; selective medium is solid agar medium containing sodium chloride

  (5 – 10 % NaCl) and yolk – yolk-salt agar.

Stapylococcus sp. – grape-like clusters



- Biochemical and metabolic characteristics:

  facultative anaerobes, catalase positive and produce coagulase (coagulase-positive), possess high proteolytic activity.

- Antigenic composition:

  species-specific antigens are protein A and teichoic acids (ribitol- and glycerol teichoic acids), S.aureus contains cross-reactive antigens reacting with human erythrocytes, skin cells and kidney cells.

Staphylococci: virulence (pathogenicity) factors

- **Virulence enzymes**:
  - coagulase (main virulence factor): reacts with prothrombin and converts it into thrombin→ causes production of fibrinogen → clotting of mammalian plasma with appearance of fibrin-containing cover around bacterial cell → the means of defence against phagocytosis,
  - hyaluronidase,
  - fibrinolysin (together with hyaluronidase adds additional invasiveness to Staphylococcus),
  - deoxyribonuclease,
  - lecitinase and others.

Staphylococci: virulence (pathogenicity) factors

- **Protein A** (agglutinogen A) – is a surface protein, which is bound covalently with PG of the cell wall:
  - interacts nonspecifically with Fc-fragment of IgG, the result is a disturbance in the process of complement fixation and phagocytosis,
  - strong allergen,
  - mitogen for T- and B-lymphocytes.

Staphylococci: virulence (pathogenicity) factors

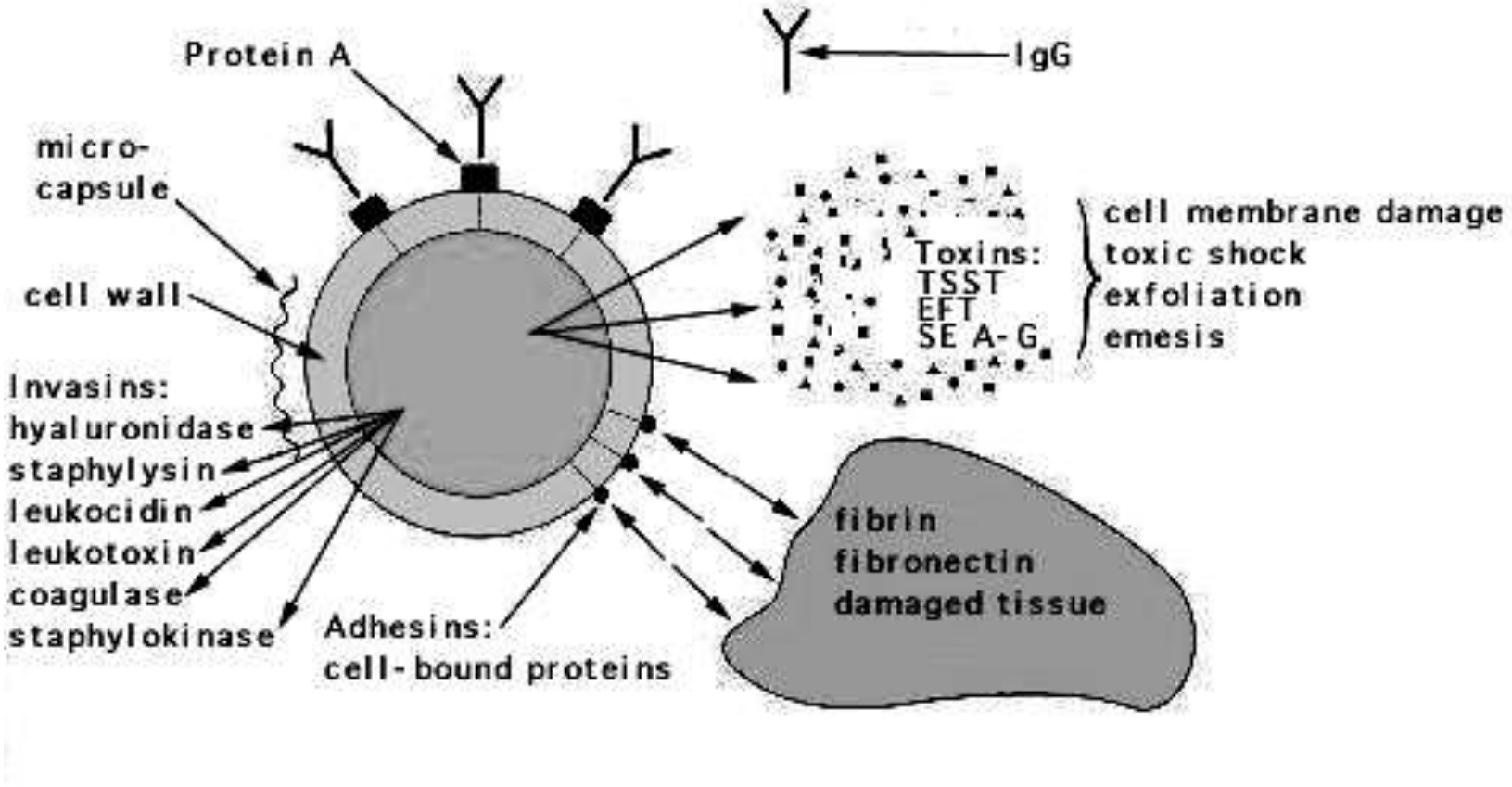
Exotoxins (protein toxins):

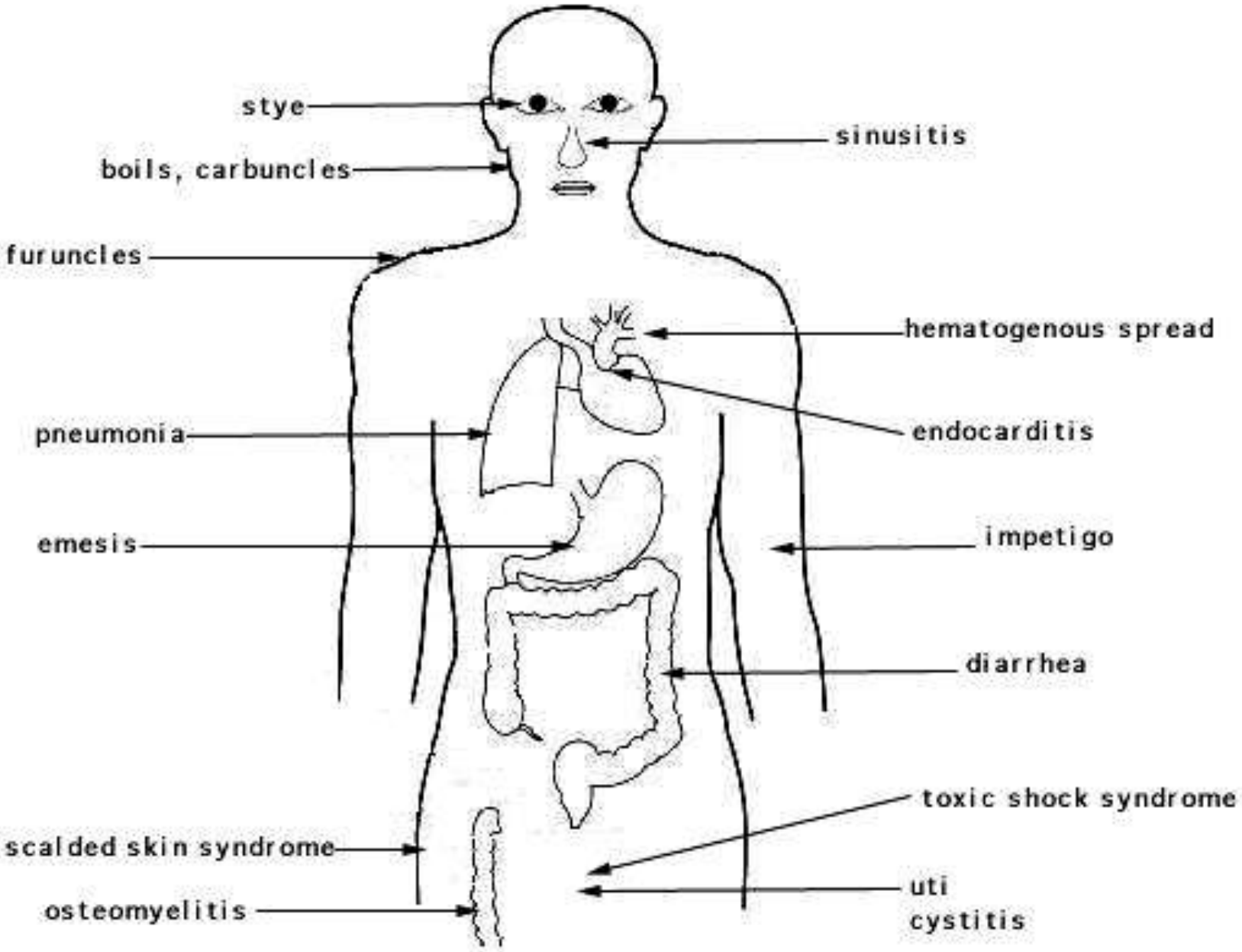
•*Membrane toxins (main is* ***α-toxin****).* When it is injected to laboratory animals (biotest) causes the breakdown of the cells and death of the animals. Can cause the same effect on blood cells including erythrocytes (so could be characterised as haemolysin) and mast cells.

•*Exotoxin* which causes ***toxic shock syndrome*** *(**TSS**).* Approximately 50 % of all S. aureus strains produce this toxin.

Exfoliatin – effects on skin cells and causes three syndromes (infants develop toxic epidermal necrosis called scalded baby syndrome, older children and adult develop localized bullae known as Lyell's disease and also scarlet fever-like rash.

Factors of pathogenicity of Staphylococci




stye
boils, carbuncles
furuncles
sinusitis
hematogenous spread
endocarditis
pneumonia
emesis
impetigo
diarrhea
toxic shock syndrome
scalded skin syndrome
osteomyelitis
uti
cystitis

Staphylococci: virulence (pathogenicity) factors

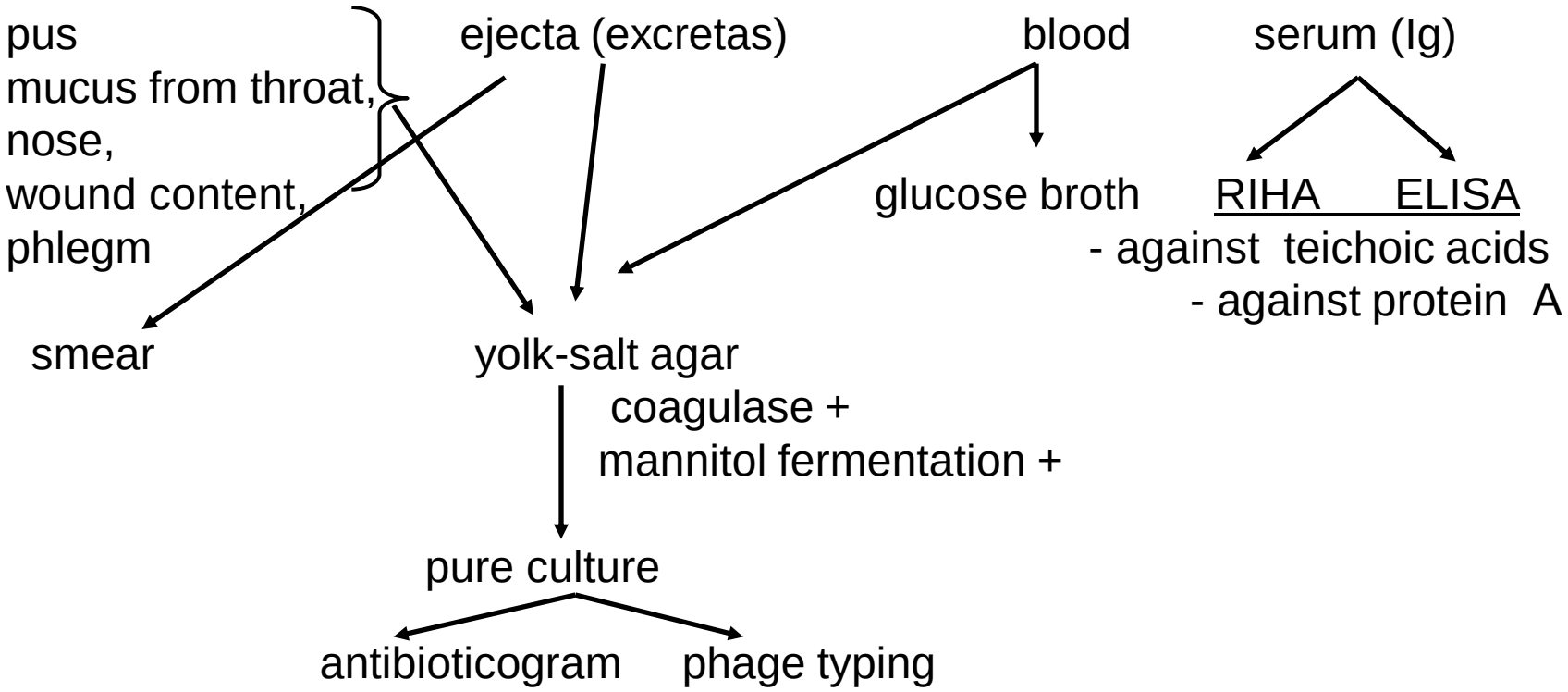
Enterotoxins (cause food poisoning as they are superantigens which stimulate overproduction of IL-2).

Allergens – usually cause ITH and DTH (result in conversion of the infections caused by staphylococci into chronic form); staphylococci frequently are etiological agents which cause allergic reactions of skin and respiratory tract.

Cross-reactive antigens of Staphylococci – cause autoimmune diseases.

Factors which inhibit phagocytosis: microcapsule, protein A, exotoxins.

Microbiological diagnostics of pyoinflammatory infections caused by Staphylococci



STREPTOCOCCI

Streptococci - classifications

Types of classification

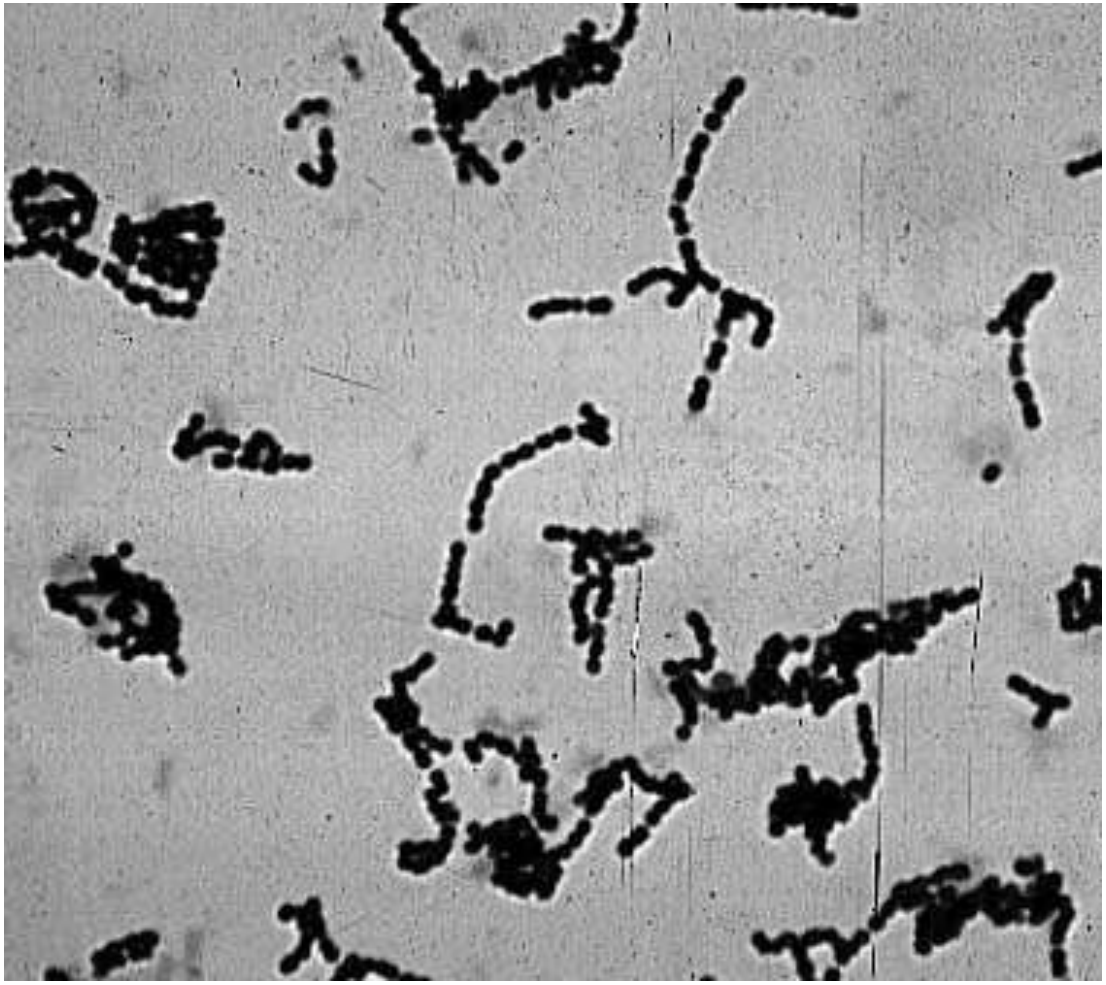
- According to ***polysaccharide antigen*** (can be identified in precipitation reaction) are classified into **20 serological groups** (A – V). In most cases Streptococci of group A are pathogenic for humans.
- According to ***the action on red blood cells*** (tested by the ability to cause hemolysis on sheep blood agar) are classified as:

  α-hemolytic – turn hemoglobin to met-hemoglobin resulted in appearance of green zones (green coloration of the medium surrounding colonies) on blood agar

  β-hemolytic – cause β-hemolysis - complete lysis of erythrocytes (appearance of distinct clear zone). Most of them are belonging to serological group A

  γ- hemolytic – are characterized by no visible effect on sheep blood agar; the designation indicates no hemolysis

Streptococci sp. – cocci arranged into chains



Species of Streptococci

S.pyogenes (group A streptococci)

S.agalactiae (group B streptococci)

S.viridans (α-hemolytic streptococci)

S.pneumoniae (pneumococci)

Streptococci: characteristics

- Morphology

  oval gram-positive cocci that occur in chains, never produce spores and macrocapsules, nonmotile.

- Growth characteristics

  grow on enriched media (containing glucose, serum or blood) at 37^0C during one day. Colonies on solid media are S-shaped.

- Biochemical and metabolic characteristics

  biochemically active like Staphylococci but their proteolytic activity is lower.

- Antigenic composition

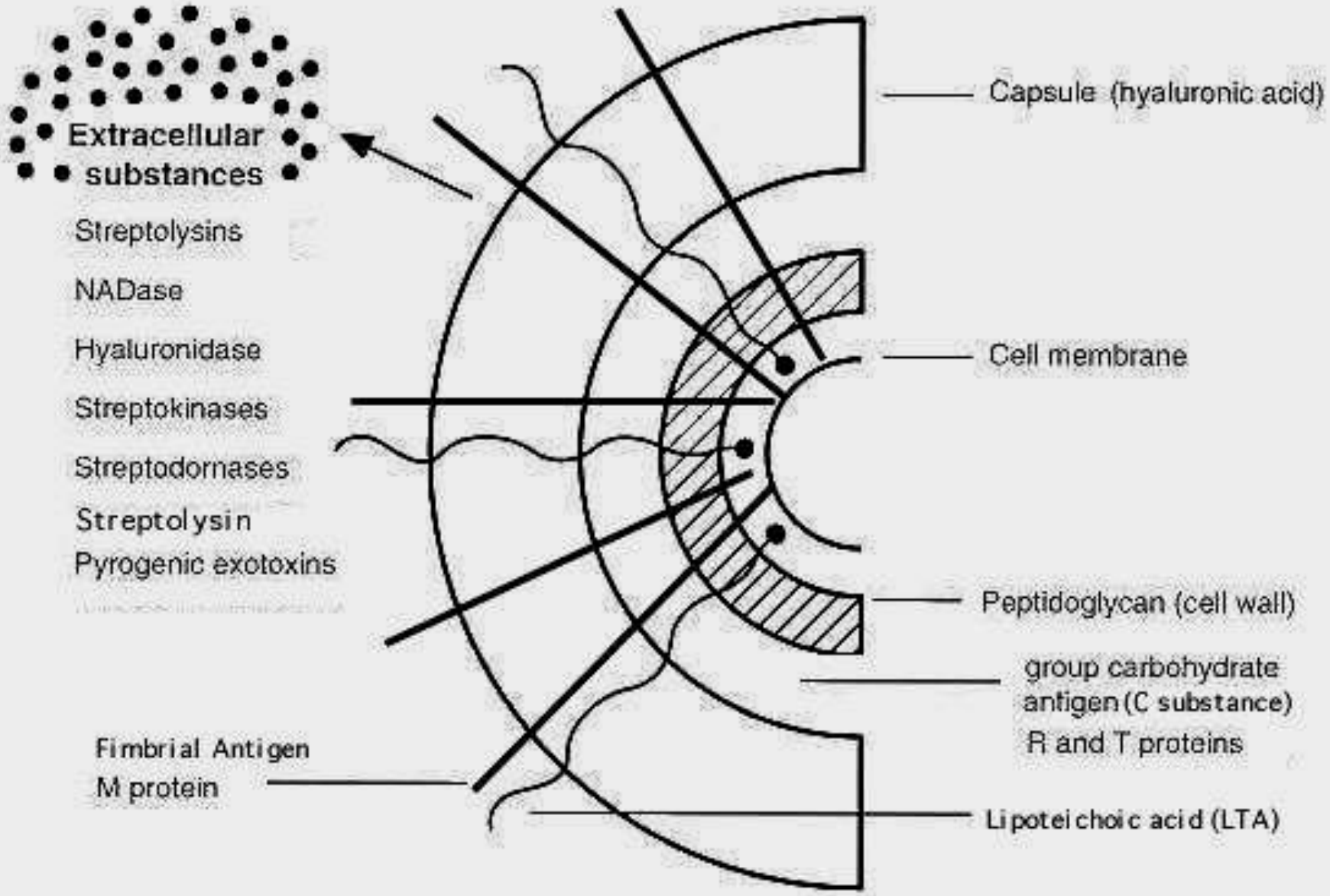
  polysaccharide antigens (or *group antigens*) are **polysaccharides** of the cell wall

  protein *type-specific antigen* (the most important for classification) is **M-protein** according to its content there are classified about 100 serological variants of Streptococci

- *High resistance to unfavourable conditions in the environment*

Streptococci: characteristics

- Virulence (pathogenicity) factors
  - Pili
    - M-protein (adhesion, inhibition of phagocytosis and super antigens)
  - Enzymes
    - streptokinase (proteolysis of fibrin)
    - aminopeptidase (suppresses chemotaxis of phagocytes)
    - hyaluronidase (spreading factor – factor of invasion)
    - DNA-ase (streptodornase – depolymerises DNA)
  - Protein toxins
    - streptolysin O and S
    - cytotoxin
    - erythrogenic toxin (occurs in streptococci causing scarlet fever – pyrogen and allergen)


Extracellular substances
Streptolysins
NADase
Hyaluronidase
Streptokinases
Streptodornases
Streptolysin
Pyrogenic exotoxins
Capsule (hyaluronic acid)
Cell membrane
Peptidoglycan (cell wall)
group carbohydrate antigen (C substance)
R and T proteins
Fimbrial Antigen
M protein
Lipoteichoic acid (LTA)

Streptococcal infections

Clinical forms of infection

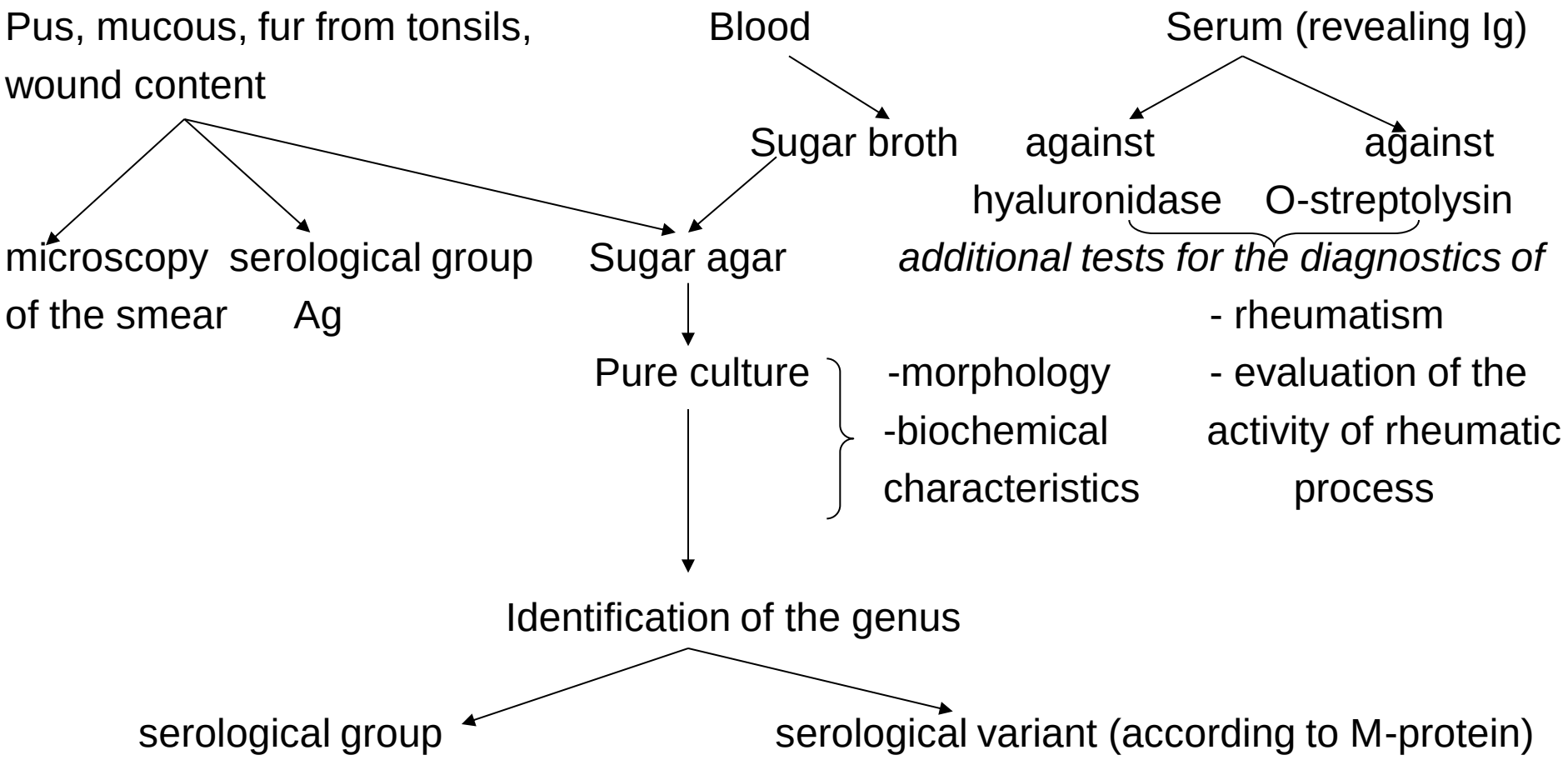
Pharyngitis **Scarlet fever** **Skin infections:**

- **cellulites (adiposities)**
- **erysipelas**
- **piodermia**

Complications

Endocarditis **Rheumatic fever** **Glomerulonephritis** **Toxic shock syndrome**

Microbiological diagnostics of the infections caused by Streptococci



PNEUMOCOCCI

Pneumococci: main characteristics

- **Morphology**

  lancet-shaped gram-positive cocci, arranged in pairs in smear, never produce spores, possess polysaccharide capsule, nonmotile

- **Growth characteristics**

  are best grown on agar media containing blood or serum + 0,1% glucose at 37^0C during a day, form S-shaped colonies with zone of α-hemolysis

- **Biochemical and metabolic characteristics**

  average metabolic activity, inulin fermentation is main metabolic characteristic

Pneumococci: main characteristics

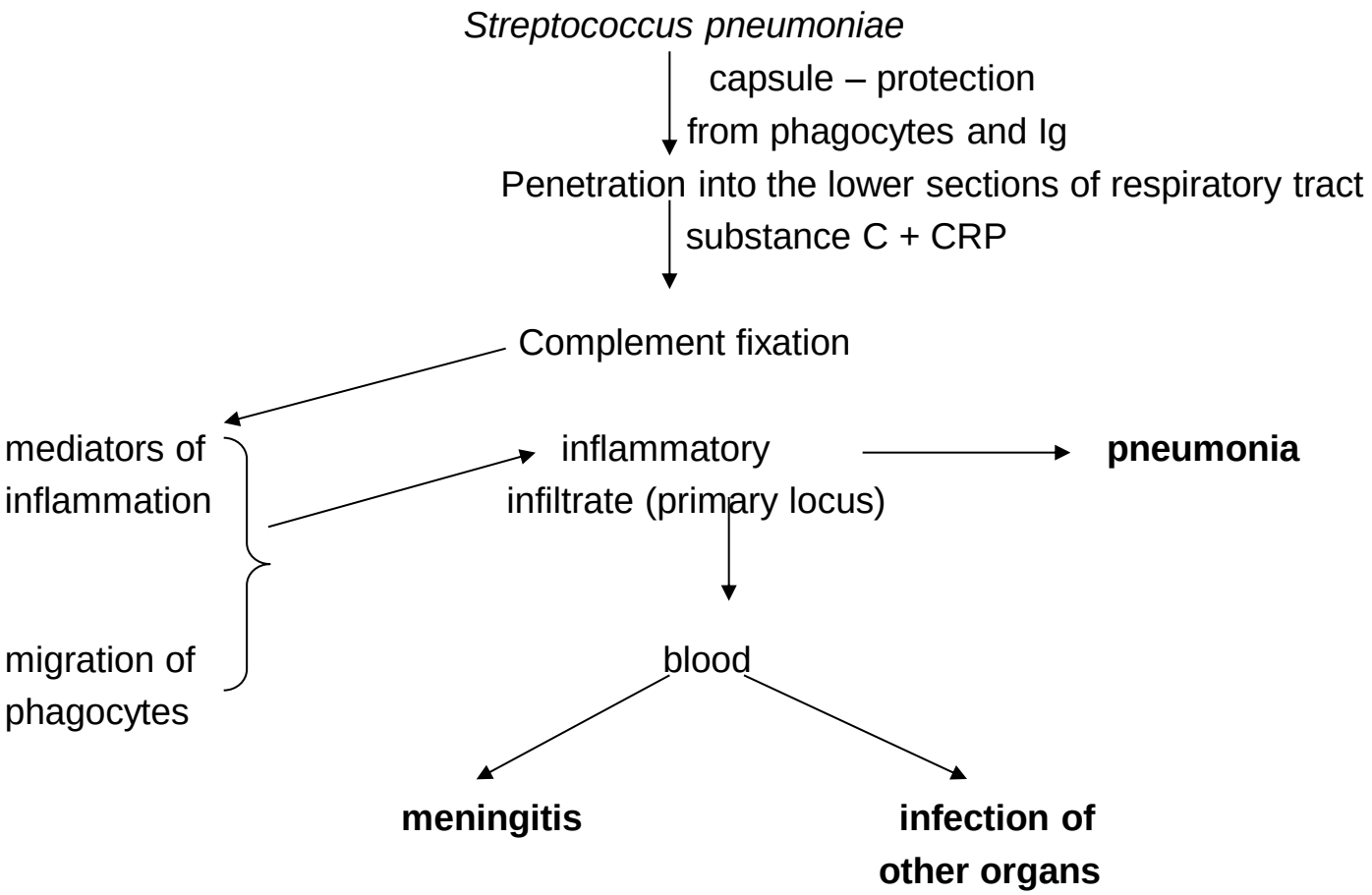
- **Antigenic composition**

according to K-antigen (capsule polysaccharide) are classified into more then ***80 serological variants***

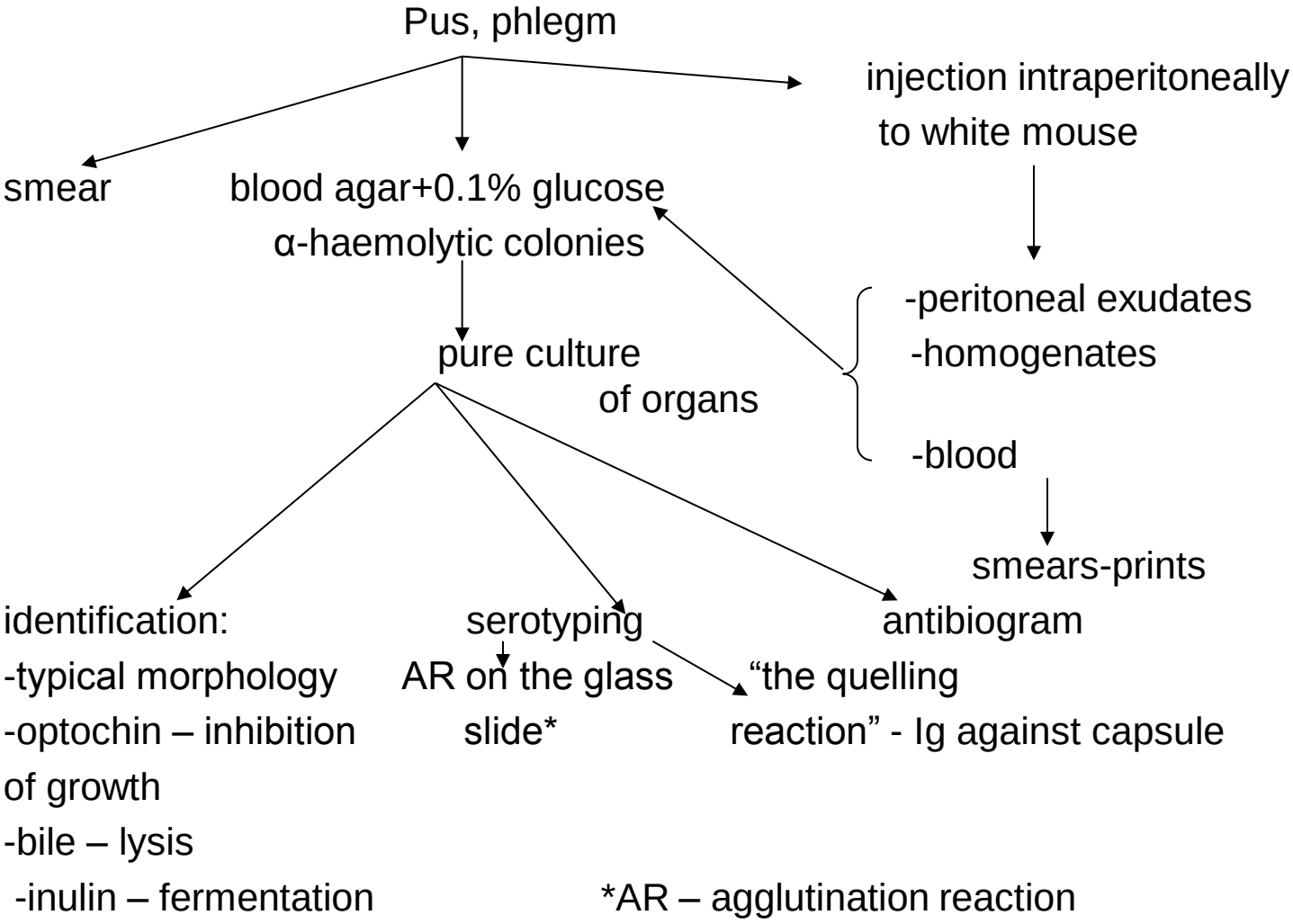
- **Virulence (pathogenicity) factors**
  - capsule
  - substance C (teichoic acid of the cell wall)

- *Not resistant to unfavorable conditions of environment*

Pneumococcal infections: patogenesis



Microbiological diagnostics of the infections caused by Pneumococci



MENINGOCOCCI

Neisseria meningitidis

Meningococcus: main characteristics

- **Morphology**

  gram-negative cocci, usually occurring in pairs, individual cocci are kidney-shaped, nonmotile and nonsporeforming, possess capsule that is difficult to reveal.

- **Growth characteristics**

  grow on enriched media (containing protein, for example, serum agar) at 37^0C (in an atmosphere containing 5% CO_2), in 24-36 hours they form small S-shaped colonies

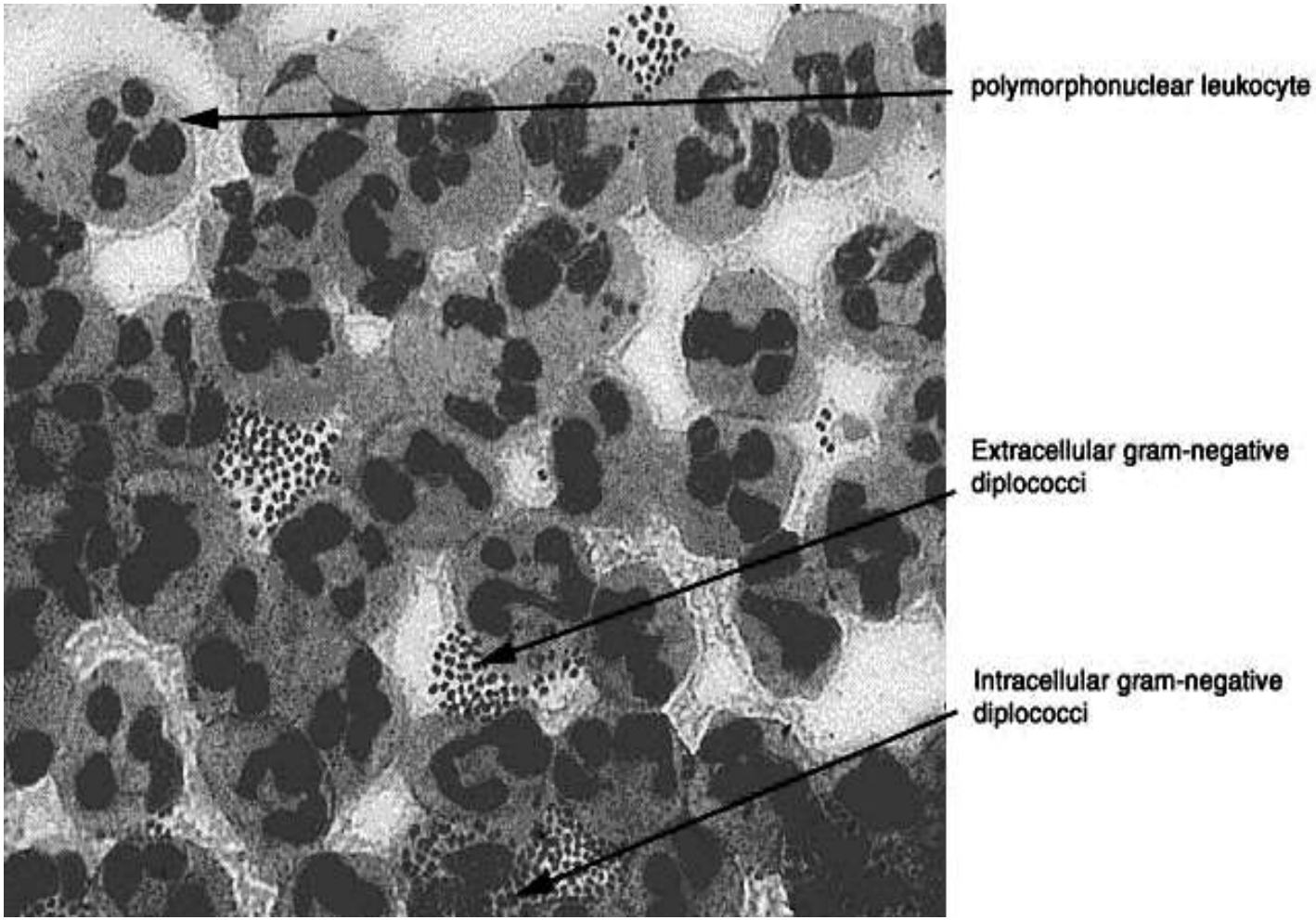
- **Biochemical and metabolic characteristics**

  produce oxidase (genus characteristics), ferment glucose and maltose forming acid but not gas (species characteristics)

Meningococcus: main characteristics

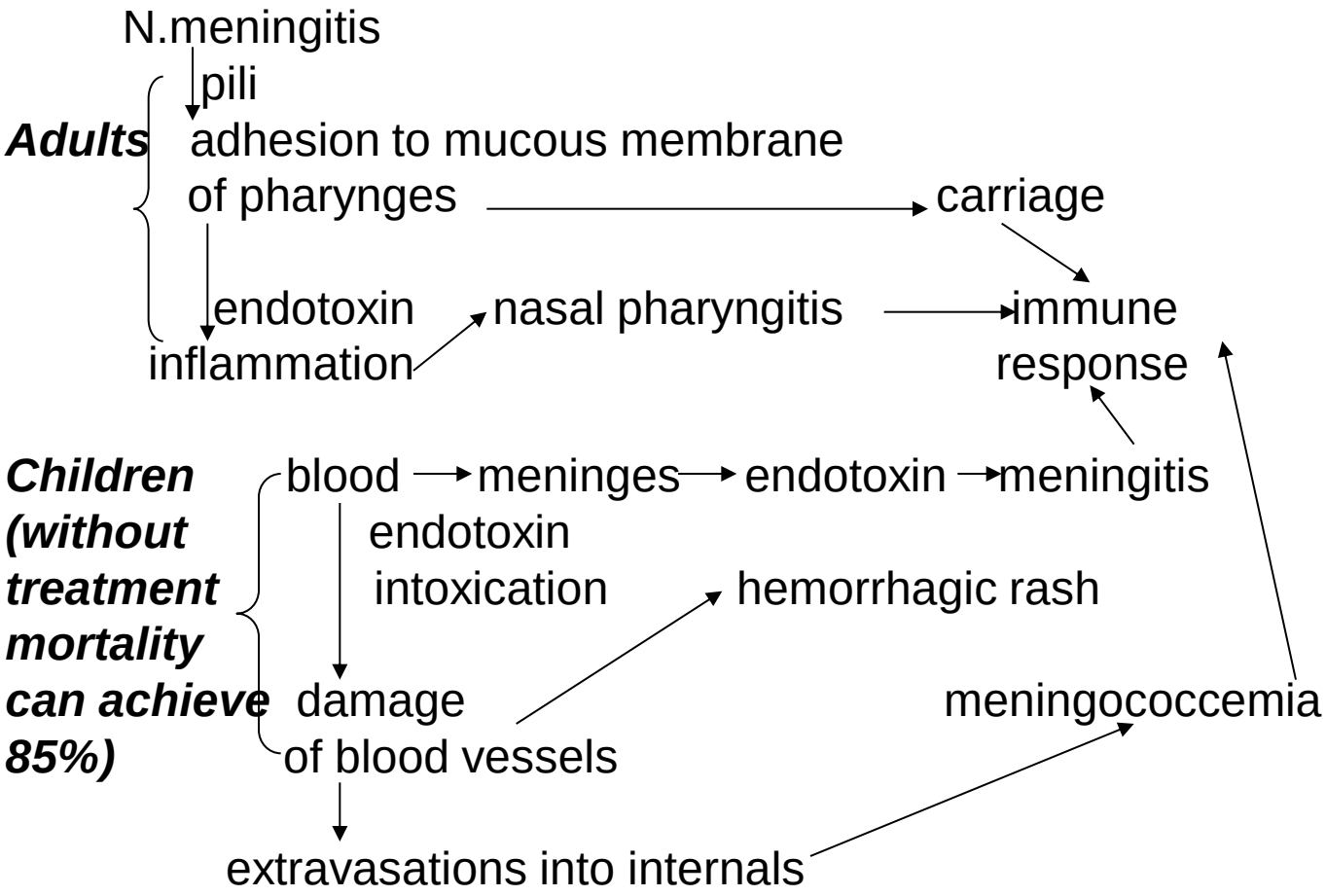
- **Antigenic composition**
  - ✓according to the specificity of *capsule polysaccharides* are classified into serological groups (epidemics are caused by serological groups A – C, ***most virulent are meningococci of the group A***)
  - ✓according to the *proteins of outer membrane* they are classified into ***serological types***
  - ✓according to *LPS* – into ***serological variants***
- **Virulence (pathogenicity) factors**
  - ✓capsule
  - ✓endotoxin
  - ✓pili
  - ✓IgA-proteases
- *Low resistance to unfavorable conditions of environment*

Neisseria meningitidis in pathological material – noncompleted phagocytosis

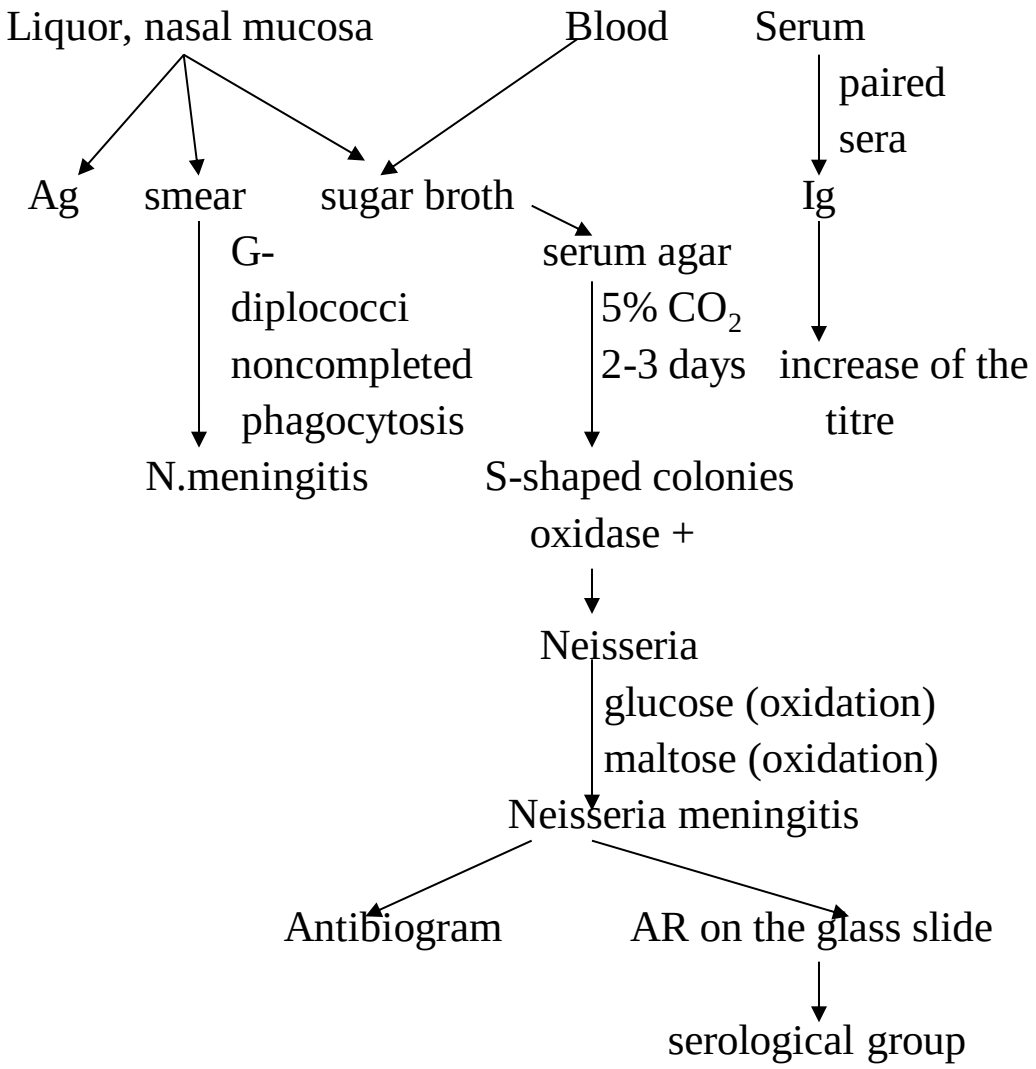


Meningococcal infection

- Pathogenesis



Microbiological diagnostics of the infections caused by meningococci



GONOCOCCI

Neisseria gonorrhoeae

Gonococcus: main characteristics

- Morphology

  gram-negative cocci, usually occurring in pairs, individual cocci are kidney-shaped, nonmotile and nonsporeforming, don't possess macrocapsule.

- Growth characteristics

  grow on enriched media (containing protein, for example, serum agar) at 37^0C (in an atmosphere containing 5% CO_2), in 24-36 hours they form small (virulent variants which have pili) or large (nonvirulent ones which have no pili) S-shaped colonies.

- **Biochemical and metabolic characteristics**

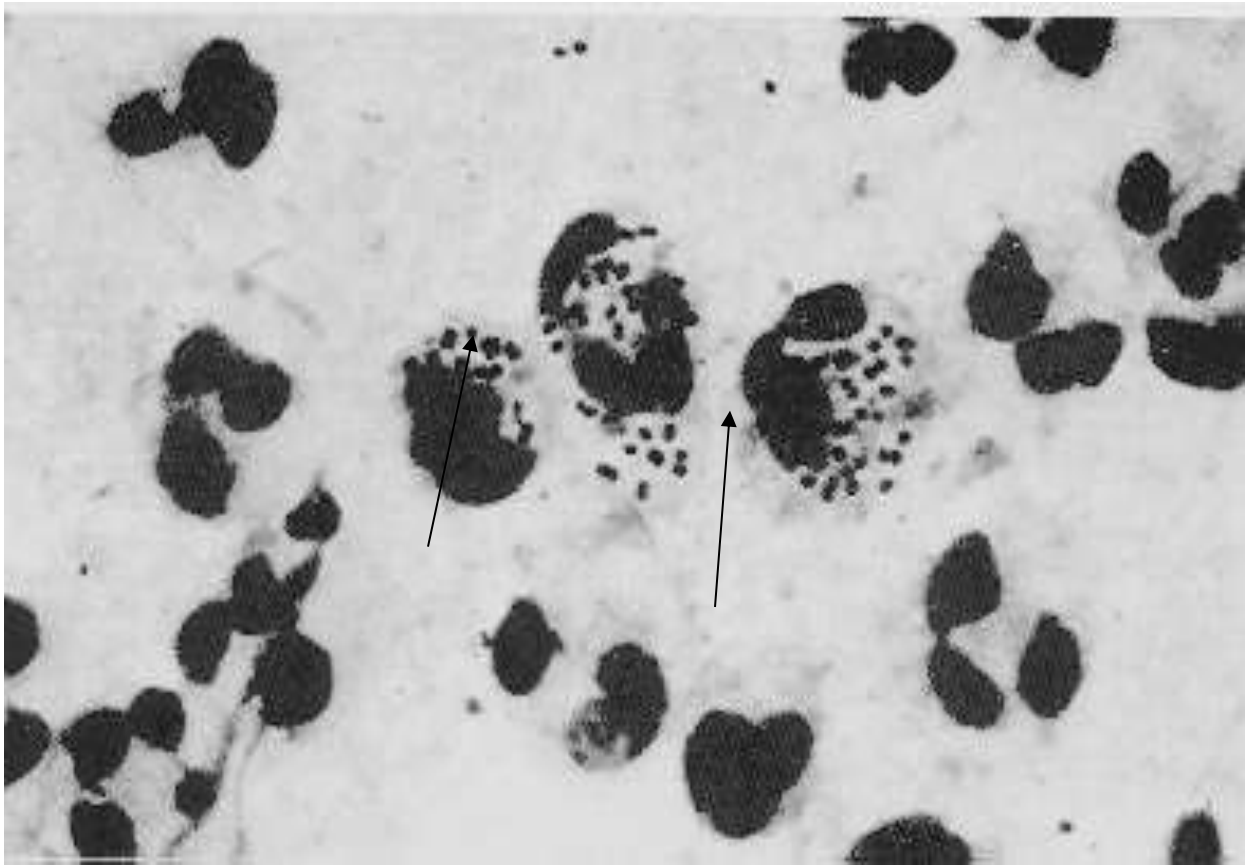
  produce *oxidase* (main characteristics of the genus), ferment *glucose* producing acid but not gas (main characteristics of the species).

- Antigenic composition

antigens are the *proteins of outer membrane* (but the serological classification of gonococci is not widely used because of high variability of the antigen structure) and *LPS*

- Virulence (pathogenicity) factors
  - pili (participate in adhesion and colonisation)
  - LPS (endotoxin)
- *Low resistance to unfavorable conditions of environment*

Neisseria gonorrhoeae in pathological material – noncompleted phagocytosis



Gonococcal infection

Pathogenesis:

The portals – cylindrical epithelium of:

- urethra
- uterine cervix (neck womb)
- rectum (straight intestine)
- conjunctiva (ophthalmia neonatorum)

↓

inflammation – acute gonorrhea

↓

deeper genital organs – chronic gonorrhea

↓

blood – bacterimia, sepsis

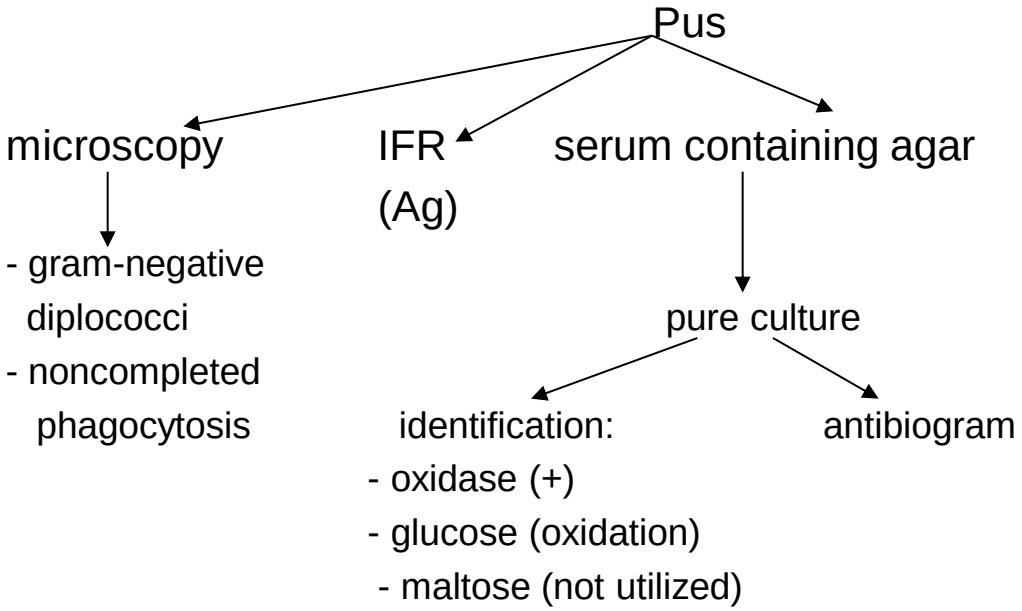
↓

dissemination – complications of chronic gonorrhea

Microbiological diagnostics of the infections caused by Gonococci

- **Laboratory diagnostics**

A. Acute gonorrhoea



B. Chronic gonorrhoea:

1. Revealing of Ig (RIHA , CF reaction)
2. Revealing of sensitization (skin test)

Enterobacteriaceae: Escherichia, Shigella and Salmonella

Theme N19

The Enterobacteriaceae Family is a group of bacteria that cause primary infections of the human gastrointestinal tract. They are referred to as enterics.

The main characteristics of the family of Enterobacteriaceae: classification

Division: Gracilicutes.

- Family: Enterobacteriaceae.

  a) pathogenic genera:
    - Shigella,
    - Salmonella,
    - Escherichia (pathogenic or diarrheagenic variants);

  b) opportunistic genera:
    - Escherichia (opportunistic variants),
    - Klebsiella,
    - Proteus,
    - Yersinia.

The main characteristics of the family of enterobacteriae: morphology

- Gram-negative facultative anaerobic short polymorphic nonsporeforming rods some of them don't possess a macrocapsule.
- Frequently they are motile (peritrichous flagellation) and have no special arrangement in the smear.
- Klebsiellae and shigellae are nonmotile.
- Motility of yersiniae is dependent on the temperature (they loss motility at 37^0C).
- Macrocapsule is always produced by klebsiellae (even when growing on ordinary natural media).
- Klebsiellae form pairs in the smear (diplococci).

The main characteristics of enterobacteriae: growth requirements

- The bacteria grow on ordinary nutritive media at 37^0C, and produce S-shaped colonies after 24 hours of incubation.
- Yersiniae grow at low temperatures (even at 4^0C).
- They are often isolated from faecal matter on agar containing lactose and a pH indicator.
- Endo-agar is selective medium for isolation of escherichiae.

Characteristics of the colonies of pathogenic enterobacteriae grown on differential solid media

Medium	Colour of the colonies		
	Escherichia	Shigella	Salmonella
Endo	pink	uncoloured	uncoloured
Levin	blue	uncoloured	uncoloured
Plosive	red	uncoloured	uncoloured
Bismuth-sulfite agar	uncoloured	uncoloured	black

The main characteristics of enterobacteriae : biochemical and metabolic characteristics

- utilize glucose (glucose +)
- produce catalase (catalase +)
- don’t produce oxidase (oxidase –)

- other biochemical characteristics are genus- or species - specific.

The main characteristics of enterobacteriae : antigenic structure

- **O**-antigen (lipopolysaccharide part of the bacterial cell wall) or ***somatic antigen*** – occurs in all species
- **H**-antigen (flagellar antigen) occurs in most of the species (which are motile)
- **K**-antigen (capsular antigen) occurs in some species (which possess capsule)
- **Vi**-antigen (virulence antigen) occurs in Salmonella typhi

---

- according to the structure of O-, H- и K-antigens every species could be classified into tens or even hundreds of serological variants

The main characteristics of enterobacteriae: virulence (pathogenicity) factors

- **Endotoxin**
- **Factors of invasion** (flagella and ability to produce some chemical substances which allow the invasion of bacteria)
- **Factors of adhesion** (pili, surface proteins of the cell wall)
- **Factors of aggression** (provide the possibility for bacteria to survive in the cytoplasm of phagocytes and in the blood serum)
- **Protein toxins** (found in some variants of escherichiae and shigellae).

The main characteristics of enterobacteriae: resistance to unfavorable conditions of environment

- Highly resistant

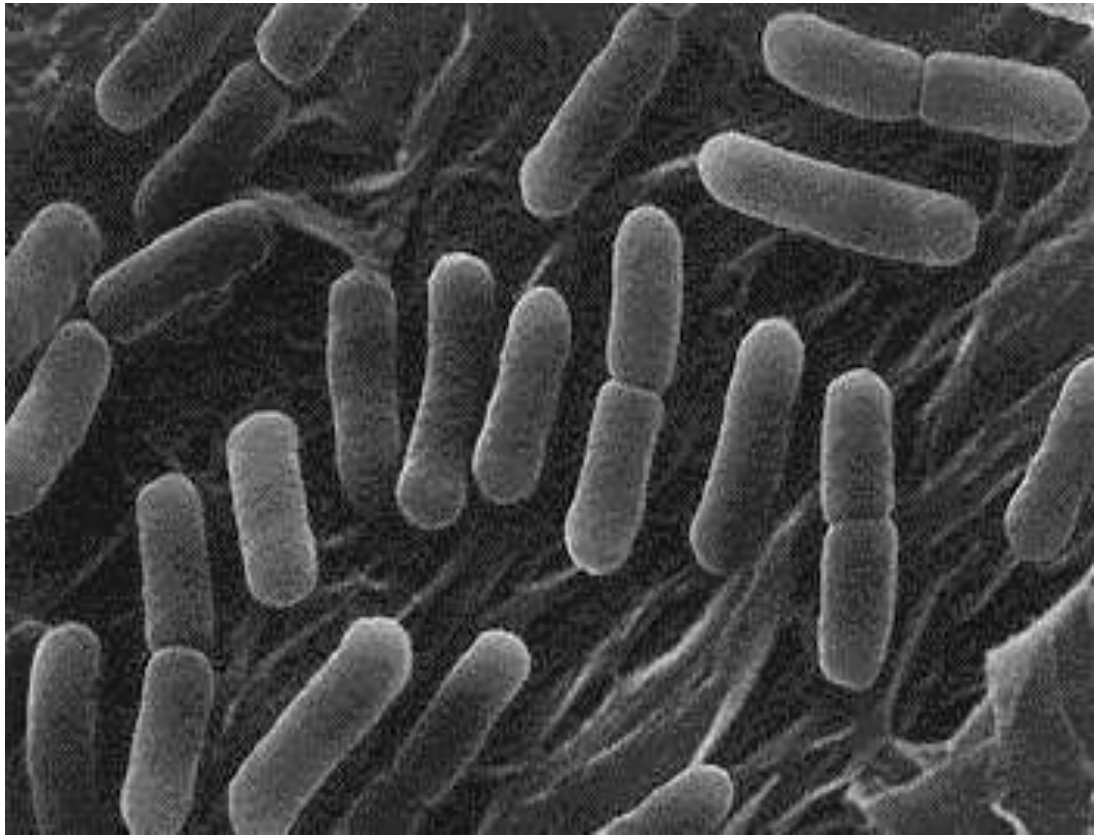
- The most resistant are bacteria which belong to the genus ***Proteus***
- ***Yersiniae*** are able to live and multiply at low temperatures (even in fridge)

Escherichia

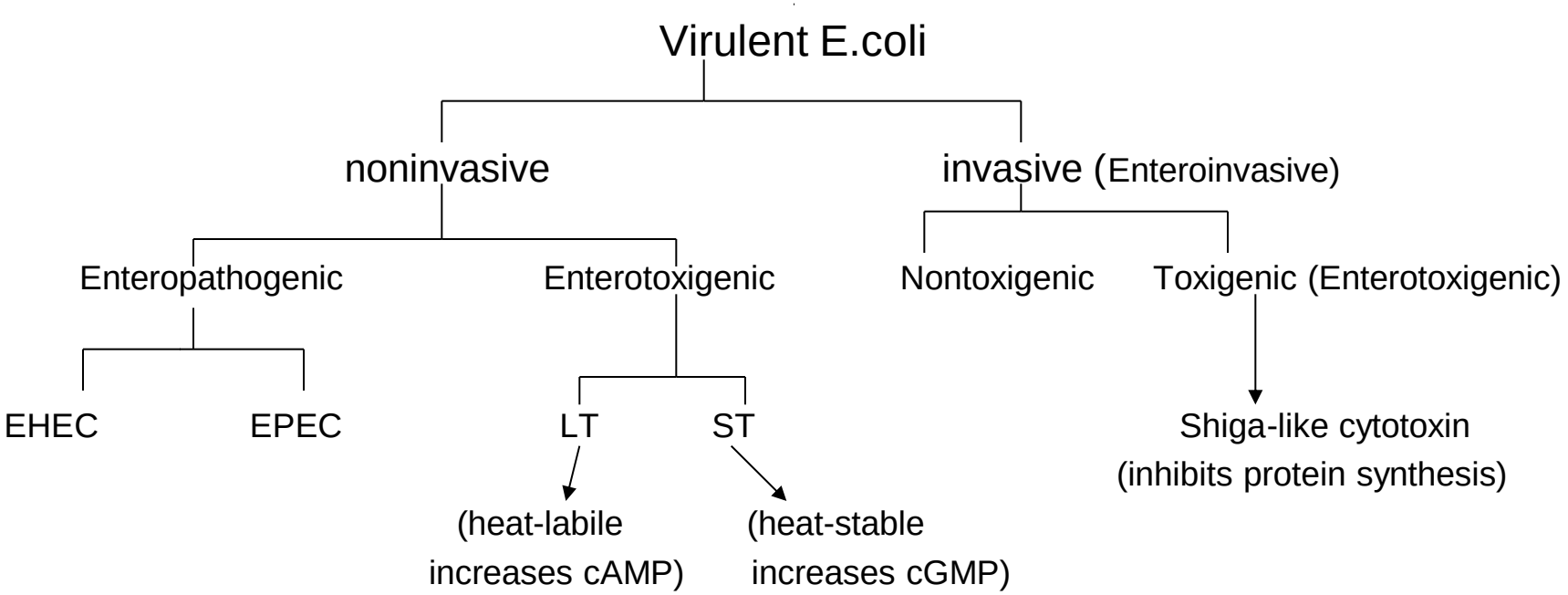
The genus Escherichia : species

- **E.coli**
- E. fergusoni
- E.hermannii
- E.vulneris

E. coli – scanning electron microscopy photo



Infections caused by escherichiae


Virulent E.coli
noninvasive
invasive (Enteroinvasive)
Enteropathogenic
Enterotoxigenic
Nontoxigenic
Toxigenic (Enterotoxigenic)
EHEC
EPEC
LT
ST
Shiga-like cytotoxin
(inhibits protein synthesis)
(heat-labile
increases cAMP)
(heat-stable
increases cGMP)

Infections caused by escherichiae: clinical manifestations

Enteric infections (the source of the infection is exogenous) are classified into:

- enterotoxigenic (ETEC) – gastroenteritis: traveller's diarrhoea, and infantile diarrhoea in developing countries
- enteroinvasive (EIEC) – dysentery-like infections in adults and children
- enteropathogenic (EPEC) – cause of acute infantile diarrhoea in developing countries, disease is rare in adults
- Enterohemorrhagic (EHEC) – cause sporadic cases and outbreaks of hemorrhagic colitis characterized by bloody diarrhoea and may cause haemolytic uremic syndrome.

Infections caused by escherichiae: clinical manifestations

Extraintestinal Infections

- Infections of urinary tract (the source of infection is the intestine)
- Bacteremia (the sources are urinary tract, intestine but frequently the source of the infection could not be established)
- Meningitis could develop in newborns (the source of the infection is exogenous)

Infections caused by escherichiae : pathogenesis

- ***Enterotoxigenic*** (O1, O15, O148)

  enterotoxins (two types of plasmid-encoded toxins are produced) ⇨ *secretary diarrhea*

- ***Enteroinvasive*** (0124, 0144)

  ulcerative colitis with dysentery-like syndrome ⇨ *inflammatory diarrhea*

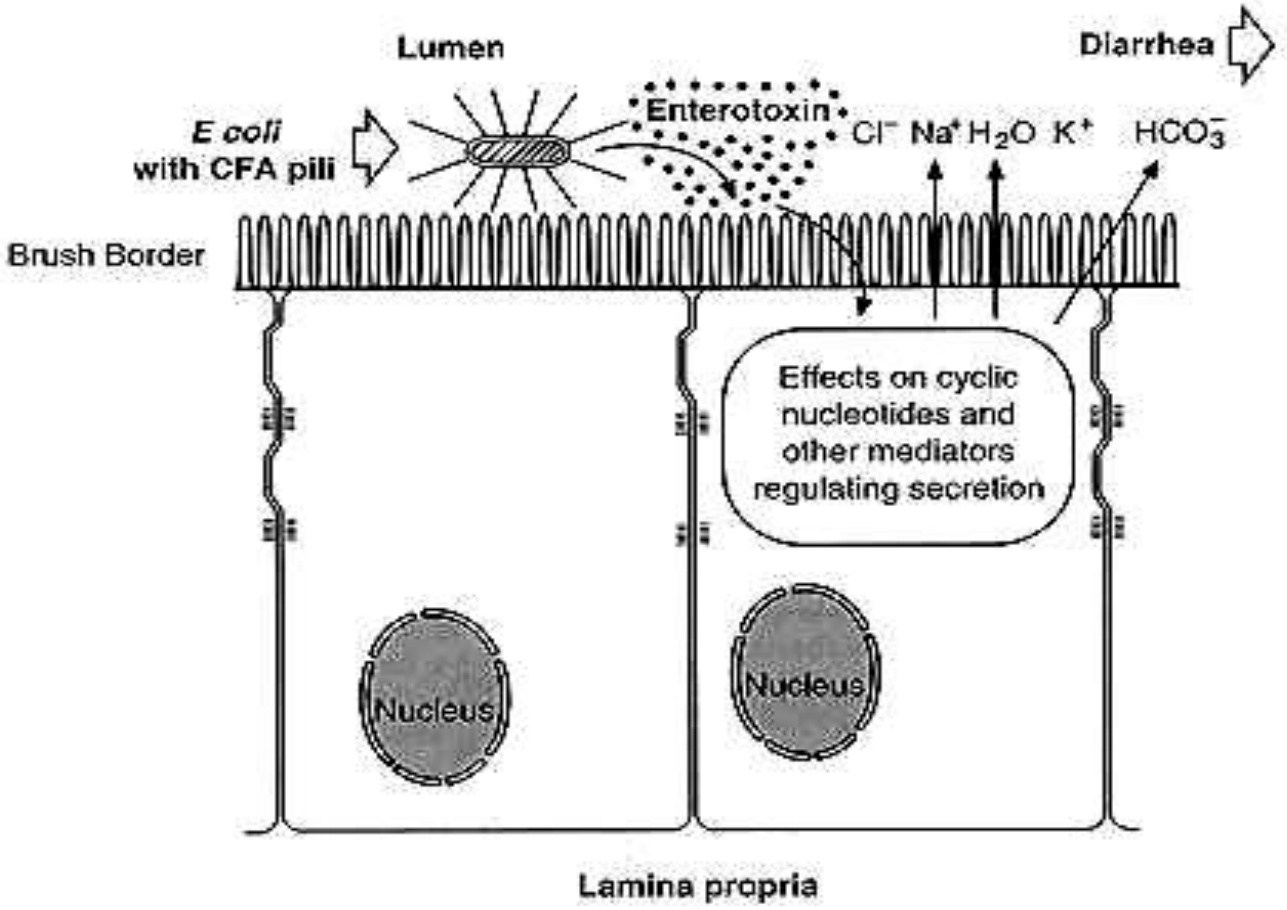
- ***Enteropathogenic*** (026, 055, 0111)

  vacuolisation and destruction of vili ⇨ smoothing out the surface of the mucous membrane ⇨ resulting in the appearance of *erosions and bacteraemia* (spreading by macrophages)

- ***Enterohemorrhagic*** (O157)

  possess cytotoxin (shigella-like toxin encoded by a lysogenic phage.) ⇨ *hemorrhagic colitis*

  Can disseminate into the bloodstream ⇨ systemic haemolytic-uremic syndrome (haemolytic anaemia, thrombocytopenia and kidney failure).


Lumen
Diarrhea
E coli
with CFA pili
Enterotoxin
Cl⁻ Na⁺ H2O K⁺ HCO3⁻
Brush Border
Effects on cyclic nucleotides and other mediators regulating secretion
Nucleus
Nucleus
Lamina propria

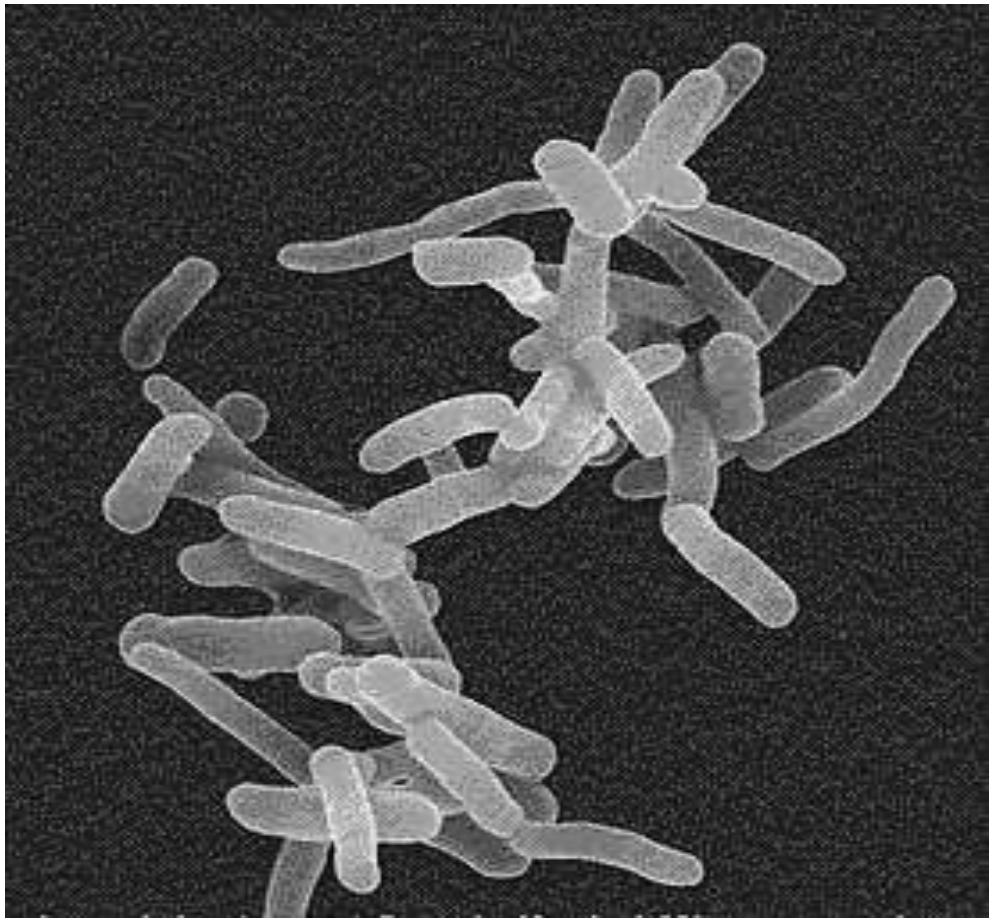
Infections caused by escherichiae: laboratory diagnostics

Microbiological tests

- Specimens of pathological material that is taken for diagnosis is dependent on the clinical form of the disease that could be stool (faeces), blood, urine, etc.
- Endo agar is usually used to grow the colonies of the bacteria.
- Biochemical and serological characteristics are used for the final identification.

Shigella

Shigella dysenteriae - scanning electron microscopy photo



International classification of the genus Shigella

- Shigella dysenteriae (serological group A)
- Shigella flexneri (serological group B)
- Shigella boydii (serological group C)
- Shigella sonnei (serological group D)

Factors of pathogenicity of Shigella

- ***Specific surface proteins*** provides *invasiveness* of shigellae (the information about the toxin is coded in transmissive plasmids).
- ***Endotoxin*** is similar with the endotoxin of other gram-negative bacteria.

Factors of pathogenicity of Shigella

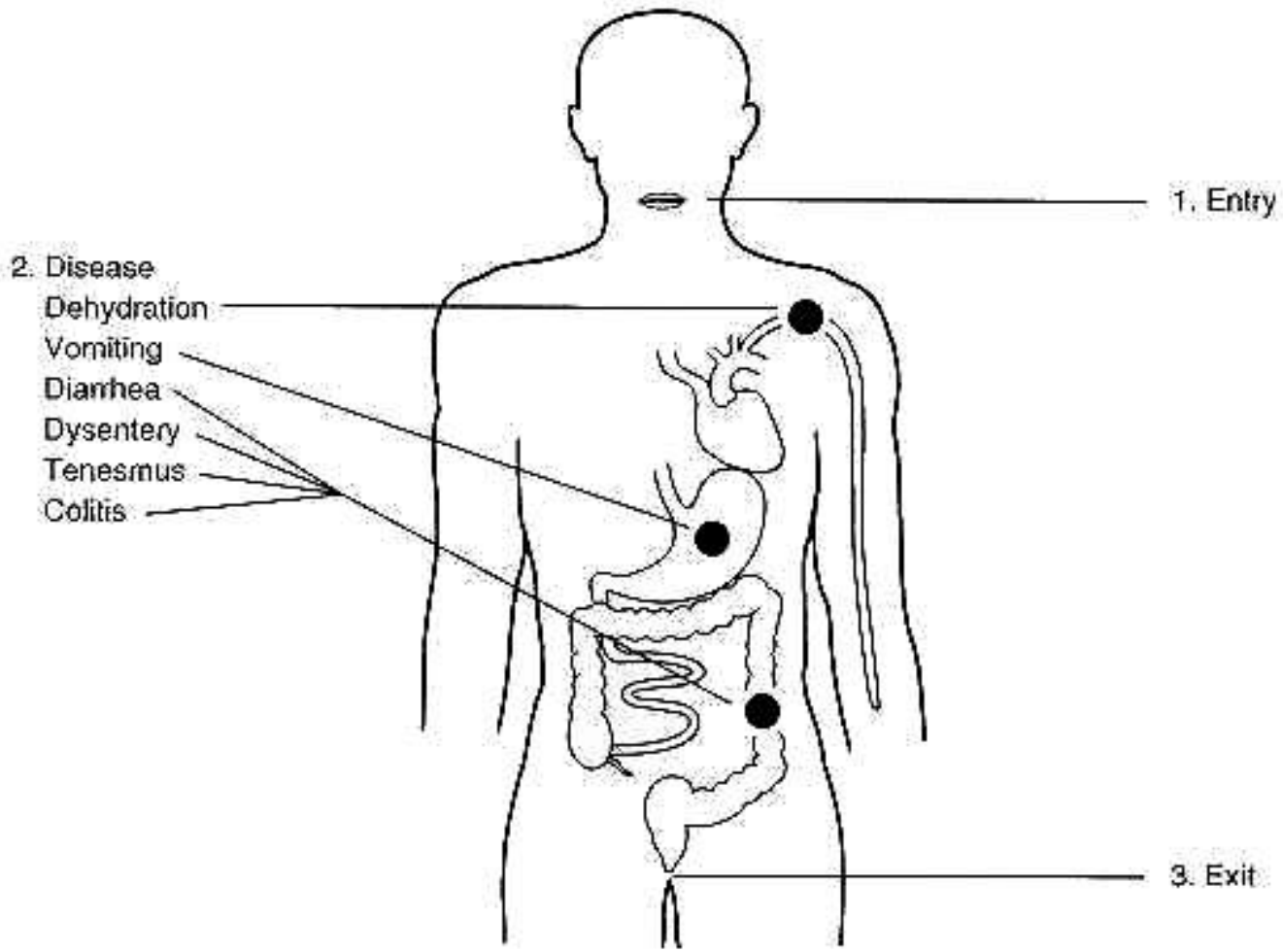
- ***Shiga's toxin***

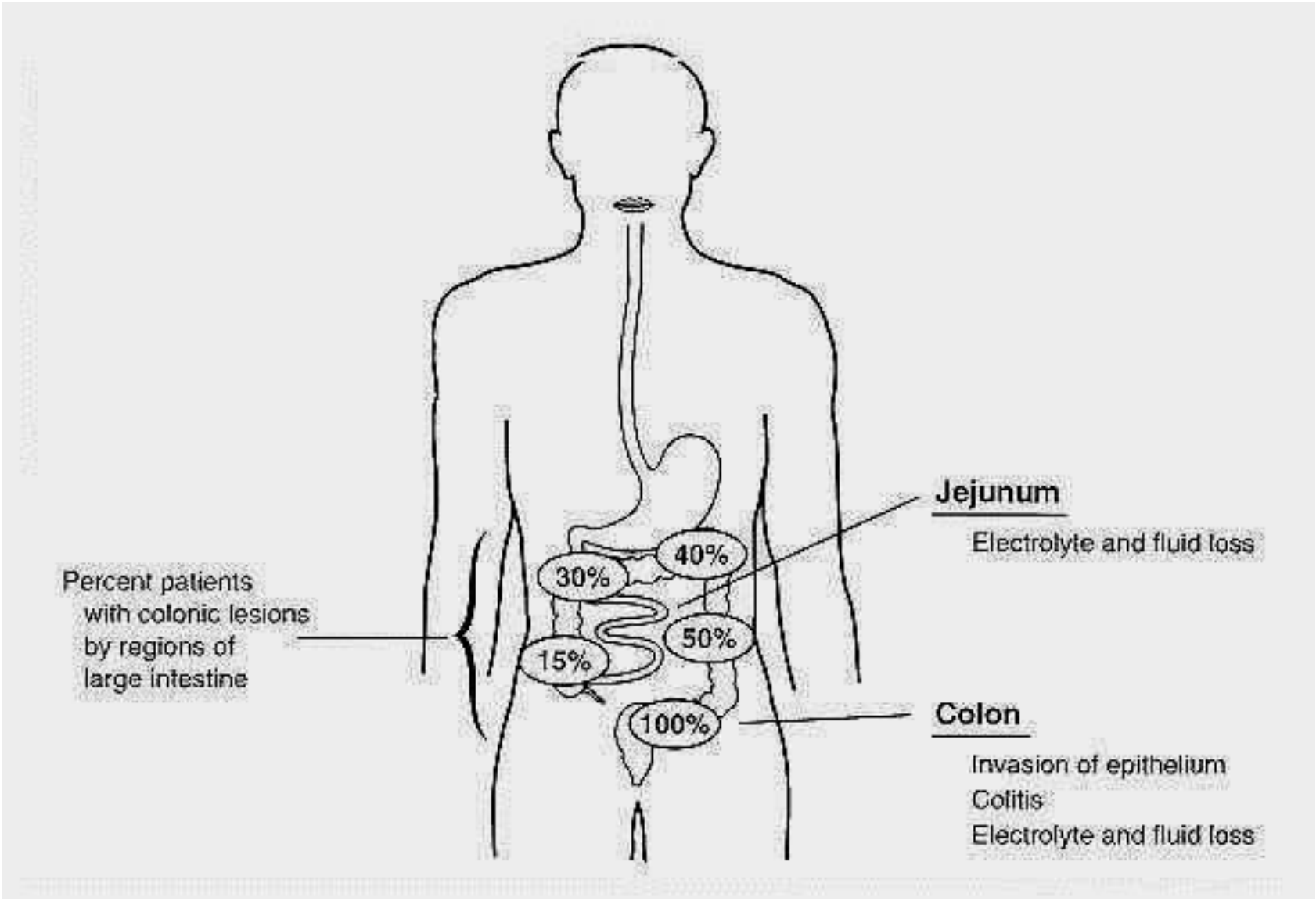
- ✓ The toxin is ***cytotoxic***, ***neurotoxic*** and ***enterotoxic.***
- ✓ It causes **death of the intestinal cells** and the **hypersecretion of water and electrolytes into the gut lumen** resulting in ***diarrhoea (***highly pronounced in the serological type 1 of S.dysenteriae).
- ✓ **Damage of the epithelial layers** lining the intestine is followed by release of mucous and blood (found in the faeces) and attraction of leukocytes (also found in the faeces as "pus").

Bacterial dysentery

Bacterial dysentery : epidemiology

- **Source** ("reservoir“) of the infection is human. This is primarily a disease of young children occurring by faecal-oral contact. Adults can catch this disease from children or infected adults.
- **Mechanism of transmission** of the infection is faecal-oral **and the ways are**
  - ► alimentary (with contaminated food: it can be transmitted by infected adult food handlers who contaminate food)
  - ► water (with contaminated water)
  - ► contact (the source is unwashed hands)


1. Entry
2. Disease
Dehydration
Vomiting
Diarrhea
Dysentery
Tenesmus
Colitis
3. Exit


Jejunum
Electrolyte and fluid loss
Percent patients with colonic lesions by regions of large intestine
40%
30%
50%
15%
100%
Colon
Invasion of epithelium
Colitis
Electrolyte and fluid loss

Bacterial dysentery : pathogenesis

invasion of the intestinal mucosal epithelium by shigellae

⇓

multiplying in the cells

⇓

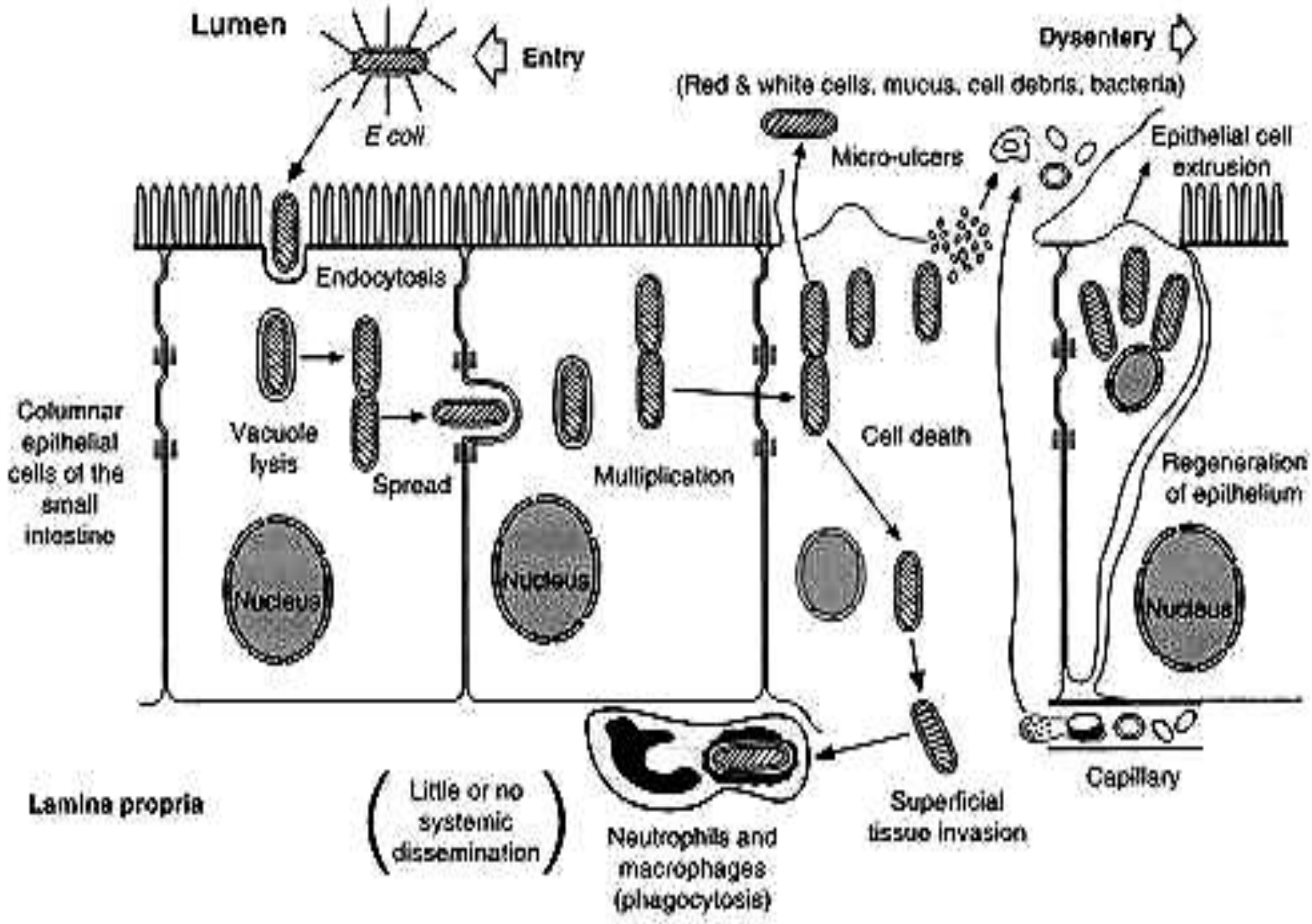
spread of the infection to other cells

⇓

death of the infected cells and invasion of bacteria into the underlining tissues through mucous membranes

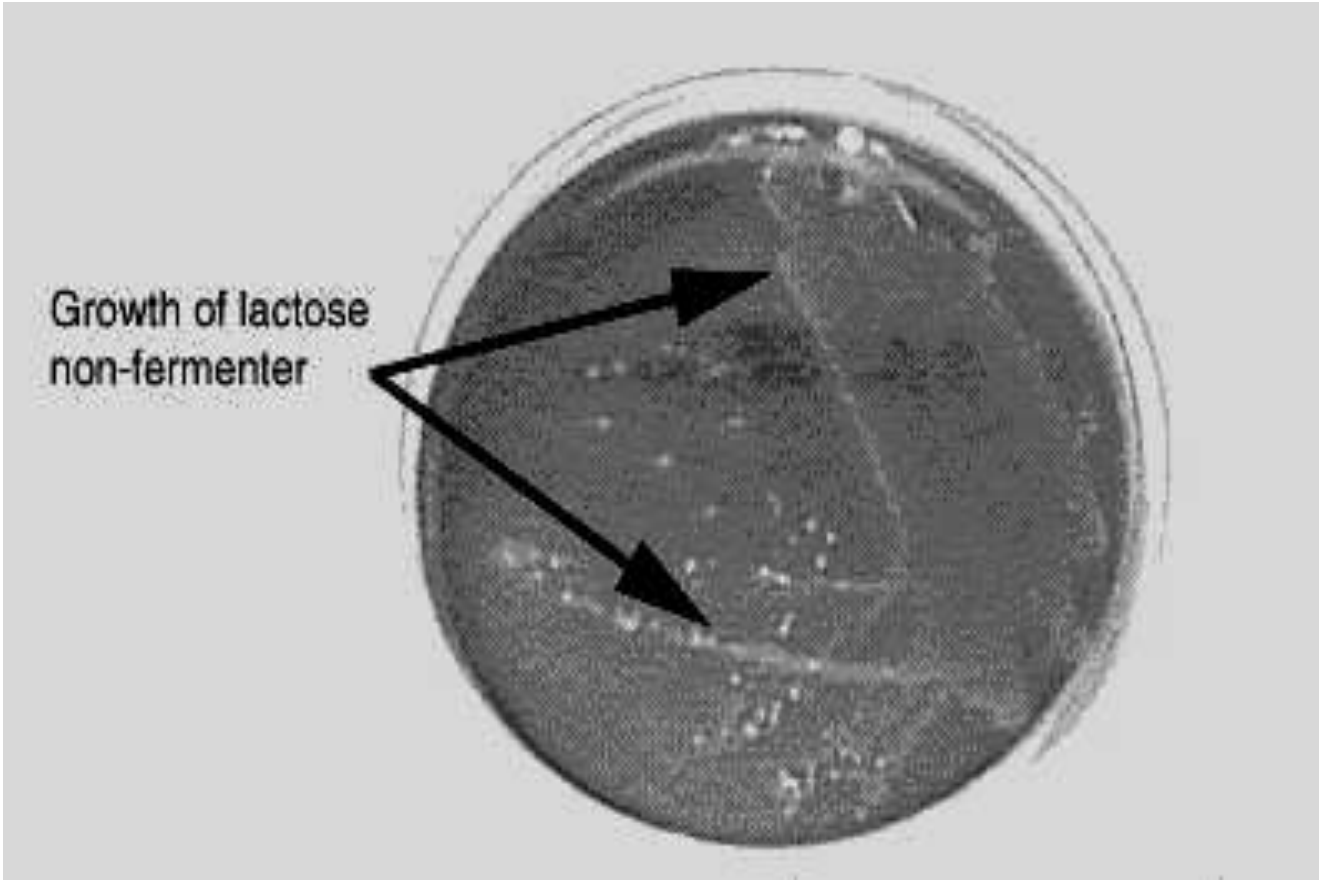
⇓

appearance of defects of the mucous membranes (microabcesses, necrosis) and development of inflammation


Lumen
Entry
E coli
Dysentery
(Red & white cells, mucus, cell debris, bacteria)
Micro-ulcers
Epithelial cell extrusion
Endocytosis
Columnar epithelial cells of the small intestine
Vacuole lysis
Spread
Multiplication
Cell death
Regeneration of epithelium
Nucleus
Nucleus
Nucleus
Capillary
Lamina propria
Little or no systemic dissemination
Neutrophils and macrophages (phagocytosis)
Superficial tissue invasion

Bacterial dysentery: laboratory diagnostics

- Pathological material (faeces) is plated on the solid Ploskirev's media.
- Colourless colonies have been chosen for isolation of pure culture.
- Biochemical and serological characteristics are used for the final identification.
- Study of the sensitivity of the bacteria to antibiotics is important.


Growth of lactose
non-fermenter

Bacterial dysentery: prophylaxis (prevention)

- **Nonspecific prophylaxis** is similar with the preventive measures used for all intestinal infections (to wash fruits and vegetables as well as hands before meals, etc)
- **Specific prophylaxis** - use of specific bacteriaphage (urgent prophylaxis)

Salmonella

Classification of the genus Salmonella

A. Salmonellae belonging to **typhoid-paratyphoid group:**

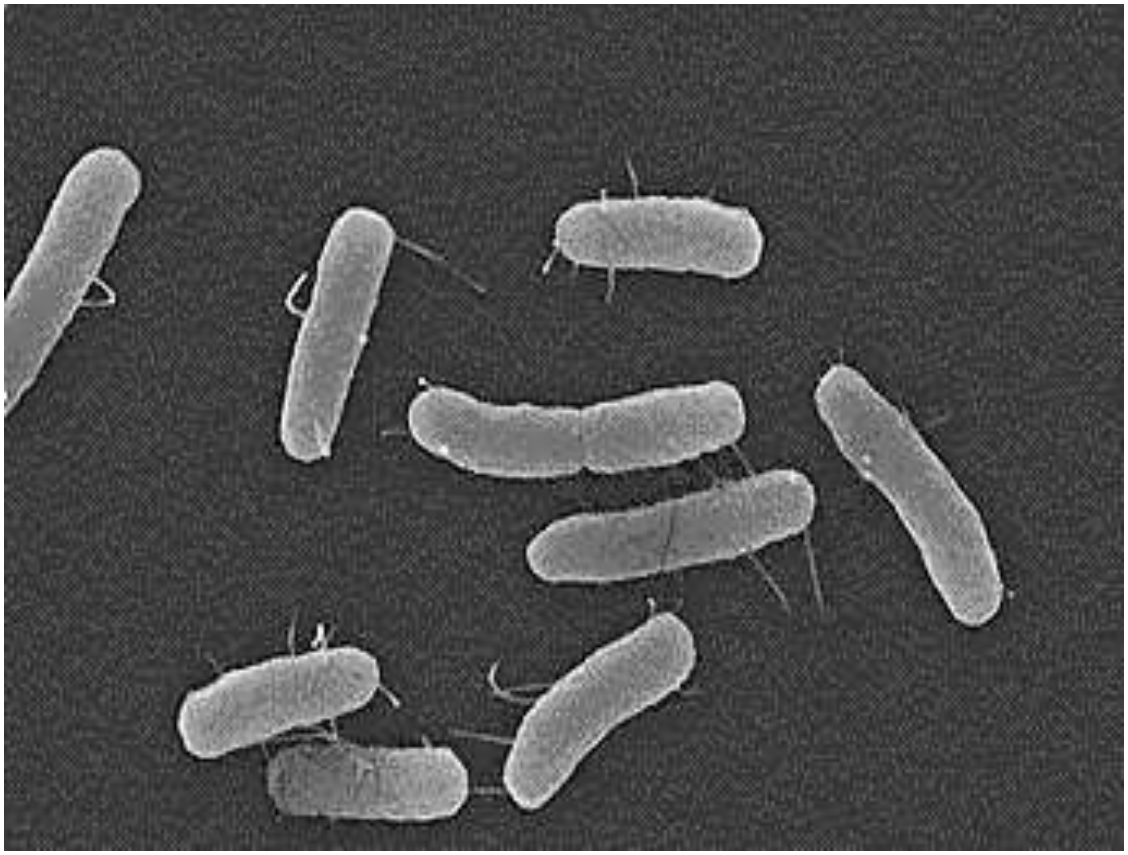
- Salmonella typhi
- Salmonella paratyphi A
- Salmonella schottmuelleri

B. Salmonellae which cause **salmonellosis (gastroenteritis):**

- Salmonella typhimurium
- Salmonella enteritidis
- Salmonella choleraesuis

C. other species (more then 2500)

Salmonella - scanning electron microscopy photo



The factors of pathogenicity of salmonellae

- **endotoxin**
- **proteins of outer membrane** (factors of adhesion)
- **microcapsule** (resistance to phagocytosis)

Typhoid fever and enteric fever

Typhoid fever and enteric fever: epidemiology

- *It is one of the historical causes of widespread epidemics but still it is happens in the third world.*
- **The source of infection** is **human** (ill individual or carrier of the infection). In enteric fever type B the source of the infection could be also domestic animals
- **The mechanism of the infection spreading** is **faecal-oral** and the next ways could be involved :

✓ contaminated with faeces *water* is the most frequent one when the bacterium is transmitted from a human reservoir to the water supply if sanitary conditions are poor;

✓ contaminated *food* - alimentary way,

✓ *contact* way of transmission could also occur.

Typhoid fever and enteric fever: **clinical symptoms**

The symptoms of typhoid fever and enteric fever are practically the same :

- fever
- headache, weakness, stupor and delirium
- rose-coloured spots on the skin (in typhoid fever)
- the heaviest complication is *perforation of the intestinal wall which causes intestinal bleeding*, development of peritonitis and sometimes leading to death

Typhoid fever: pathogenesis

Salmonellae

↓

adhesion to the cells, penetration into the small intestine

↓

multiplication in lymphoid tissue (sensitisation)

↓

getting into bloodstream (bacteria are disseminated in macrophages) - bacteremia

↓

infection of parenchymatous organs, production of endotoxin (intoxication)

↓

accumulation in the gall-bladder (reservoir of salmonella)

↓

secondary invasion of the small intestine

↓

allergic reaction as a result of the secondary contact with lymphoid tissue

↓

inflammation and necrosis of the lymphoid tissue

↓

perforation of the intestinal wall

Typhoid fever and enteric fever: **laboratory diagnostics**

- Specimens of pathological material (blood, urea, faeces, bile – in the case of carriage) are plated on the solid **selenite-containing media** and put into **bile broth** to accumulate (to enrich) the culture.
- After growth bacteria are plated on the **bismuth-sulfite agar**. Black colonies (because of H_2S production by S.typhi) have been chosen for isolation of pure culture.
- Biochemical and serological tests are used for the final identification: the tube dilution agglutination – **Widal test** is used to detect the increase of the antibodies' titre in the blood serum of patient.
- Study of the sensitivity (resulting in lysis) to the specific bacteriophages – **"phage typing".** It helps in epidemiologic tracing of isolates.

Typhoid fever and enteric fever: **immunity**

- Naturally acquired (postinfectious) immunity is strong and prolonged

Typhoid fever and enteric fever : **prophylaxis (prevention)**

- **Nonspecific** prophylaxis
  - is similar with the prophylactic sanitary measures taken to prevent all intestinal infections (prevent contamination of food, water, treatment of carriers)
- **Specific** prophylaxis
  - chemical vaccine – but vaccines are not widely effective and not generally used
  - use of specific bacteriophage (urgent prophylaxis)

Salmonellosis (gastroenteritis)

Salmonellosis : epidemiology

Sources of the infection are:

- animals (mainly domestic) and birds (mainly domestic) – more frequently
- human (ill persons or carriers of the infection) – rarely

The mechanism of the infection transfer is faecal-oral (the way is alimentary – with contaminated food: meat, dairy products, eggs, poultry)

Salmonellosis : pathogenesis

Salmonellae

↓

adhesion to the cells, penetration into the small intestine

↓

multiplication in mucous membranes

↓

acute inflammation

↓

superproduction of cytokines, prostaglandins and endotoxin (intoxication)

↓

activation of adenylate cyclase

↓

increase of cAMP concentration

↓

increase secretion of liquid into the lumen of the intestine

↓

secretary diarrhea

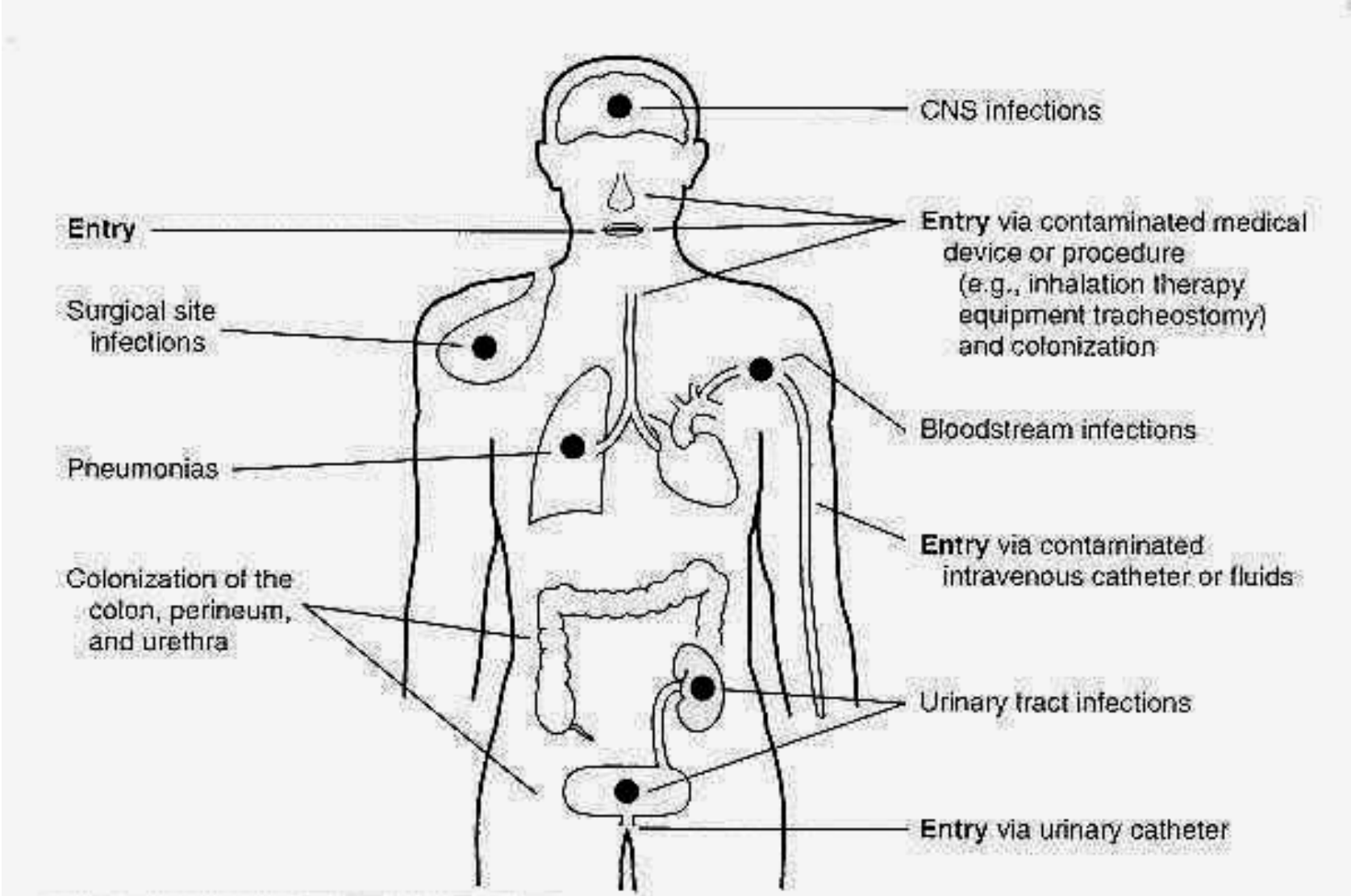
Salmonellosis : laboratory diagnostics

- Pathological material (faeces, vomit masses, stomach flushing liquids, food debris) is plated on solid selenite-containing media.
- Then the grown bacteria are plated on bismuth-sulphite agar. Black colonies have been chosen for isolation of pure culture.
- Biochemical and serological characteristics (AR and RIHA to detect the increase of the titre of antibodies) are used for the final identification.
- Biological method (biological probe): use of laboratory animals (white mice).

ENTEROBACTERIACEAE: KLEBSIELLA, YERSINIA AND PROTEUS. PSEUDOMONADACEAE. CAMPYLOBACTER AND HELICOBACTER

Theme N20

Sites of colonisation and extraintestinal disease production by opportunistic enterobacteriae and Proteus.



- *Some representatives of the family Enterobacteriaceae are **opportunistic** pathogens (not highly pathogenic).*
- *Opportunistic bacteria produce infections in **immunocompromised** patients.*
- *Klebsiella* accounts for a large percentage of ***hospital-acquired infections***.

Classification of the genus Klebsiella

- *K. pneumoniae* – the species *pneumoniae* includes the next variants:
  - var. pneumoniae
  - var. rhinoscleromatis
  - var. ozaenae
- *K. oxytoca*

The klebsiellae : main characteristics

Morphology

Gram-negative, encapsulated, nonmotile, diplobacilli (bacilli arranged in pairs).

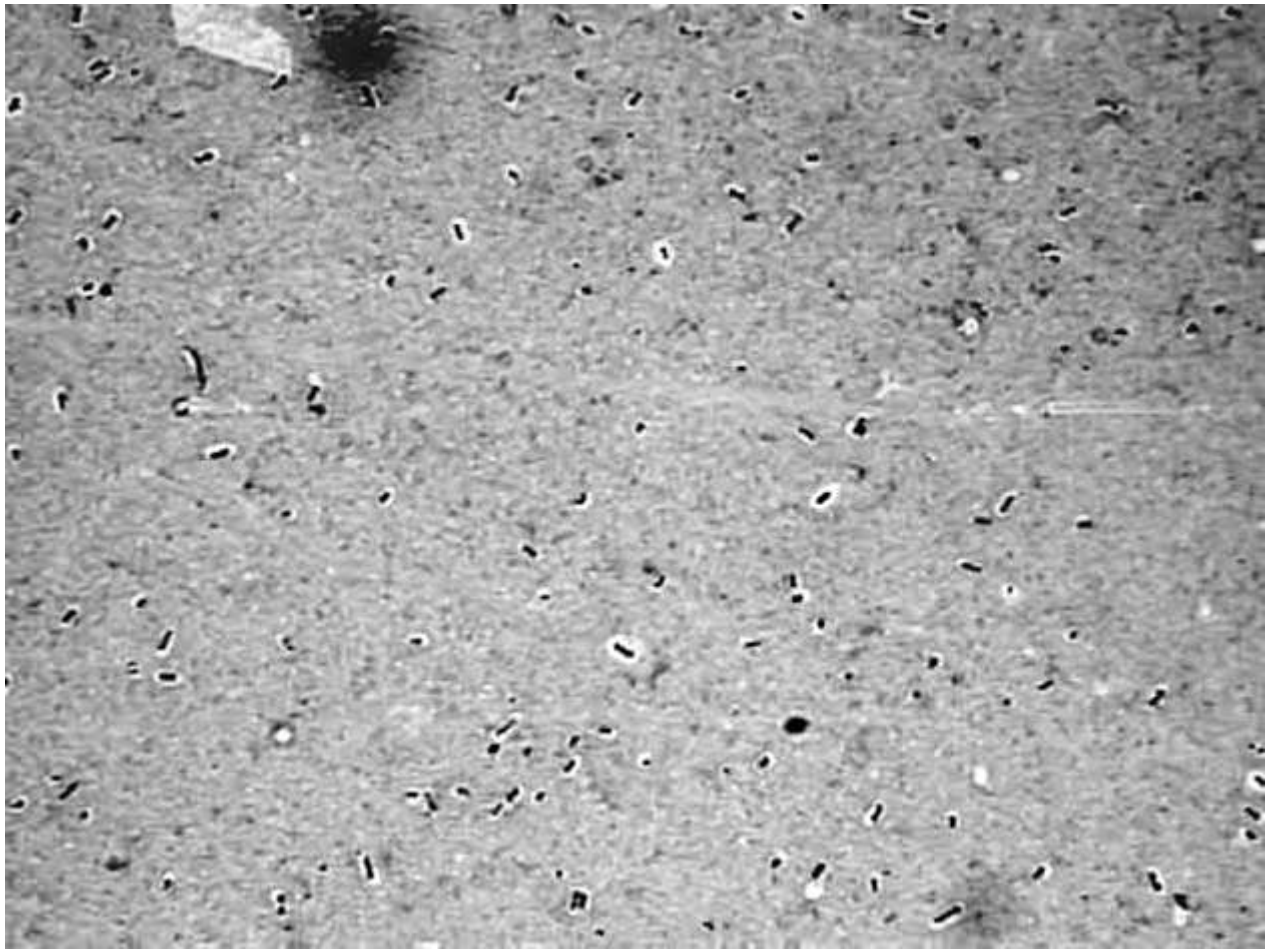
Metabolism

K. pneumoniae: indole –

K. oxytoca: indole +

Factors of pathogenicity

- endotoxin
- macrocapsule - promotes virulence by protecting the bacteria from ingestion by leukocytes



Klebsiellae: encapsulated diplobacilli

The role of klebsiellae in human disease

K. pneumoniae, var. rhinoscleromatis

Infectious agent associated with an unusual chronic granulomatous disease of the upper airways. Disease called ***rhinoscleroma*** that is characterized as:

- chronic infection;
- destructive granuloma: appearance of infiltrations leading sometimes to complete obturation (blocking) of pharynx. Granuloma develops in mucous membranes of the nose, trachea, pharynx and bronchi;
- not highly contagious but poor hygiene, crowded living conditions, and malnutrition Increase the risk for transmission.

K. pneumoniae, var. ozaenae

Infectious agent that causes development of ***ozena***. Ozena is characterized as:

- chronic disease,
- fetid, progressive atrophy of mucous membranes of upper respiratory tract + production of viscous secretions having unpleasant odor.

K. pneumoniae, var. pneumoniae and K. oxytoca

Produce extensive hemorrhagic necrotising consolidation of the lung.

A. Causes infections associated with mucous membranes of:

- intestine
- eyes
- urinary tract

B. Causes infections of:

- meninges
- joints

C. Generalisation of the infection:

- bacteremia
- septicopyemia

D. In children it causes bacterial pneumonias (proportion is about 3%).

Laboratory diagnostics of klebsiellosis

Specimens

Microscopic investigation of stained smears

•Revealing of diplobacilli having large capsule (preliminary diagnostics) - bacteria are stained by Giemsa

Isolation of pure culture (platting on differential media)

Revealing of large mucoid colonies

Final identification:

•Biochemical and serological properties

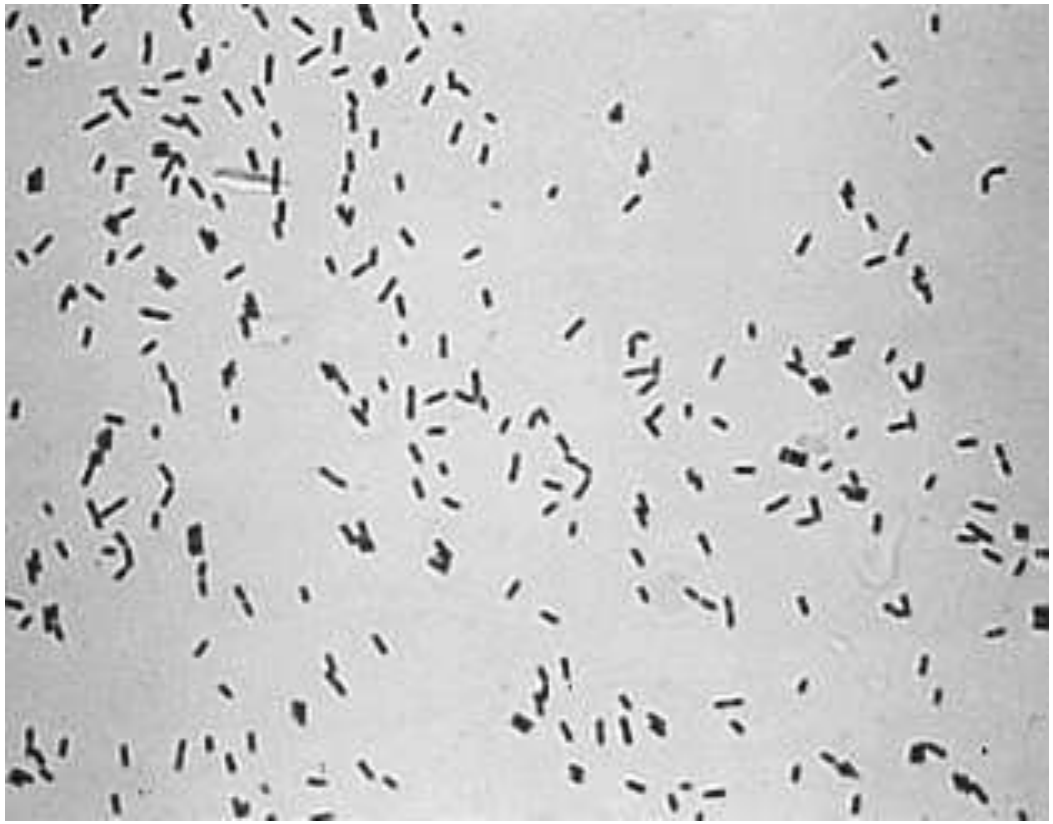
The Proteus group

Proteus: classification of the genus

- Proteus vulgaris
- Proteus mirabilis

Proteus: morphology

The proteus group bacteria are Gram-negative, motile, aerobic rods.



Proteus: main characteristics of the group

- **Proteus vulgaris is highly motile** – results in "swarming***" phenomenon:*** colonies spread rapidly over the surface of solid media.

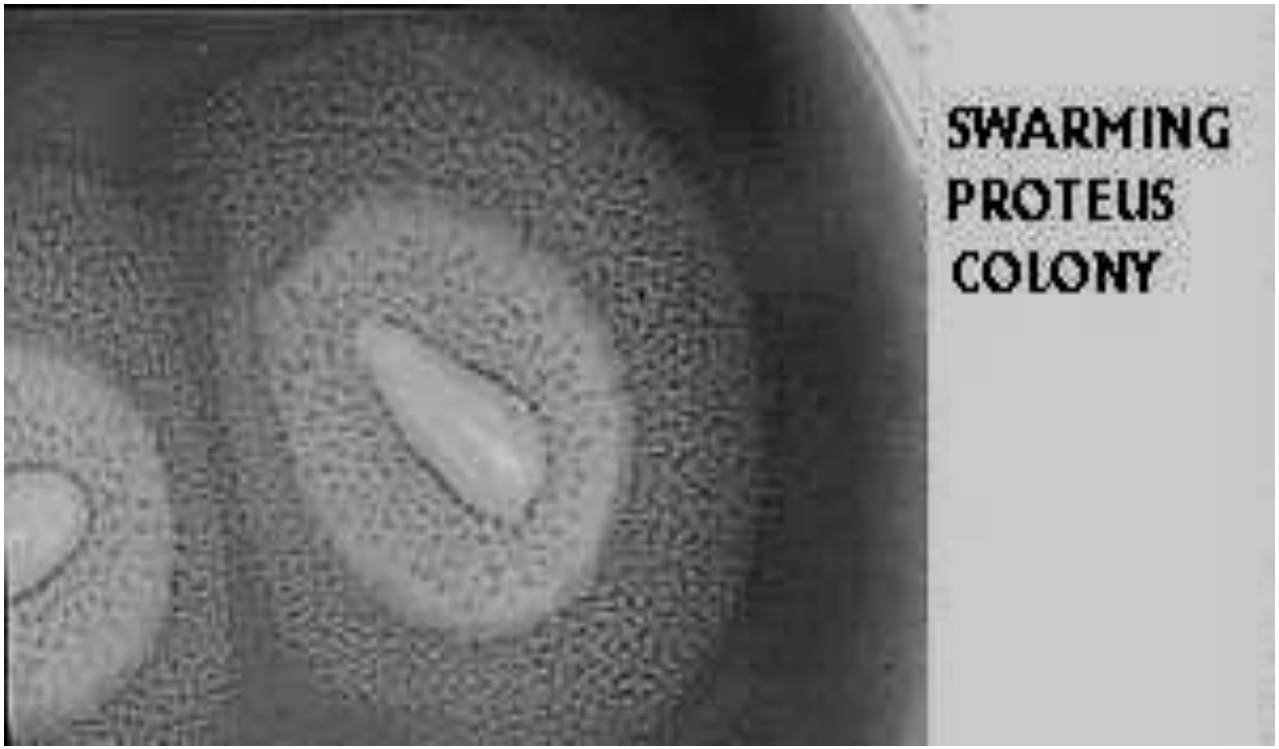
  Shukevich's method proposed to identify Proteus vulgaris:

1. Bacteria are placed into the condensed water at the bottom of the test tube with agar slant.
2. The surface of the slant is rapidly got covered by colonies.
3. Incorporation of phenylethyl alcohol into the agar results in the loss of the "swarming" and the surface of the slant stays uncovered by the colonies.

- **Metabolic characteristic important for identification:**

both species of Proteus (vulgaris and mirabilis) ***decompose urea*** *with the liberation of ammonia.*

Swarming Proteus colony



The role of Proteus in human disease

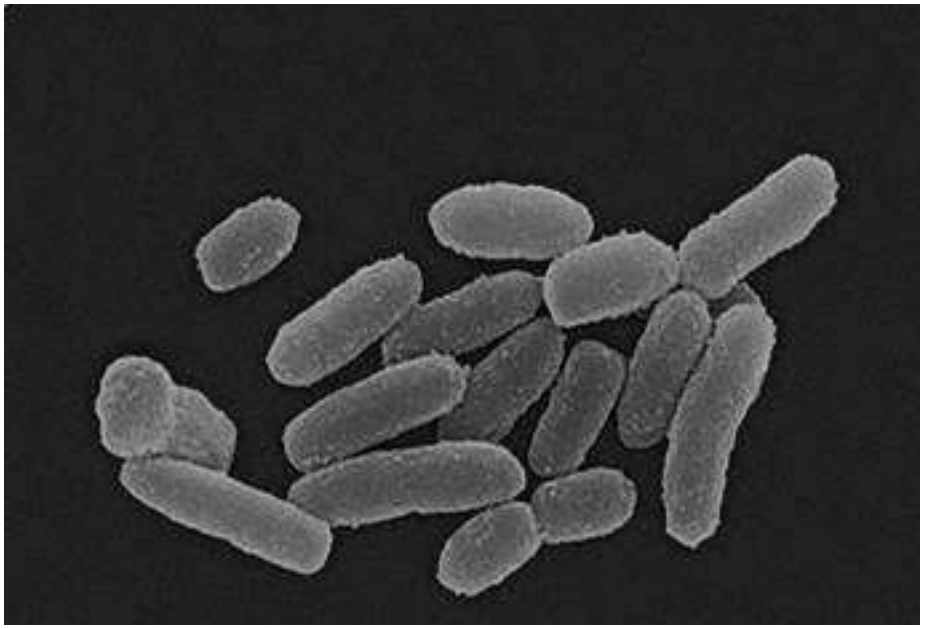
Opportunistic pathogens which produce endogenous infections in humans:

- chronic urinary tract infections,
- bacteremia and focal infections in debilitated patients.

Yersinia

Yersiniae are Gram-negative, facultatively anaerobic rods which grow best at 25^{0}C.

Yersinia enterocolitica - dividing rods



Yersinia: classification of the genus

- ***Yersinia pestis*** highly pathogenic bacterium (causes special danger infection – plague)

Opportunistic pathogens:

- *Yersinia pseudotuberculosis* (causes pseudotuberculosis)
- *Yersinia enterocolitica* (causes enteric disease - yersiniosis and bacteremia)

Infections caused by opportunistic Yersinia: characteristics of pseudotuberculosis

Yersinia pseudotuberculosis:

- causes pseudotuberculosis - infection of birds, rodents and it is rarely transmitted to humans;
- produces typical lesions consist of whitish nodules, resembling tubercles, in the intestines and the parenchymatous organs;

Clinical forms of the disease:

- ✓ appendicitis,
- ✓ enteritis
- ✓ regional lymphadenitis.

Infections caused by opportunistic Yersinia: characteristics of enterocolitis (yersiniosis)

Yersinia enterocolitica

- an occasional member of the human gut flora,
- releases a toxin that causes enteritis with pain resembling appendicitis,
- can produce febrile diarrhea in humans with contact (person-to-person) mechanism of transmission.

The symptoms of enterocolitis are:

- ✓ severe abdominal pain (may suggest appendicitis and leads to operation)
- ✓ ileitis
- ✓ mesenteric adenitis
- ✓ hepatic or spleenic abscesses
- ✓ rarely - bacteremia or endocarditis

Yersiniosis: laboratory diagnostics

specimen (faeces, urea, blood, liquor, appendix)

⇓

isolation of pure culture

⇓

identification procedure is analogical to the identification of other enterobacteriae

THE PSEUDOMONAS GROUP

The Pseudomonas group: classification

Division: *Gracilicutes*

Family: *Pseudomonadaceae*

Genus: *Pseudomonas*

Opportunistic species

- *P. aeruginosa*
- others

Genus: *Burkholderia*

Pathogenic species

- *Burkholderia mallei* (P. mallei) – human and animal pathogen causing Glanders (the Latin name of this disease – *malleus* gave name to the causative agent species).

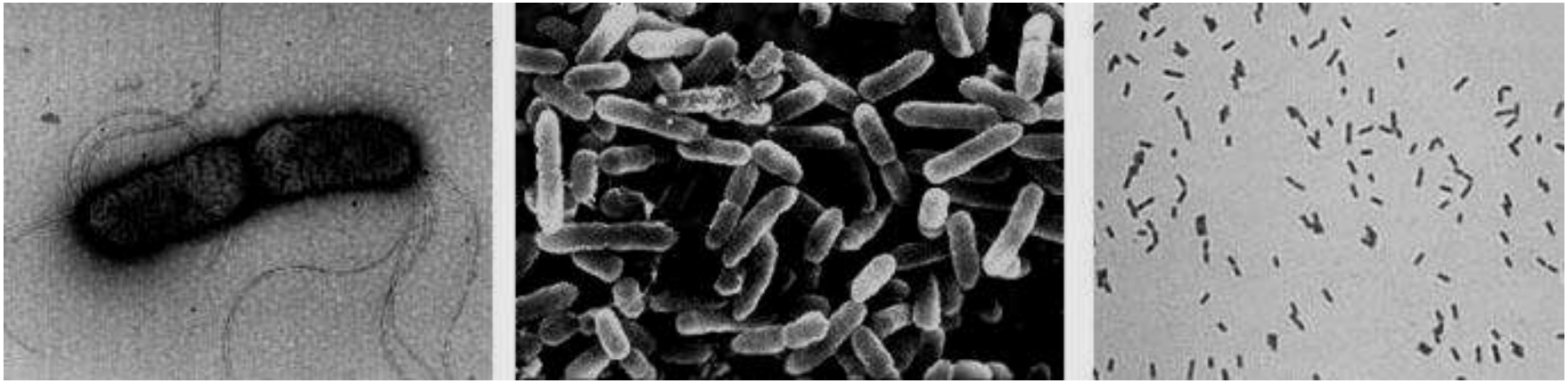
Opportunistic species

- *Burkholderia pseudomallei* (P. pseudomallei) – zoonosis
- *Burkholderia cepacia* - pyoinflammatory infections

Main characteristics of Pseudomonas aeruginosa

- Gram-negative motile rods (have polar flagella),
- grows rapidly on ordinary culture media,
- utilises glucose only in the aerated conditions of growing,
- bacterial culture produces slight aromatic sweetish odour,
- it produces water-soluble bluish-green pigment that diffuses through the medium (***pyocyanin***),
- colonies are smooth round with a fluorescent greenish colour,
- produces β-hemolysis when grown on blood-containing media,
- high proteolytic activity,
- oxidase +.

Pseudomonas aeruginosa: morphology



Infections caused by Pseudomonas aeruginosa

- It is a pathogen only when introduced into areas devoid of normal defences or when it presents in mixed infections.
- Produces infections of:

  - wounds (and burns) giving rise to blue-green pus,
  - meningitis,
  - urinary tract infections when introduce by catheters,
  - pulmonary cystic fibrosis,
  - in infants and debilitated persons may invade the blood-stream resulted in fatal sepsis.

Pseudomonas aeruginosa: factors of pathogenicity

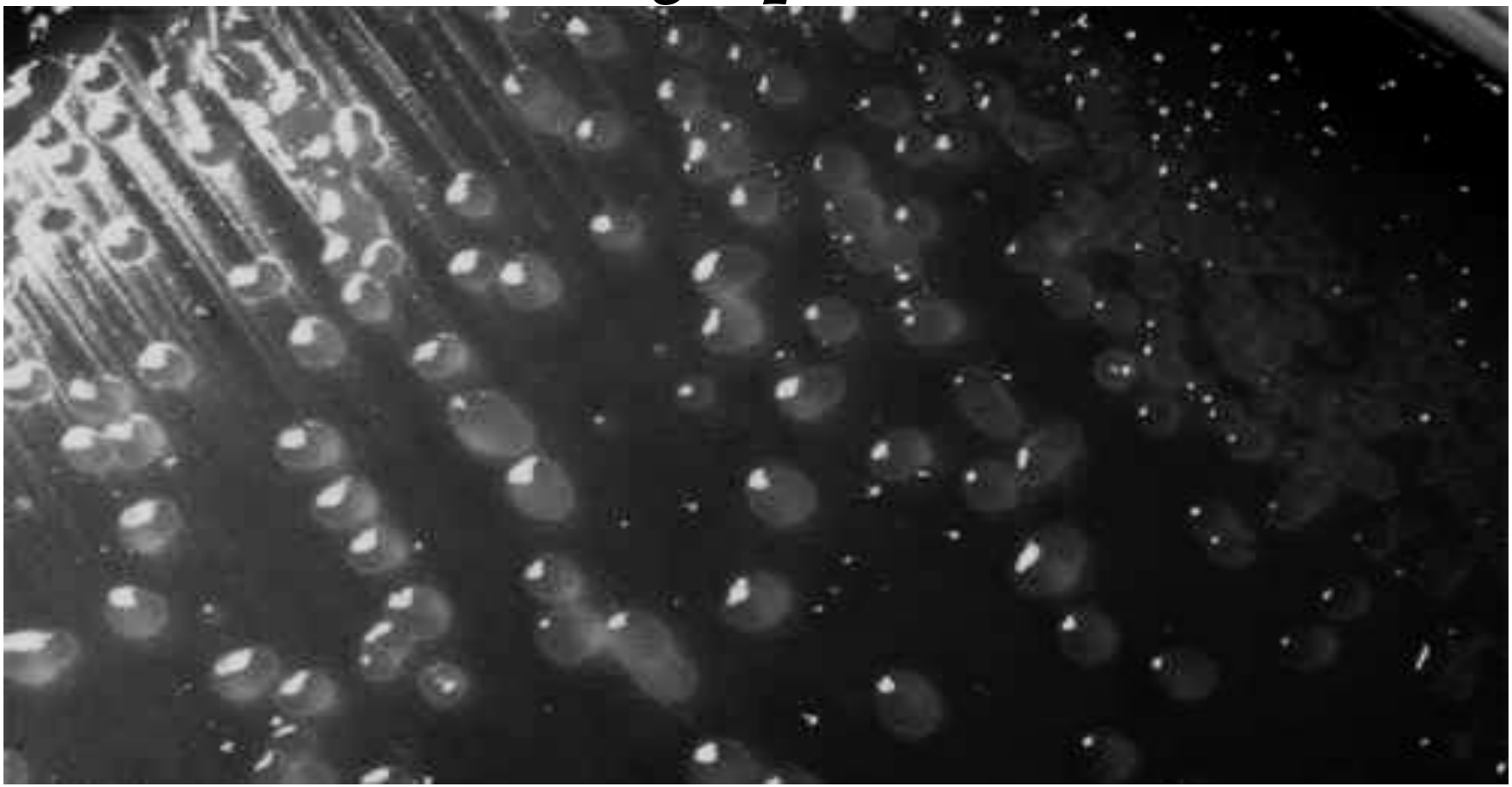
- **Fimbriae** – participate in adhesion.
- **Exotoxin A**_ – cytotoxin.
- **Mucoid capsule-like substances** – defense against phagocytosis.
- **Endotoxin**.
- **Virulence enzymes** (neuraminidase, protease and collagenase).

Pseudomonas aeruginosa: laboratory diagnostics

Includes isolation of pure culture and its identification by:

- growing characteristics,
- biochemical and serological properties.

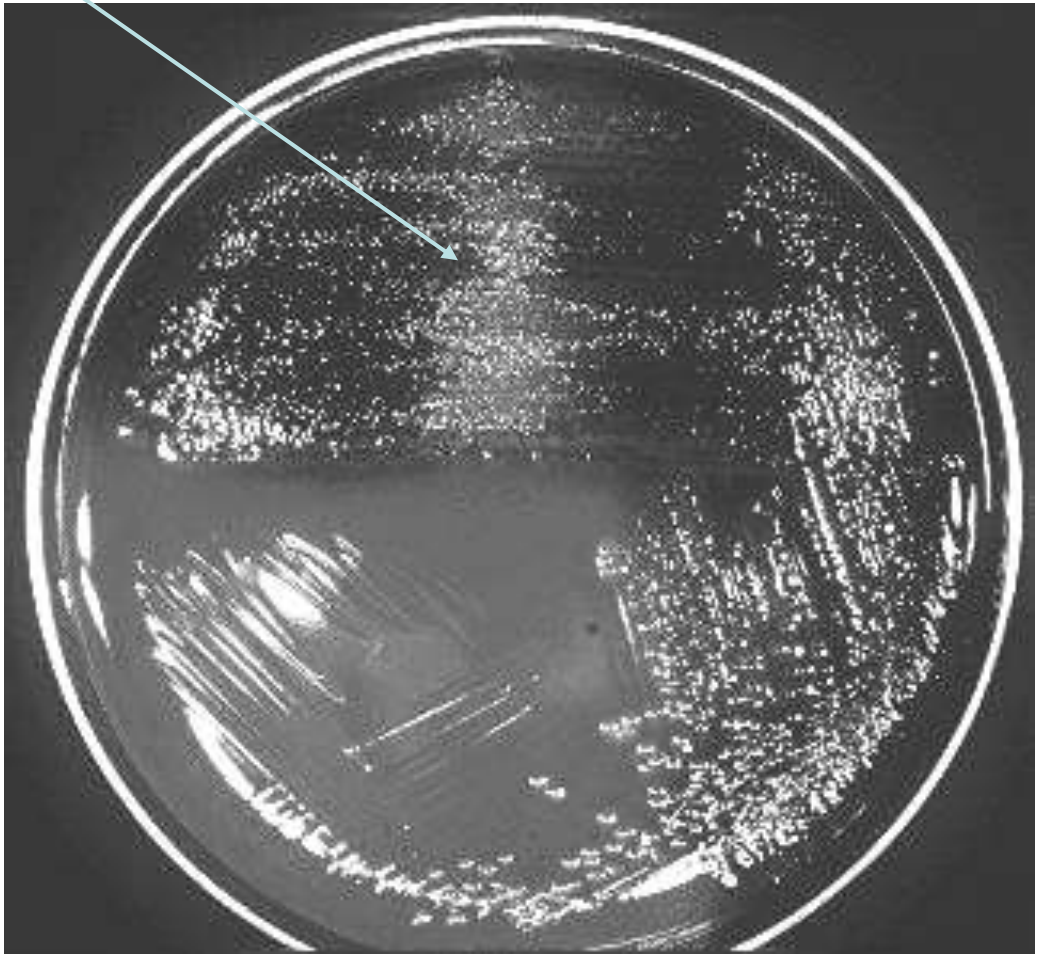
Mucous colonies formed by Pseudomonas on the agar plate



Pseudomonas aeruginosa: resistance

- It is highly resistant to unfavorable conditions of the environment.
- It could live in wound secretions for a long time.
- It is resistant to most antimicrobial agents and therefore becomes dominant when more susceptible bacteria of the normal flora are suppressed.
- Frequently it causes nosocaminal (hospital) infections. Resistant strains can cause contamination of:
  - antimicrobial preparations
  - antiseptic solutions
  - disinfectants

β-hemolysis produced by P.aeruginosa on the blood agar



Pseudomonas aeruginosa: prophylaxis and therapy

- **Vaccine** (pyoimmunogen) administrated to high-risk patients and provides protection against sepsis.
- **Antibiotics** (aminoglycosides, chloramphenicol) – are most commonly effective against P.aeruginosa.

CAMPYLOBACTER AND HELICOBACTER

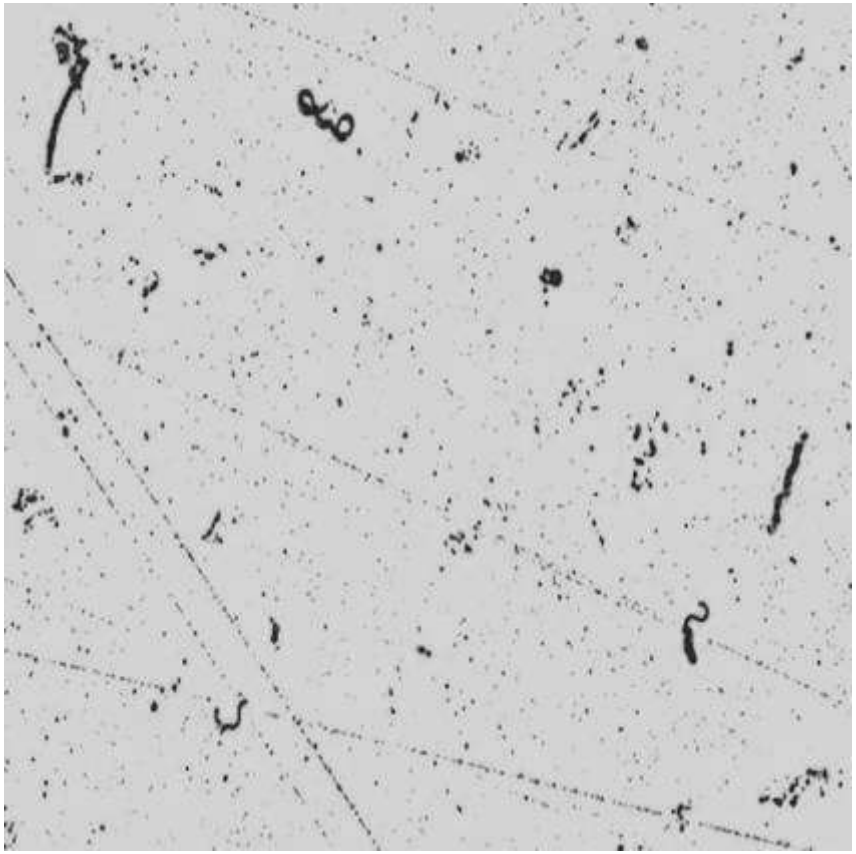
Campylobacter: classification and main characteristics

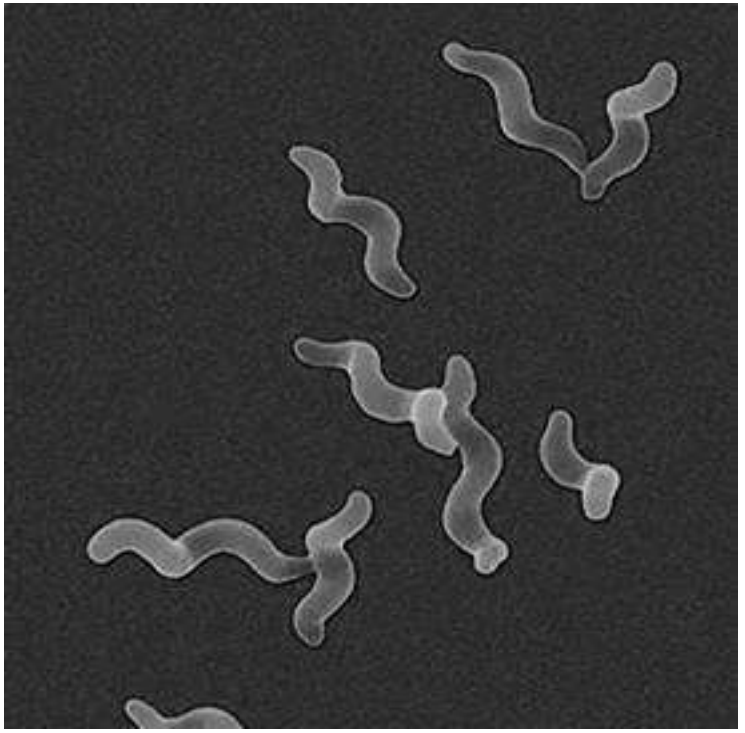
Campylobacter – main species

- C. jejuni - the most common of the *Campylobacter* causing human disease
- C. fetus
- C. coli

- ✓ gram-negative rods
- ✓ curved (resemble the letter S or primitive drawing of flying bird – gull winged shape)
- ✓ motile (single, polar flagellum)
- ✓ could be grown on the complex nutritive media (chocolate agar), grows best at 42 ^{o} C. The organism is *microaerophilic* and require the increase concentration of CO_2
- ✓ possesses factors of pathogenicity: *adhesins, cytotoxins* and *enterotoxin*
- ✓ the organism infects the intestinal tract of several animal species (including cattle and sheep), transmitted to man in milk and meat products and causes **intestinal infections** (colitis), **extraintestinal infections** (sepsis, meningitis and oral cavity infections) mostly in immune compromised persons

Campylobacter: morphology





Campylobacter: laboratory diagnostics

- Specimens (faeces) usually are grown on *chocolate or Muller-Hinton agar* in the atmosphere containing CO_2 (microaerophilic conditions).
- The next step is: isolation of pure culture that is identified using morphological (microscopic investigation of stained smears), biochemical and serological properties.
- Express-diagnostics: revealing of Ag in faeces applying IFR.
- Polymerase chain reaction (PCR) - based techniques have been developed for rapid detection and for typing of *C jejuni* strains.

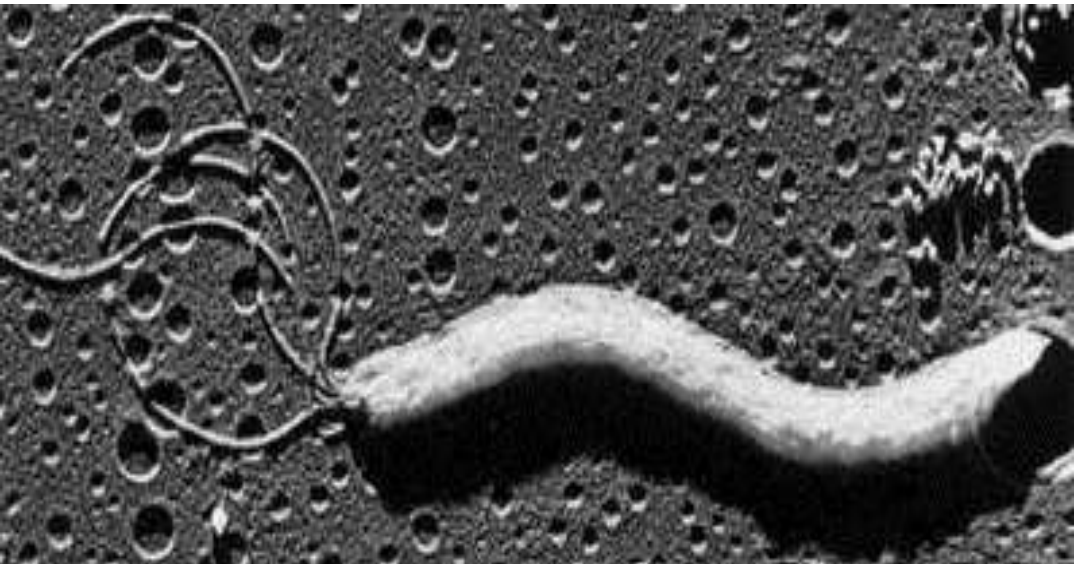
Helicobacter pylori: main characteristics

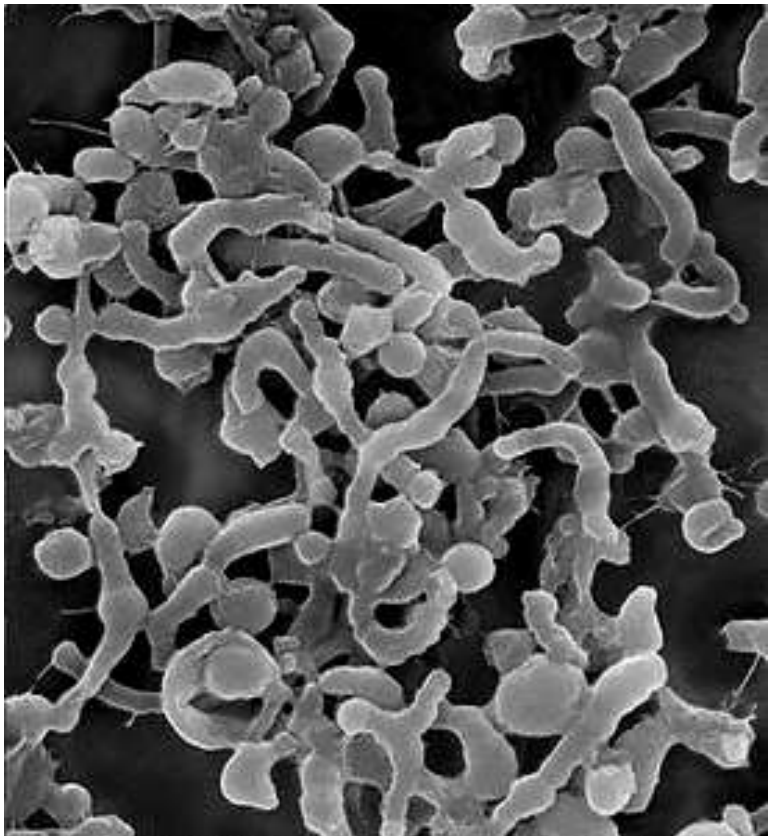
Very similar with Campylobacter but reveals some differences :

- ✓ possesses *lophotrichous* flagellation
- ✓ causes stomach and duodenum inflammatory disease leading to development of *gastritis* and *duodenitis*
- ✓ has been accepted in the last few years as the major cause of *stomach* and *duodenum ulcer disease*
- ✓ *produces a urease* which generates ammonia and carbon dioxide

H. pylori infection is important in causing gastric carcinoma and lymphoma

Helicobacter pylori: morphology





Helicobacter pylori: pathogenesis

- The bacteria are present on the luminal surface of mucus-secreting cells and within gastric pits but the microorganism does not invade tissue.
- Production of urease : ammonia, produced by urease, is known to be toxic to eukaryotic cells.
- Production of a vacuolating cytotoxin, is also associated with injury to the gastric epithelium.

Helicobacter pylori: laboratory diagnostics

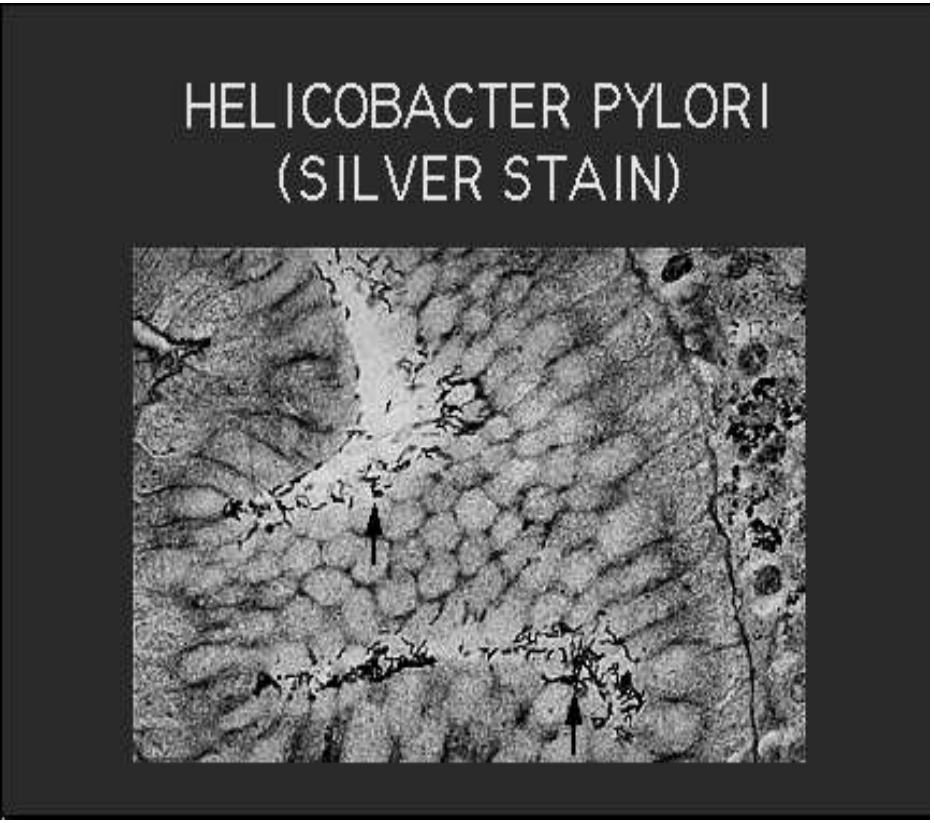
Revealed in biopsies of mucous membranes with use of the next tests:

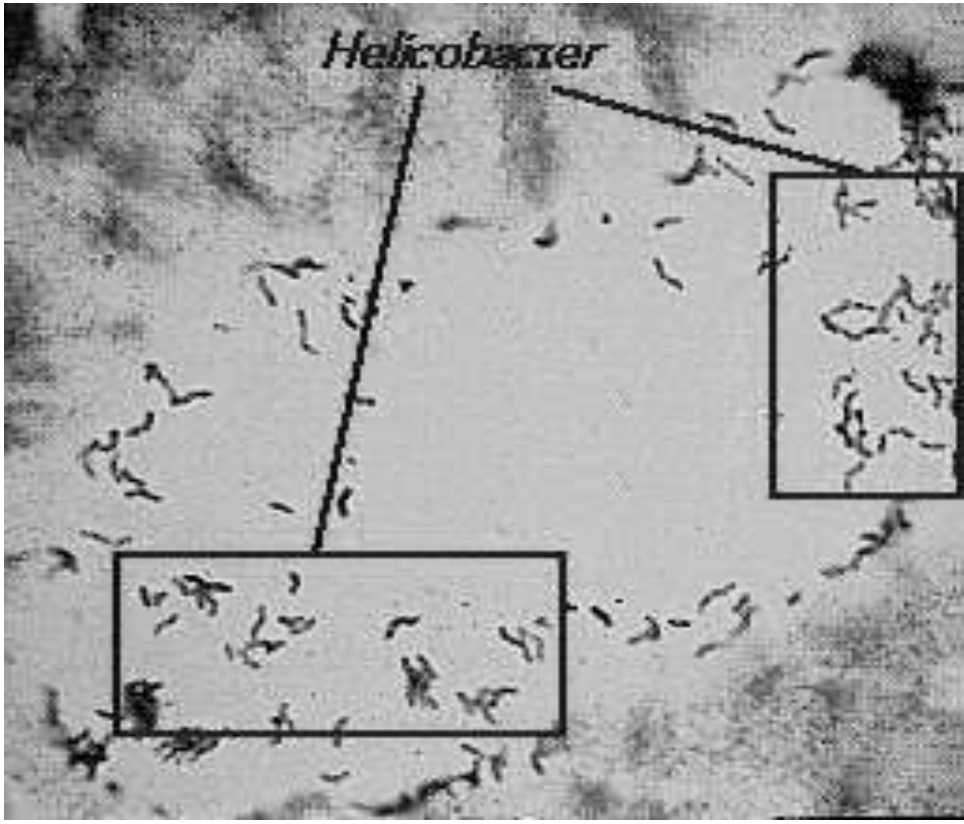
1. CLO- test (revealing of high urease activity).
2. Microscopy of stained smears.

Other methods of diagnosis:

- ✓ isolation of pure culture
- ✓ strains may be differentiated by genotypic methods including polymerase chain reaction (PCR)
- ✓ non-invasive techniques such as the urea breath test.

Helicobacter pylori: morphology





Vibrio. Brucella. Francisella. Yersinia pestis. Bacillus.

Theme N21

Infectious agents which cause special danger infections

- **Vibrio cholerae.**
- **Brucella melitensis.**
- **Francisella tularensis.**
- **Yersinia pestis.**
- **Bacillus anthracis.**

Zoonosises

(*Zoonosis* refers to a disease primarily of animals which can be transmitted to humans as a result of direct or indirect contact with infected animal populations).

Main characteristics of special danger infections

To be characterised as an infection of special danger the infection:

- should possess the tendency to pandemic distribution
- or the infectious agent has to be extremely virulent

These characteristics suppose the existence of possibility of fatal consequences for:

- all human population
- or for individual human because of:
  - high probability to get infected
  - high probability to die from this disease

All manipulations with infectious agents and pathological material containing live bacteria should be performed only in specialised laboratories (with special safety regime).

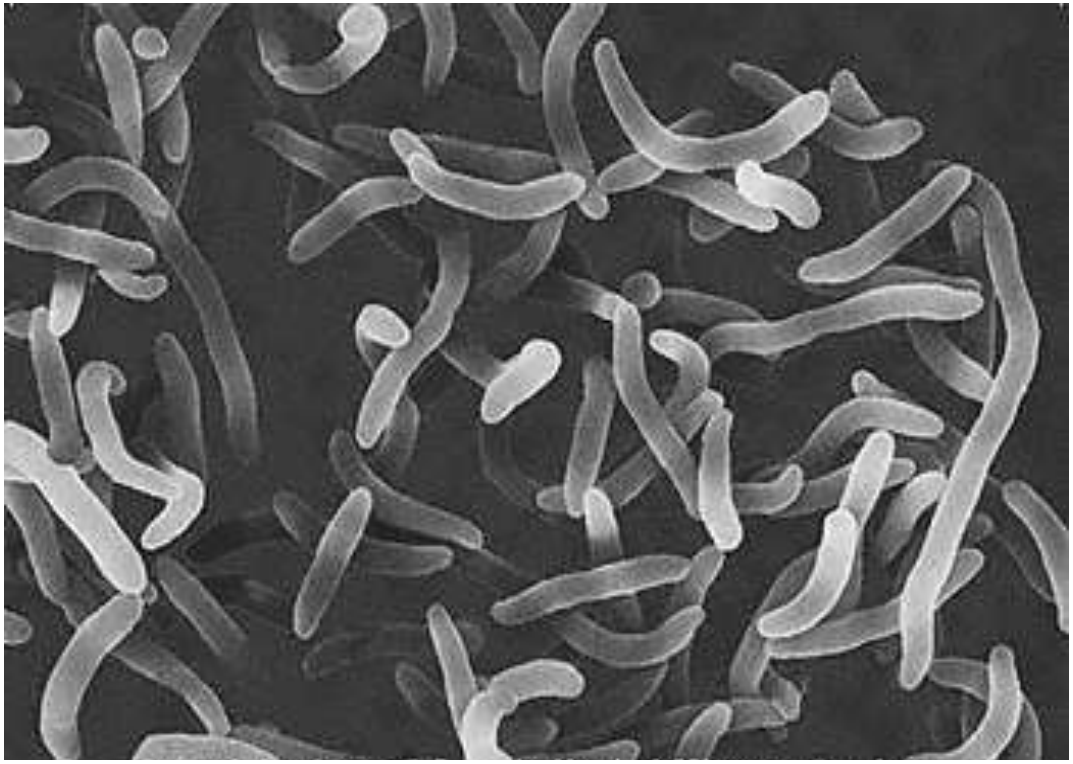
VIBRIO CHOLERAE -

infective agent that causes cholera in human – disease which was endemic for India and Southeast Asia but since 1960 has spread widely over the world

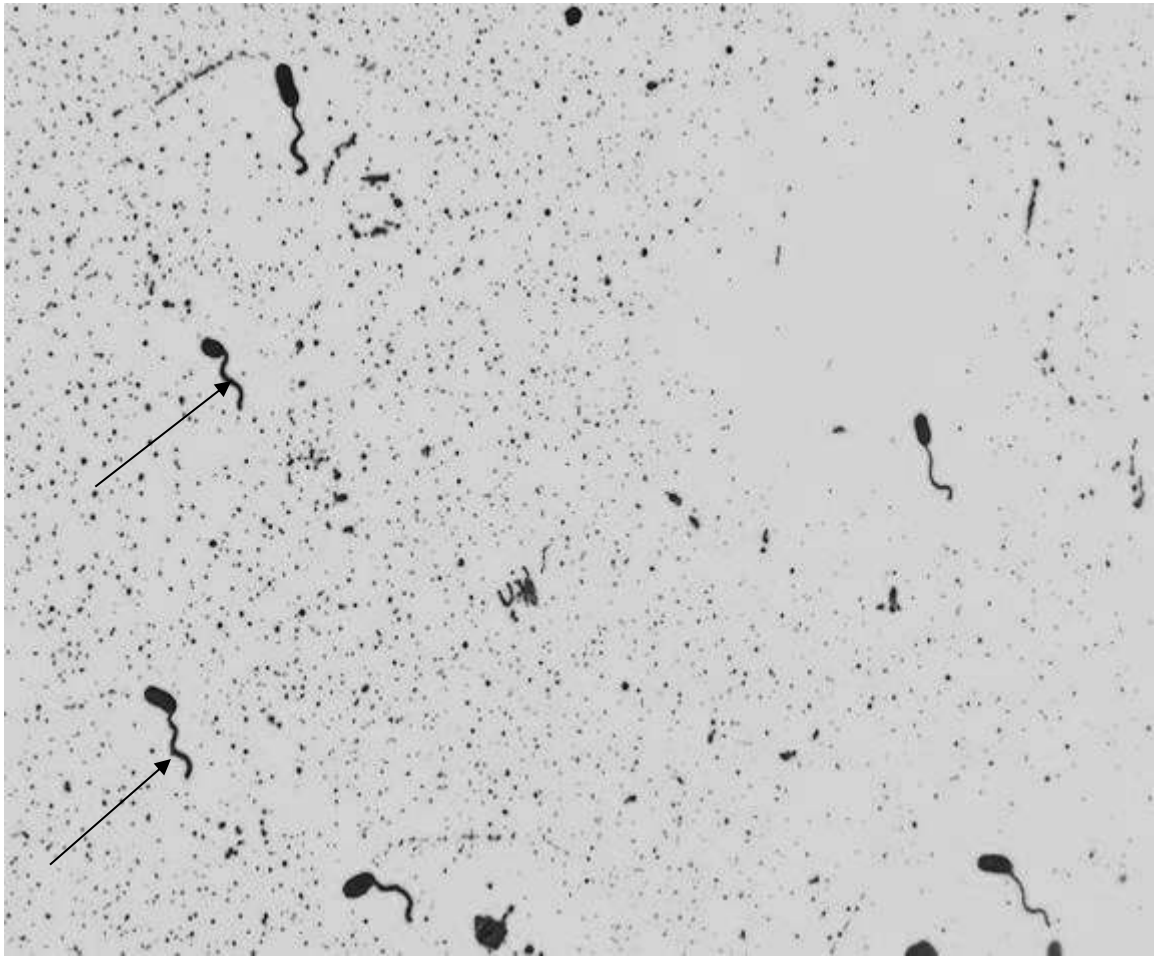
Main characteristics of Vibrio cholerae

- ✓ gram-negative rod
- ✓ actively motile (possesses a single polar flagellum - monotrichous)
- ✓ curved (comma-shaped)
- ✓ grows well on such nutritive media as thiosulfate-citrate-bile sucrose (TCBS) agar. Produces convex, smooth, round yellow colonies. When inoculated into alkaline peptone liquid medium it produces delicate pellicle on the surface after 6-8 hrs of incubation
- ✓ when grown in peptone medium containing tryptophan and nitrate it produces indole and reduces nitrate: upon addition of sulfuric acid a red colour develops (nitroso-indole reaction, “cholera red test”)
- ✓ two biological variants of V. cholerae are known (var. cholera and var. El Tor) as causative agents of cholera.

Vibrio cholerae – curved rods



Vibrio cholerae - Leifson flagella stain (digitally colorized)



Vibrio cholerae: factors of pathogenicity

- ✓ produces **enterotoxin** (choleragen)
- ✓ produces **mucinase** (the enzyme liquefies mucus which covers intestinal epithelial cells)
- ✓ produces **neuraminidase**
- ✓ produces **endotoxin**
- ✓ var. El Tor – produces soluble hemolysins

Vibrio cholerae: pathogenesis and pathology

Human

⇓

fecal-oral mechanism of spreading of V. cholerae (from feces of infected persons it contaminates water, food)

⇓

vibrios coming through acidic barrier in stomach into the small intestine

⇓

multiply and remain localised within intestinal tract (not an invasive infection)

⇓

liberate cholera toxin which binds to the receptors on villi of the small intestine

⇓

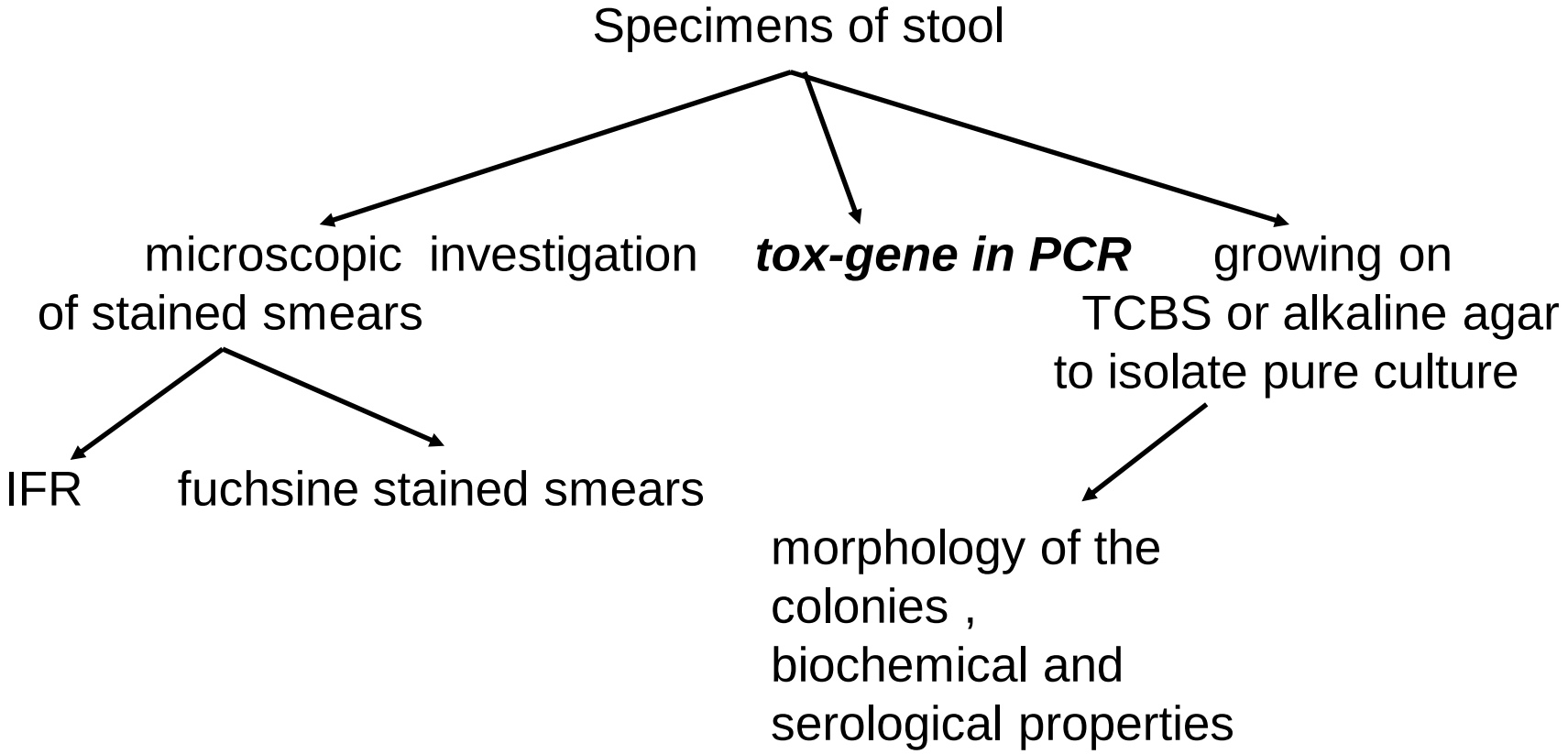
toxin stimulates marked increase in adenylate cyclase activity and cAMP concentration in small intestine

⇓

hyper secretion of water and chloride into the lumen and impaired absorption of sodium in all parts of the small intestine resulting in:

- ✓ massive diarrhoea
- ✓ dehydration (fluid loss up to 20 litres daily)
- ✓ acidosis
- ✓ shock and death

Cholera: laboratory diagnostics (permitted only in specialised laboratories)



Cholera: specific prophylaxis

It is not effective as an epidemic control measure. Provides limited protection to heavily exposed persons (in case of repeated injections). Commonly uses:

1. Killed vaccine
2. Combined vaccine which contains:
   - cholera anatoxin
   - extracted lipopolysaccharides (O-antigens)

Cholera: nonspecific prophylaxis

- Improvement of sanitation, particularly food and water
- Isolation of patients
- Disinfection of surroundings and excreta of ill patients
- Chemoprophylaxis with antimicrobial drugs

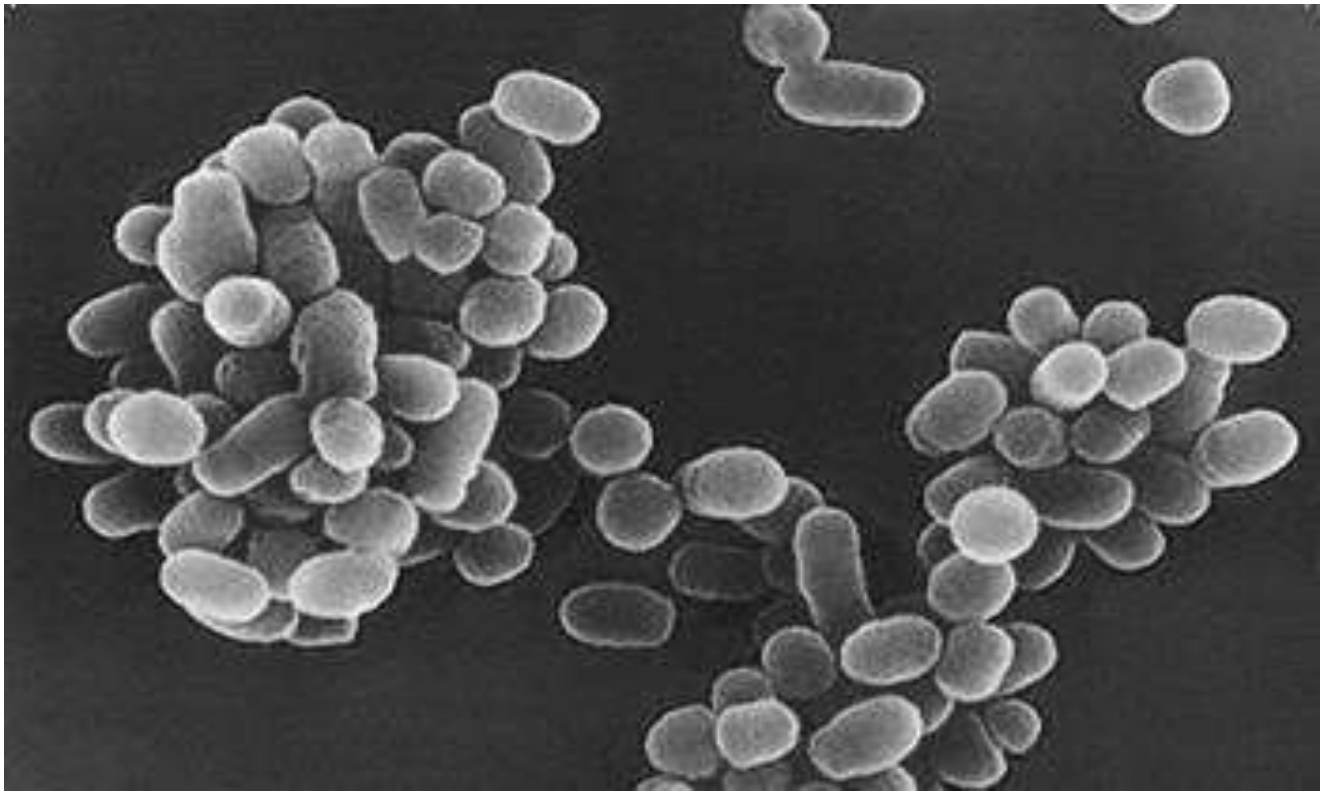
Cholera: treatment

1. The most important is water and electrolyte replacement to correct the severe dehydration and salt depletion
2. Many antimicrobial agents are effective against V. cholerae. Oral tetracycline tends to reduce stool output and shortens the period of excretion of vibrios.

The brucellae: classification and main characteristics

- Brucella
  - B. melitensis (typically infects goats) and it is the cause of most severe prolonged recurring disease in humans.
  - B. abortus (infects cattle)
  - B. suis (infects swine)
- Small coccobacilli
  - gram-negative
  - nonmotile
- Grow:
  - on enriched media (nutritional requirements are complex)
  - slowly
  - B. abortus – requires increased concentrations of CO_2

Brucella abortus - causes bovine spontaneous abortion



Brucellosis – main characteristics of the disease

- The brucellae infects wide range of animals (zoonotic infection) and all species are infective for humans
- Transmitted by the next ways:
  - alimentary (unpasteurised milk and milk products)
  - contact (contacts of farmers, veterinarians etc with infected animals)
- ***Infected humans are not infective for others.***

Brucellosis: pathogenesis and pathology

penetration through mucous membranes of intestinal tract and skin (contact way)

↓

getting via lymphatic channels into regional lymph nodes – lymphatic dissemination of bacteria

↓

getting into the bloodstream

↓

reticuloendothelial system (infections of liver, spleen, bone marrow) → Can exist intracellulary for a long time (chronic brucellosis)

↓

bloodstream

- intoxication (endotoxin)
- sensitasation (symptoms usually appear under the secondary contact with brucellae)

Brucellosis: laboratory diagnostics

specimens of blood, urea, biopsy of bone marrow

↓

isolation of pure culture

↓

identification:

- biochemical and serological properties

serum

↓

Ig

- **Agglutination tests**
- **Opsono-phagocytic test**
- **Coombs antiglobulin method**

brucellergen

↓

sensitisation

(Burne skin test)

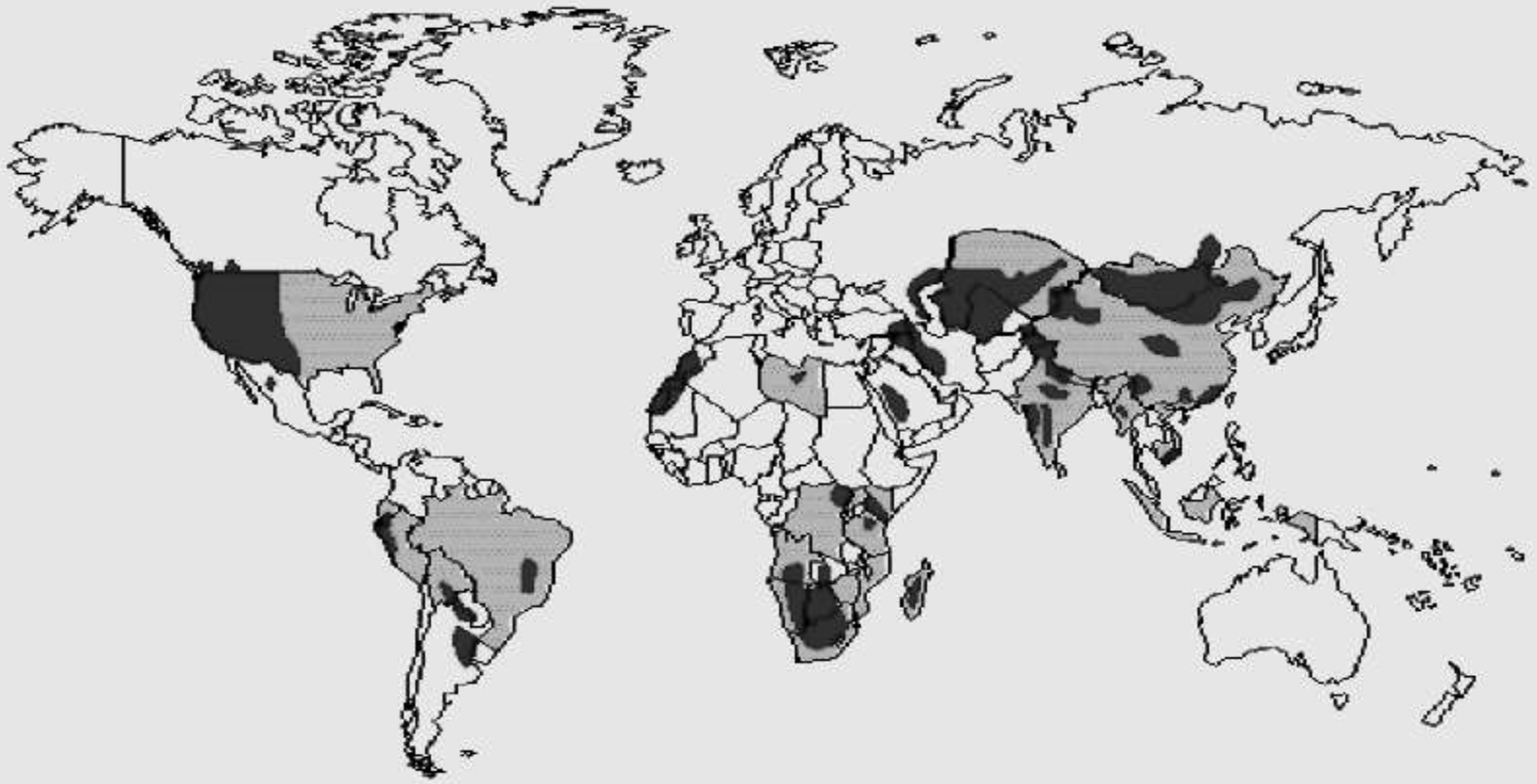
Brucellosis: specific prophylaxis

- Active immunisation by *avirulent live strain* of brucellae.

Arnold Boecklin: The Plague 1898.
Tempera on wood, Kunstmuseum, Basel

- Plague is caused by *Yersinia pestis* and the disease is known in the middle ages as the black death. This is because it frequently leads to blackening of various parts of the body. Capillary fragility results in hemorrhages in the skin which result in black patches.

World Distribution of Plague, 1998



Countries reported plague, 1970-1998.

Regions where plague occurs in animals.

Yersinia pestis: main characteristics

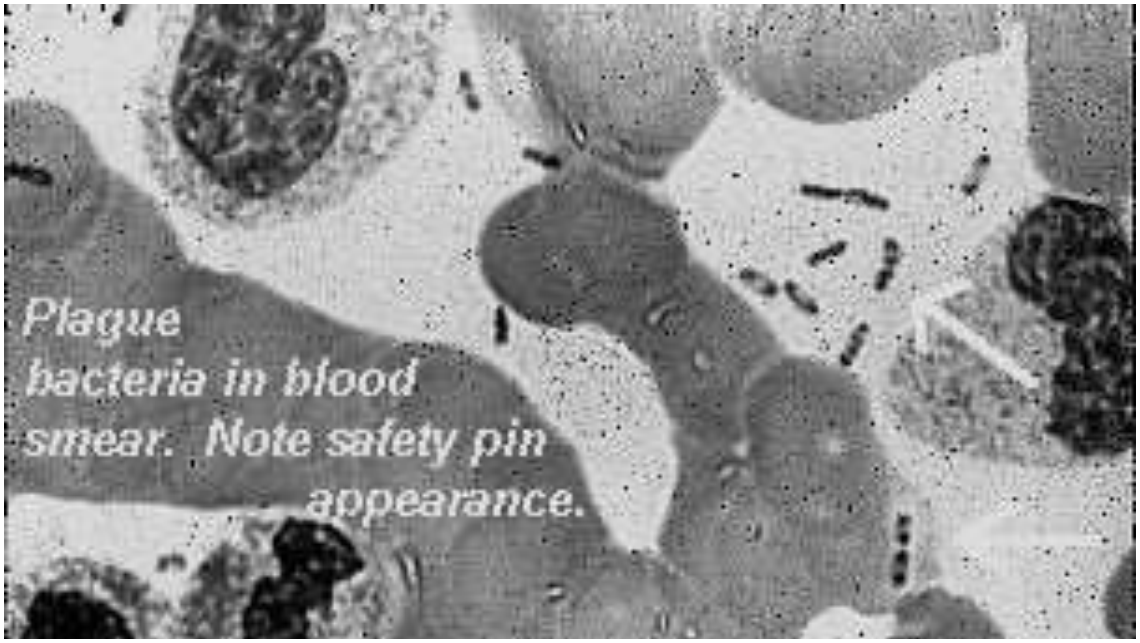
Yersiniae are rods:

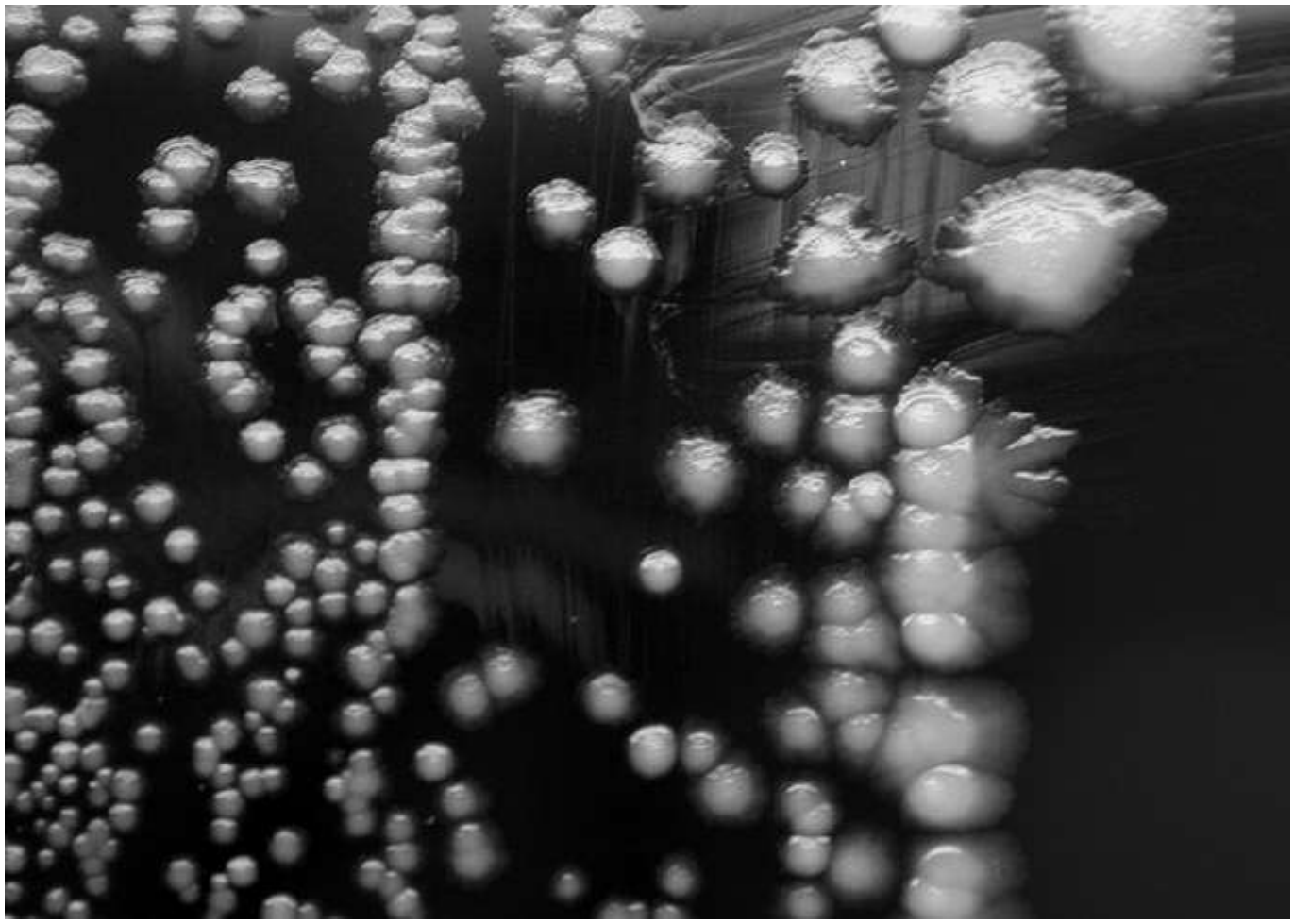
- small
- ovoid coccobacilli
- gram-negative
- show bipolar staining (when stained by methylene blue)

Grows:

- on ordinary nutritive media
- produces R-shaped colonies
- when grown in broth it produces pellicle on the surface with thread-like growth resembling stalactites and a flocculent precipitate

Yersinia pestis – blood smear.





Yersinia pestis after 48-72 hours – the colonies having irregular “fried egg” morphology

Plague: pathogenesis and pathology

Animals (rodents, rats, squirrels)

⇓

transmitting of bacteria through bite of fleas to humans

⇓

skin bubonic plague

⇓

spreads to the regional lymph nodes (buboes are formed)

⇓

getting into the bloodstream
(hemorrhagic plague)

⇓ ⇓

getting into the lungs | **entering parenchymatous organs**

⇓ ⇓

secondary pneumonic plague | **hemorrhagic inflammation and focal necrosis**

⇓ **the airborne route**
from ill human by respiratory discharges

⇓

lungs

⇓

pneumonia
(primary pneumonic plague)

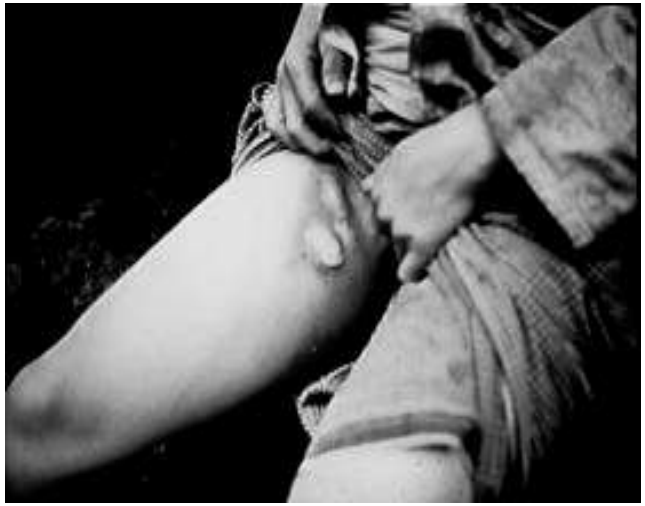
⇓

epidemic

⇓

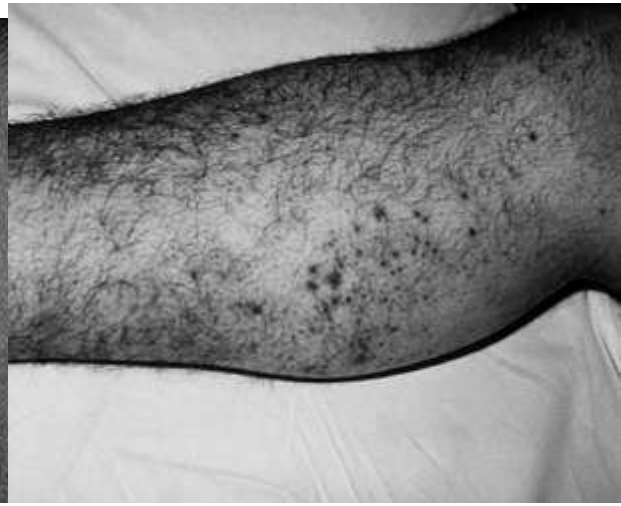
pandemic

Manifestations of a plague infection



Swollen lymph glands (buboes)

Gangrene



Capillary fragility

Plague: laboratory diagnostics

Specimens of blood, biopsy material from lymph nodes, sputum

express diagnostics

⇓

revealing of Ag (RIF)

growing on selective media

(+sodium sulfate, gentian violet)

⇓

pure culture

⇓

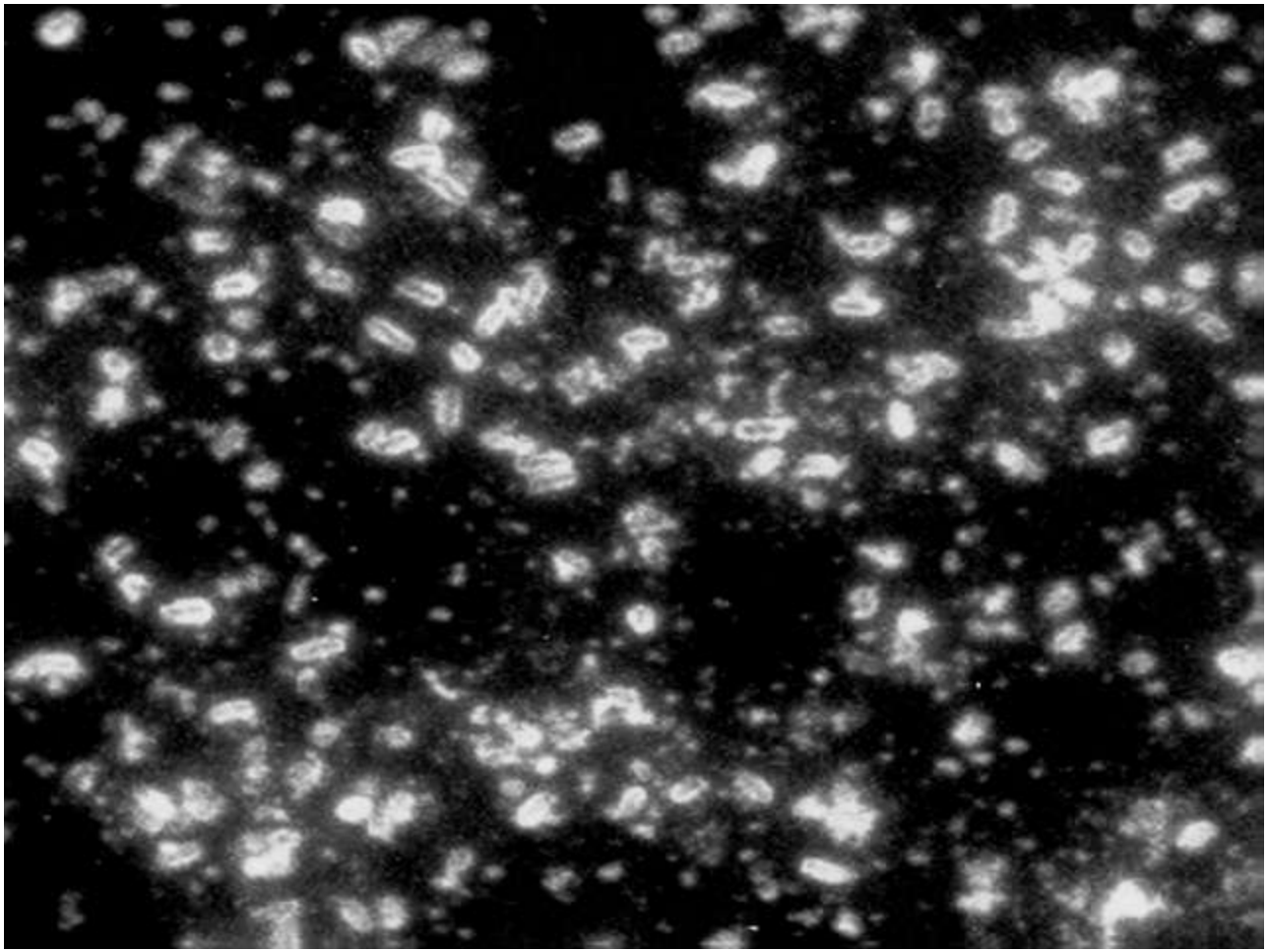
identification

- morphology
- serologic tests
- phage typing
- biological method

inoculation of guinea pig

⇓

- microscopy of the specimens obtained from lesions
- Ag detection in serologic tests



Yersinia pestis - fluorescent antibody identification

Plague: specific prophylaxis

- Vaccine prepared from avirulent live bacteria: EV

Francisella tularensis

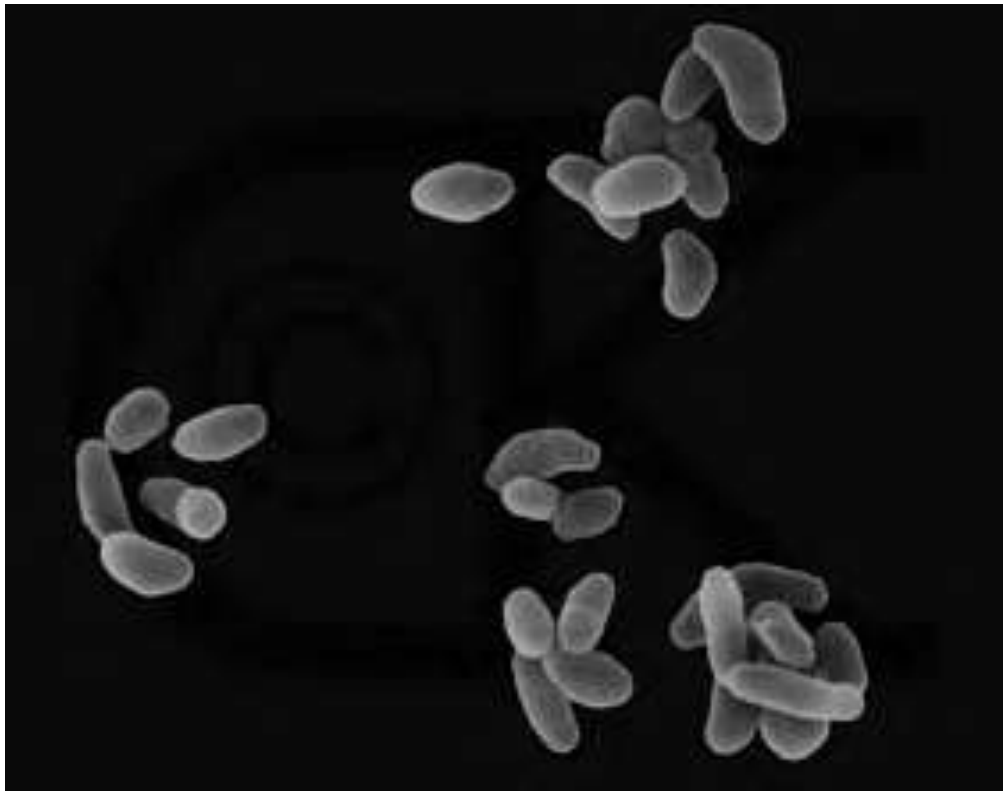
Coccobacilli

- gram-negative

Grow:

- on complex solid media containing blood, tissue extracts and cystine
- in yolk sac of chick embryo

Francisella tularensis - *coccobacilli*



Tularemia: main characteristics of the disease

Zoonotic infection occurring in wild nature. It is transmitted by the next routes:

- contact,
- alimentary,
- air-borne.

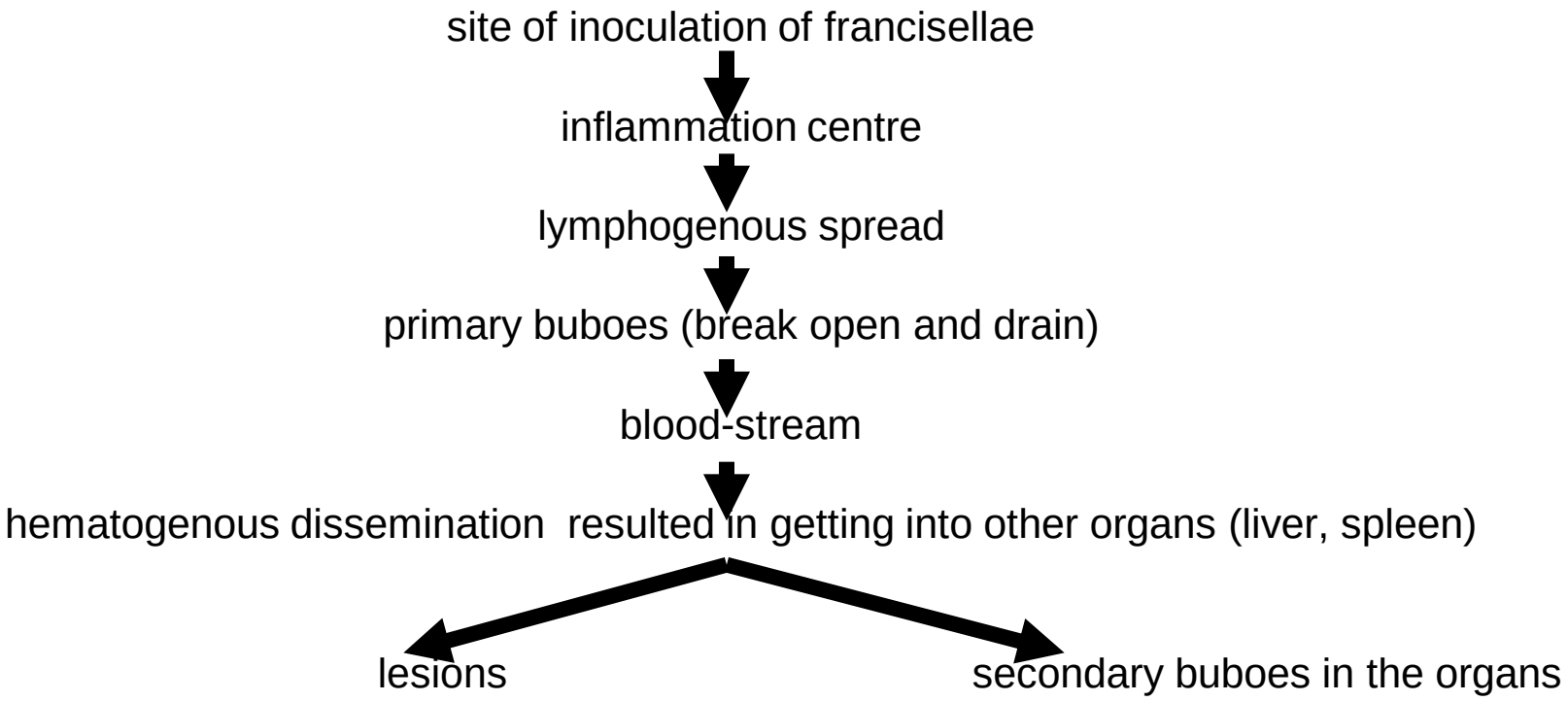
It is characterised by:

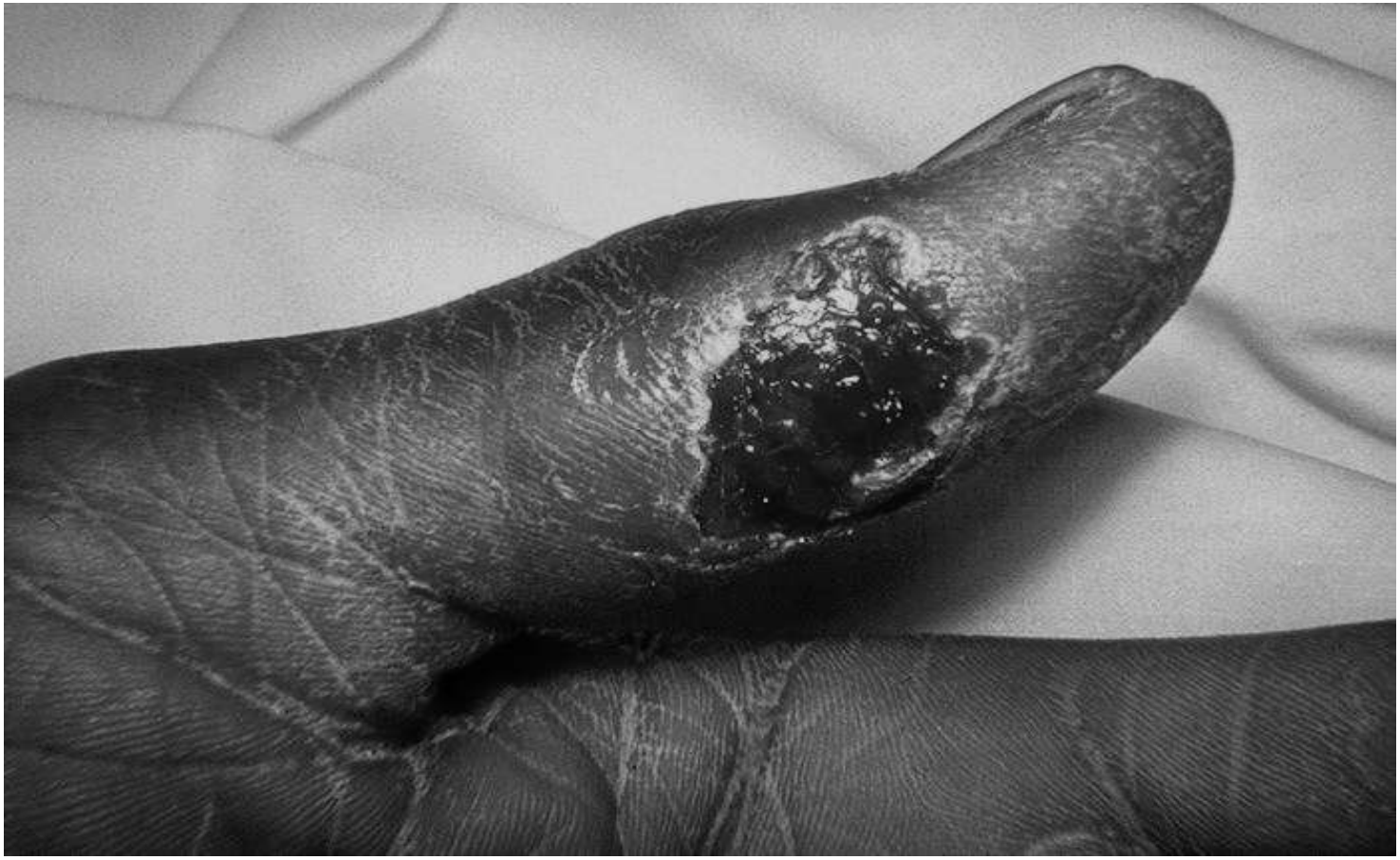
- prolongued period of disease (about a month)
- variable clinical symptoms

Several clinical forms could exist:

- Primary lesions (develop at the site of inoculation, usually in association with bites of insect)
- Pneumonic tularemia (result of hematogenous spread of bacteria to the lung or inhalation of aerosolised bacteria)
- Typhoid tularemia (is acquired by the ingestion of contaminated food or water) – the symptomatology resembles enteric fever caused by S.typhi

Tularemia: pathogenesis and pathology





Thumb with skin ulcer of tularemia

Tularemia: laboratory diagnostics

specimens (depend on the clinical form of the disease)

⇓

inoculation of guinea pig

⇓

infected organs

⇓

culturing on nutritive media containing cysteine

⇓

isolation of pure culture

⇓

identification

- **RA**
- **RIF**

serum

⇓

Ig

- RA – revealed with agglutinating antibodies
- RIF (revealed with fluorescently labelled antibodies

an extract of F.tularensis

⇓

skin test (intradermal injection)

⇓

sensitisation

(test is positive starting from the 5th day of the disease)

Tularemia: specific prophylaxis

- Vaccine prepared from avirulent live bacteria (Gajski-Elbert vaccine)

Bacillus anthracis: main characteristics

Streptobacilli:

- large, gram-positive rods
- have square ends and are arranged in long chains
- form spores and polypeptide capsules

Grow:

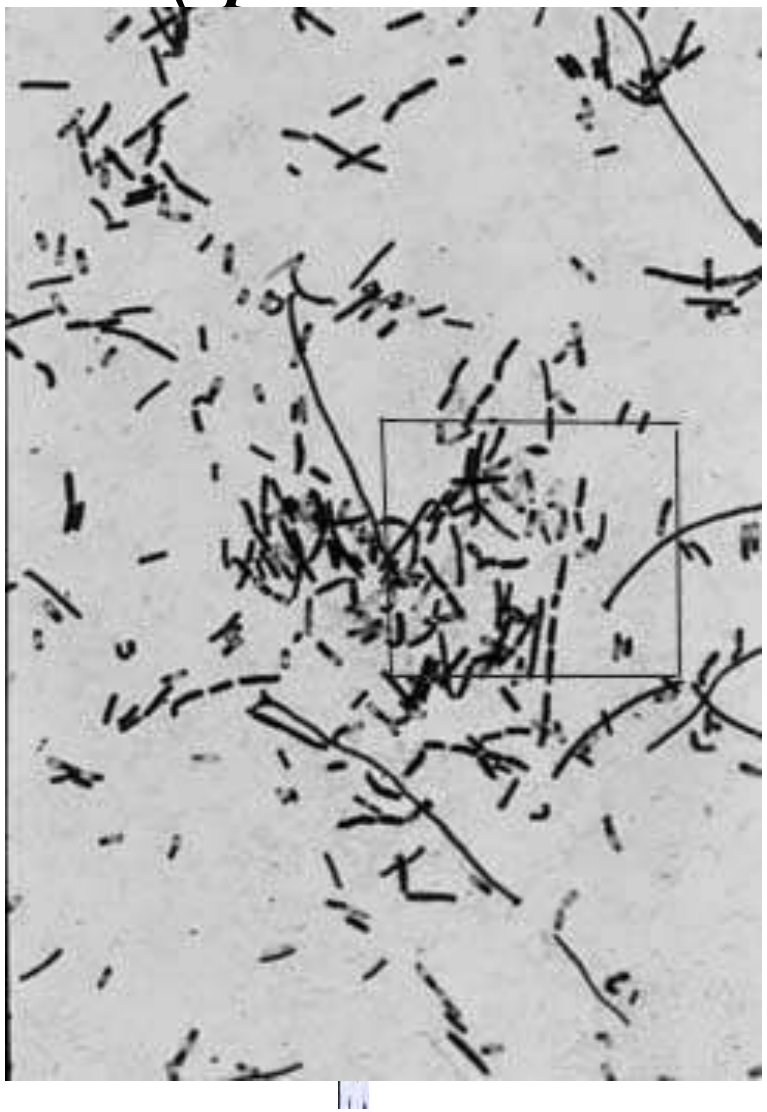
- on ordinary nutritive media
- produce rough R-shaped colonies (Medusa-head appearance colonies)
- possess anthrax toxin (cause anthrax)

Pathogenic characteristics:

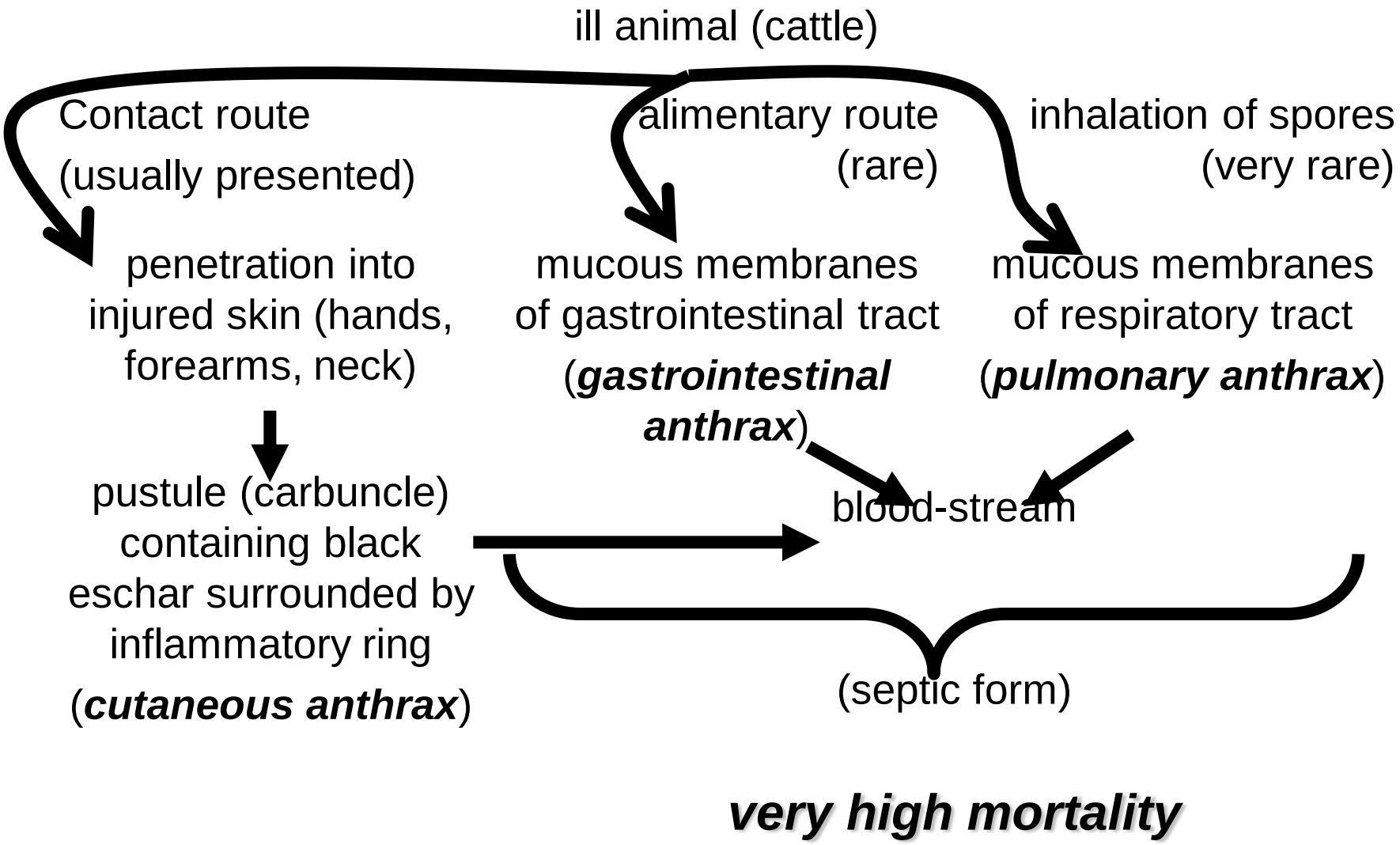
the virulence is dependent upon both factors:

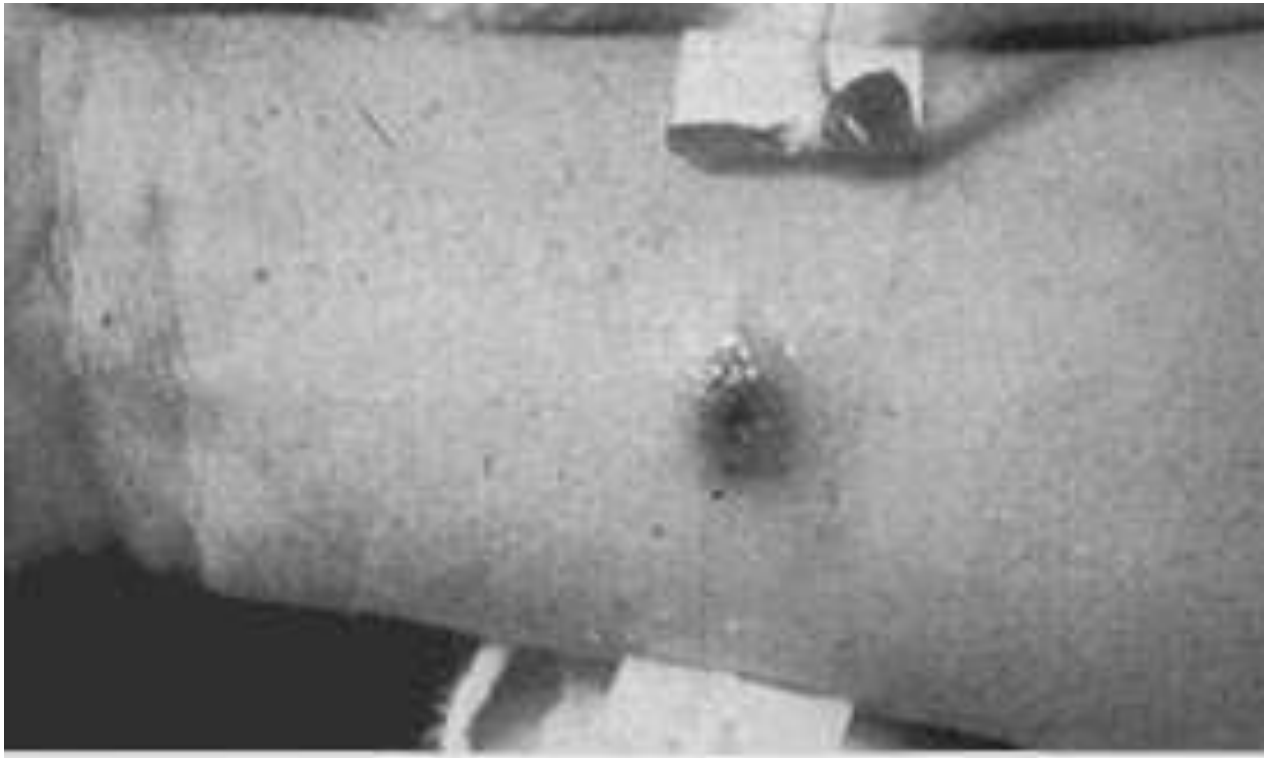
1. presence of the capsule
2. production of the toxin

Bacillus anthracis – large rods arranged in chains (spores are uncoloured oval structures)

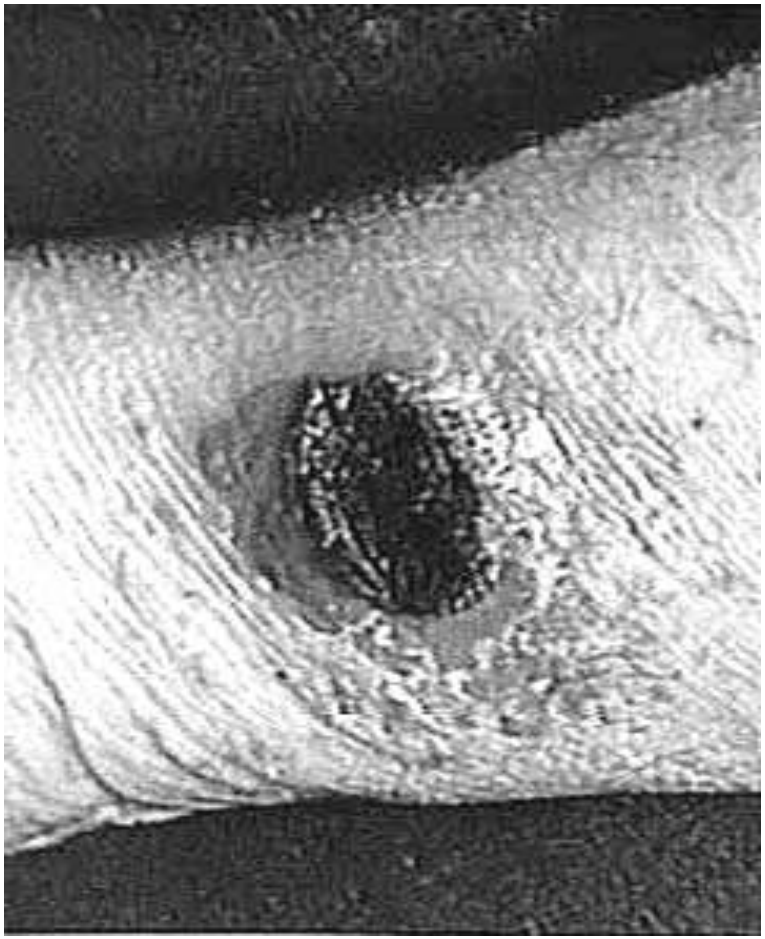


Anthrax: pathogenesis and pathology

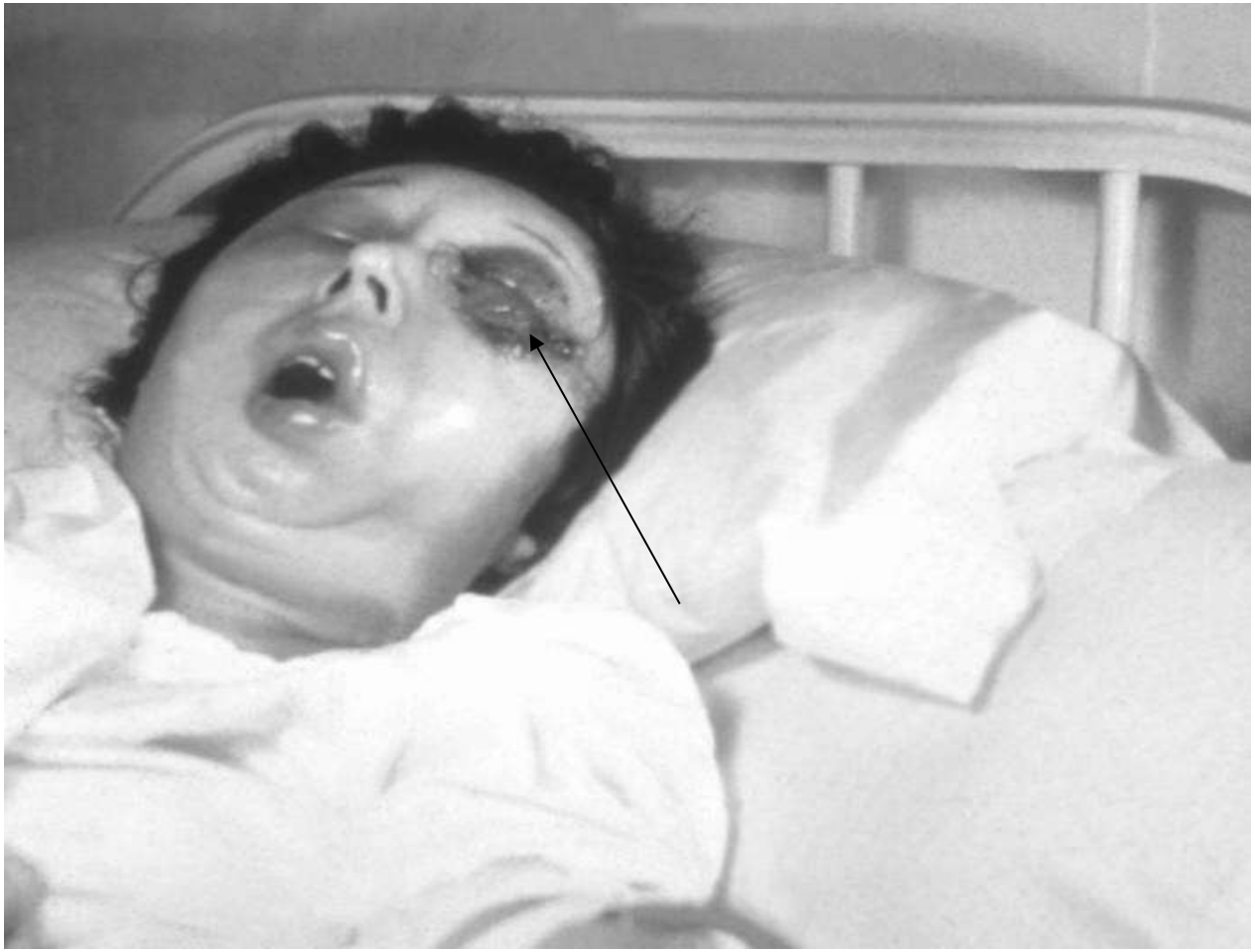




A cutaneous pustule of Anthrax on a human arm

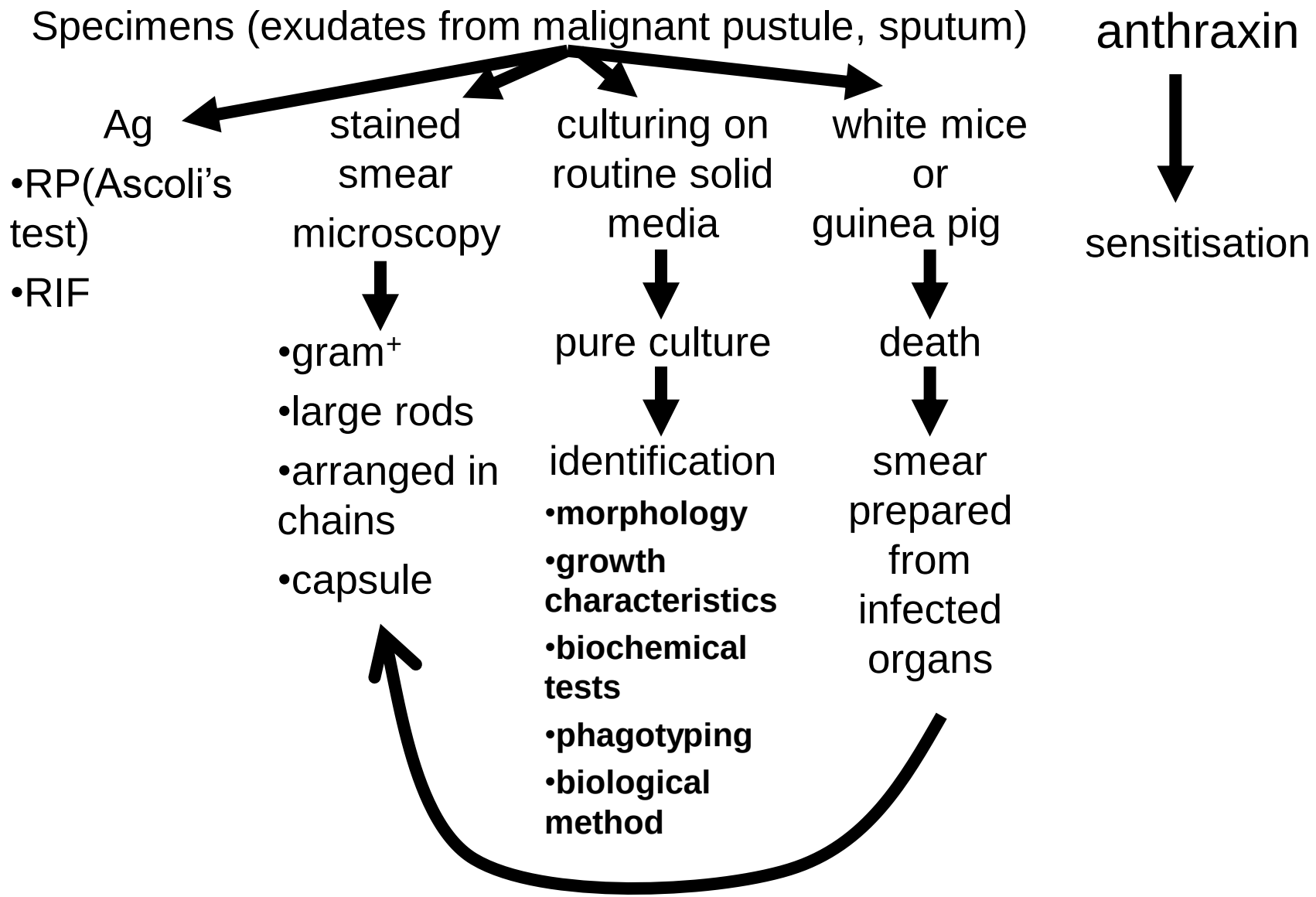


Eschar surrounded by inflammatory ring



The female patient on the 5th day of a *Bacillus anthracis* infection involving her left eye.

Anthrax: laboratory diagnostics



Anthrax: specific prophylaxis

- Living spore vaccine STI prepared from non-capsulated strain of B.anthracis (sanitary-medical technical institute)
- Specific gamma-globulin is used for urgent prophylaxis

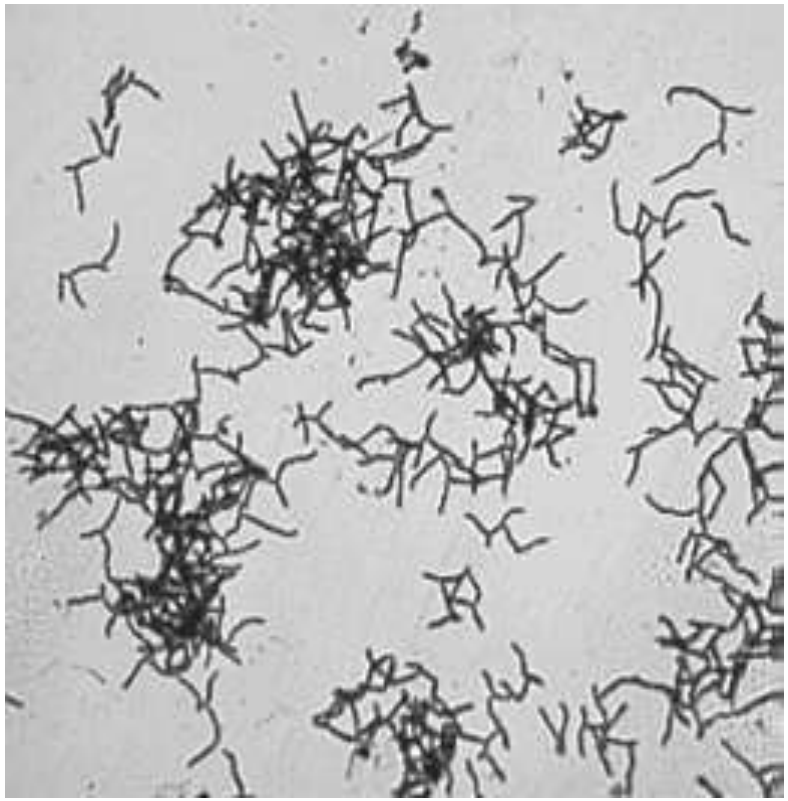
Actinomycetes. Mycobacteria. Listeria.

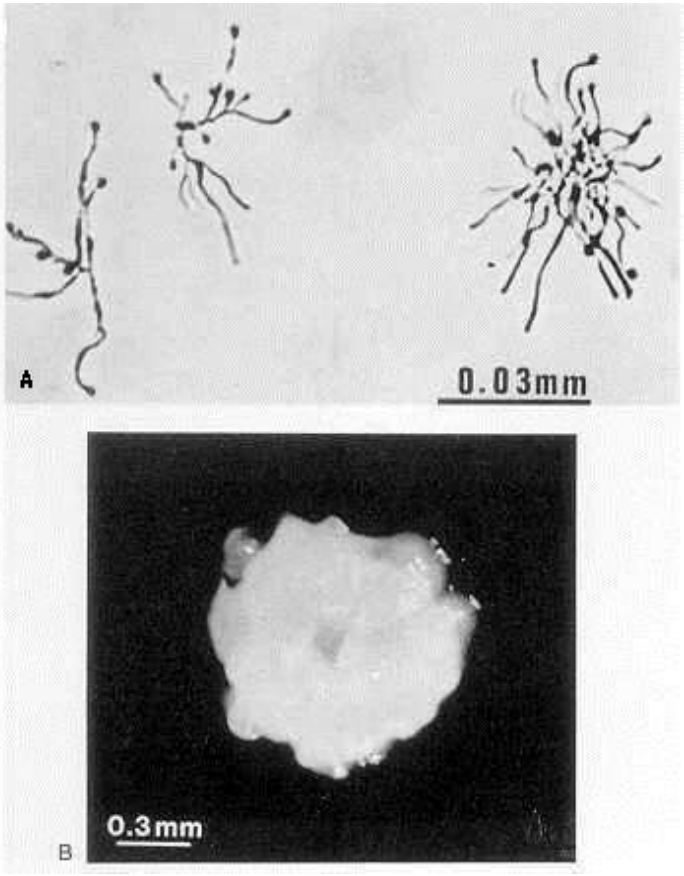
Theme N22

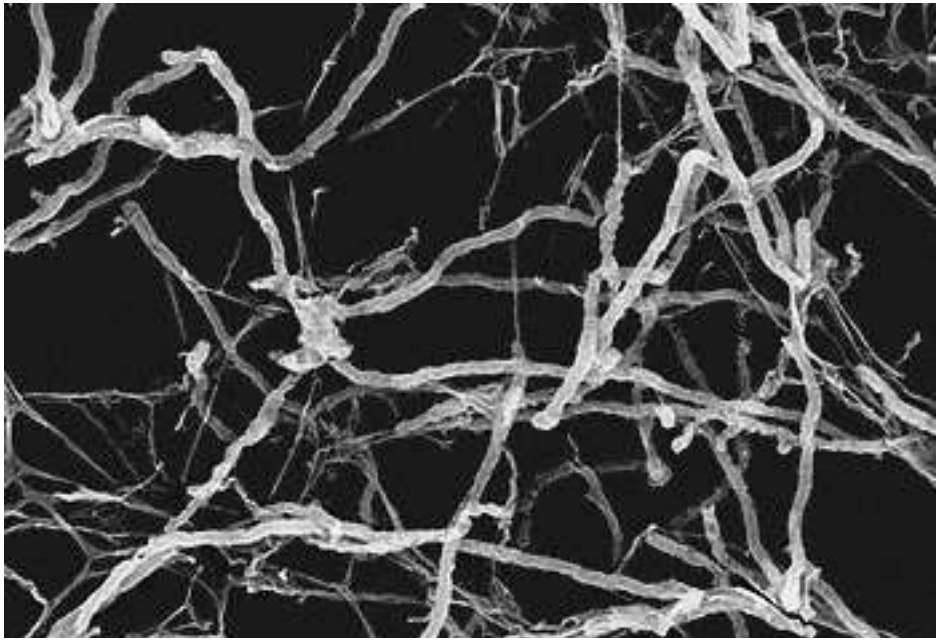
Actinomycetes : main characteristics

- Actinomyces (genus). The main pathogenic species are:
  - A. israelii
  - A. bovis
- Rods
  - gram-positive
  - filamentous (show characteristic V or Y branching) resembling fungi
  - at the ends of the rods retort-shaped bulges are found (called “clubs”)
  - in tissue Actinomyces sp. occur as “sulfur granule” that consists of a colony of gram-positive filaments surrounded by eosinophilic “clubs”.
- Grow:
  - slowly
  - only on complex media (brain-heart infusion agar)
  - produce R-shaped colonies: small and “spidery”
- ***Actinomyces israelii causes actinomycosis in human – a chronic suppurative and granulomatous disease.***

Actinomyces spp: morphology of the bacteria and the colonies produced by them

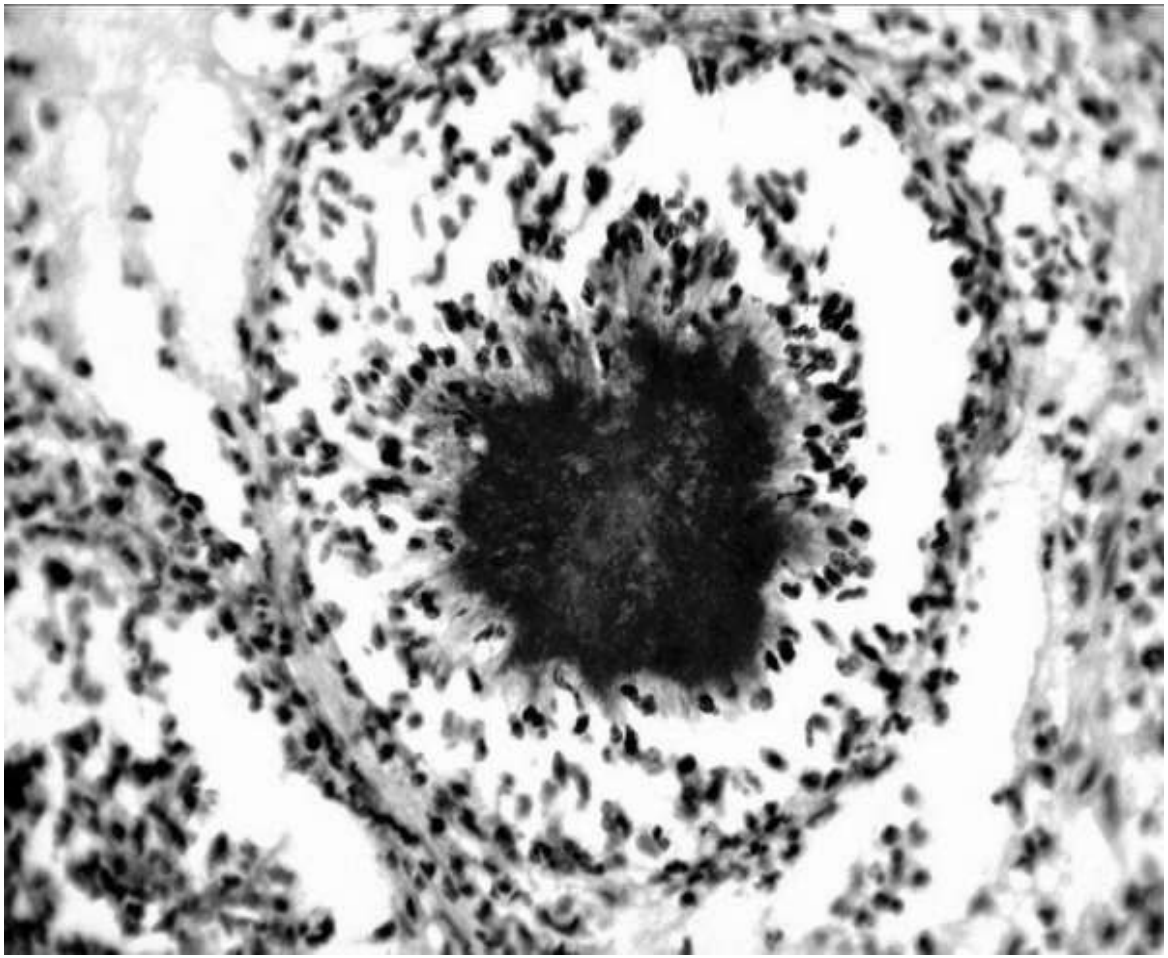




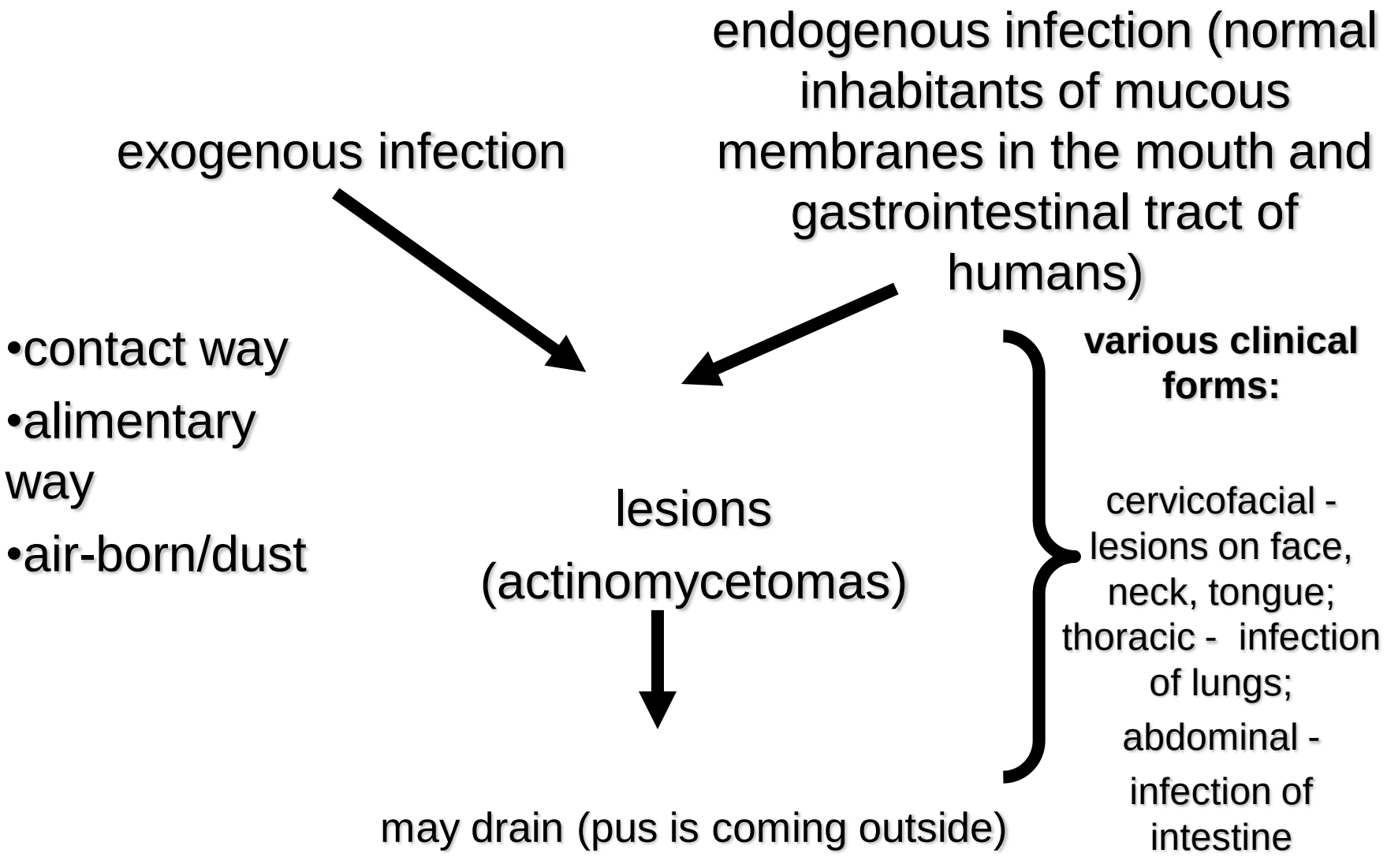


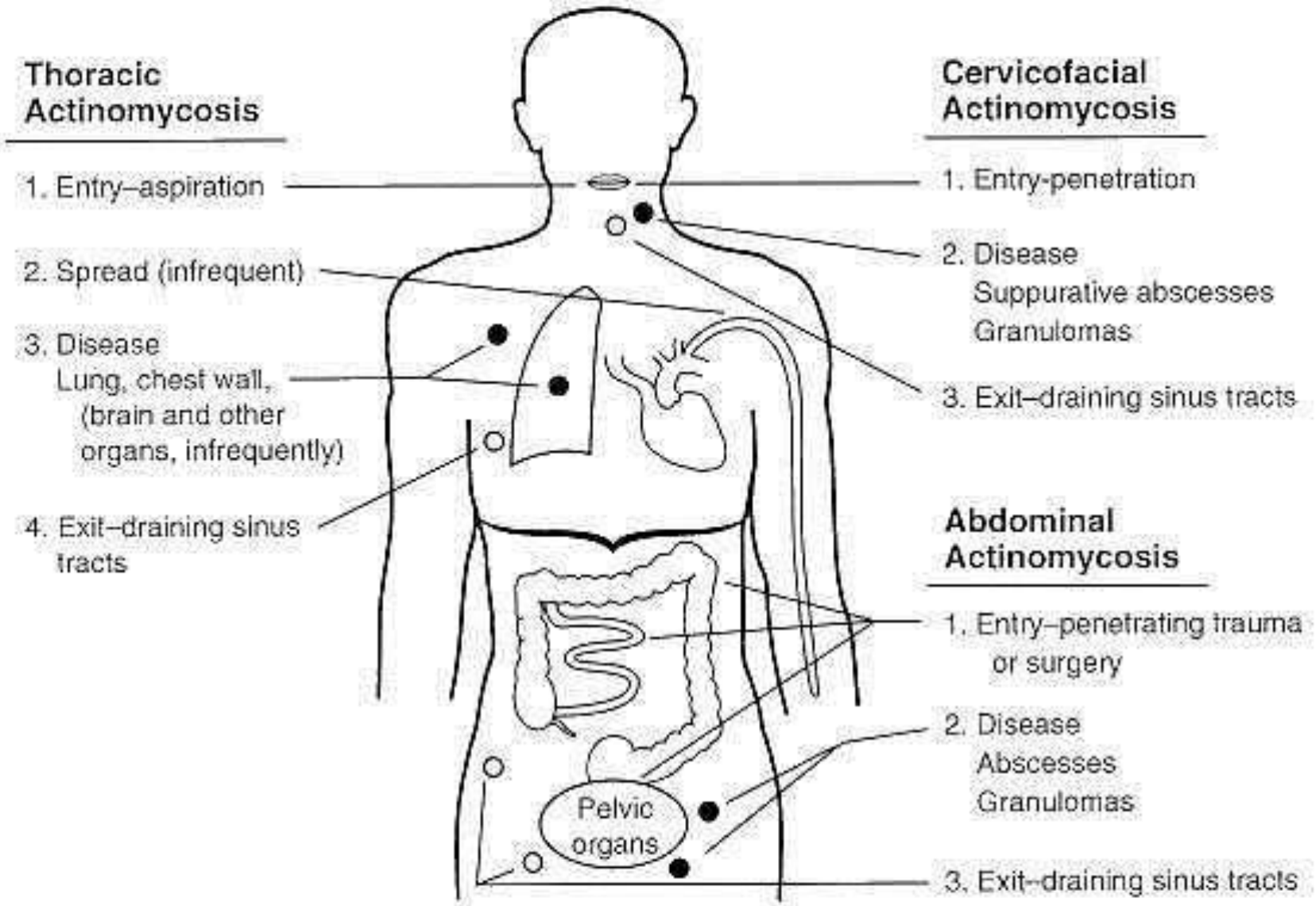
***Streptomyces* spp.**

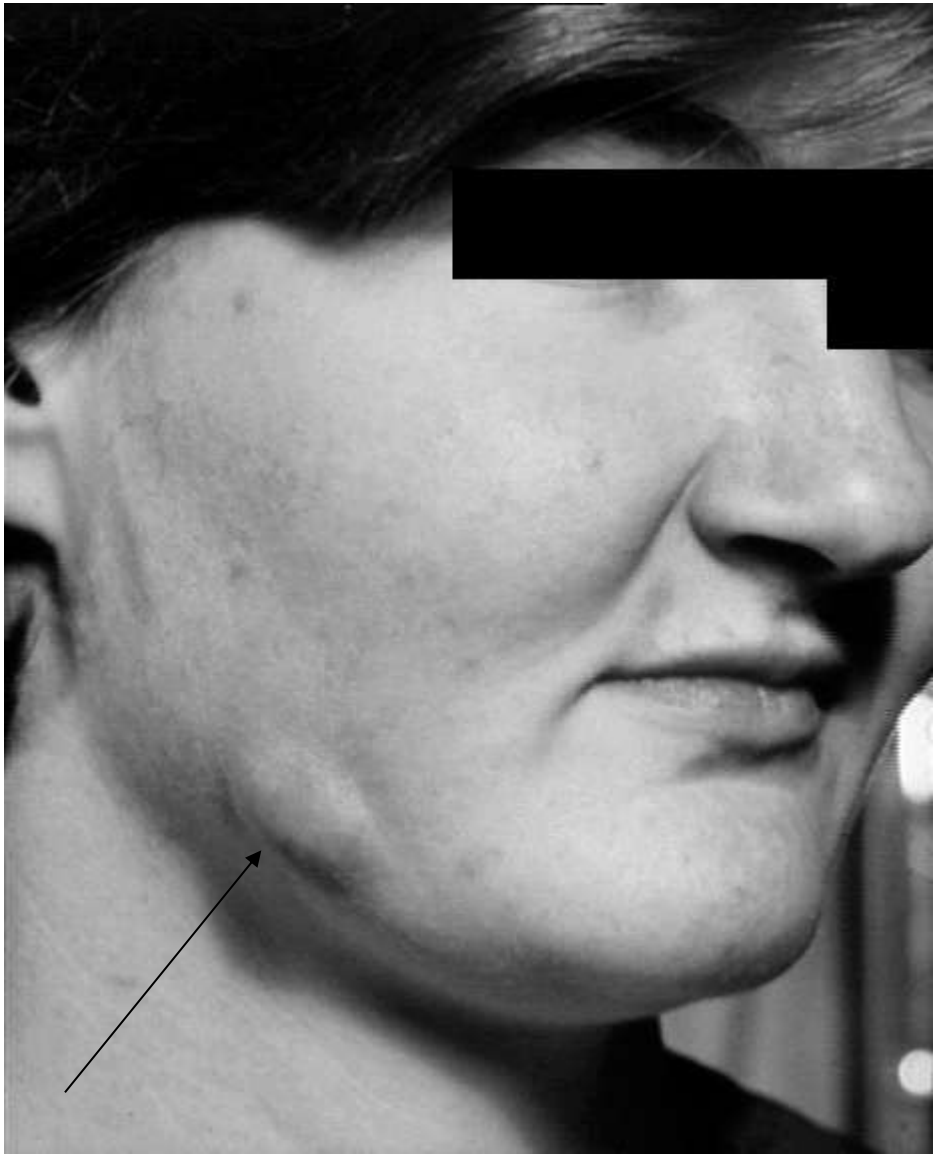
“Sulfur granule” : gram-positive filaments surrounded by eosinophilic “clubs”



Actinomycosis: pathogenesis and pathology




Thoracic Actinomycosis
1. Entry–aspiration
2. Spread (infrequent)
3. Disease
Lung, chest wall,
(brain and other
organs, infrequently)
4. Exit–draining sinus
tracts
Cervicofacial Actinomycosis
1. Entry-penetration
2. Disease
Suppurative abscesses
Granulomas
3. Exit–draining sinus tracts
Abdominal Actinomycosis
1. Entry–penetrating trauma
or surgery
2. Disease
Abscesses
Granulomas
3. Exit–draining sinus tracts
Pelvic organs



Cervicofacial actinomycosis

Actinomycosis: laboratory diagnostics

pus from drained lesions

microscopic examination

•gram-positive branching rods

•“sulfur granules”

isolation of pure culture by platting onto brain-heart infusion agar (incubation for 2 weeks)

serum

Ig (complement fixation reaction)

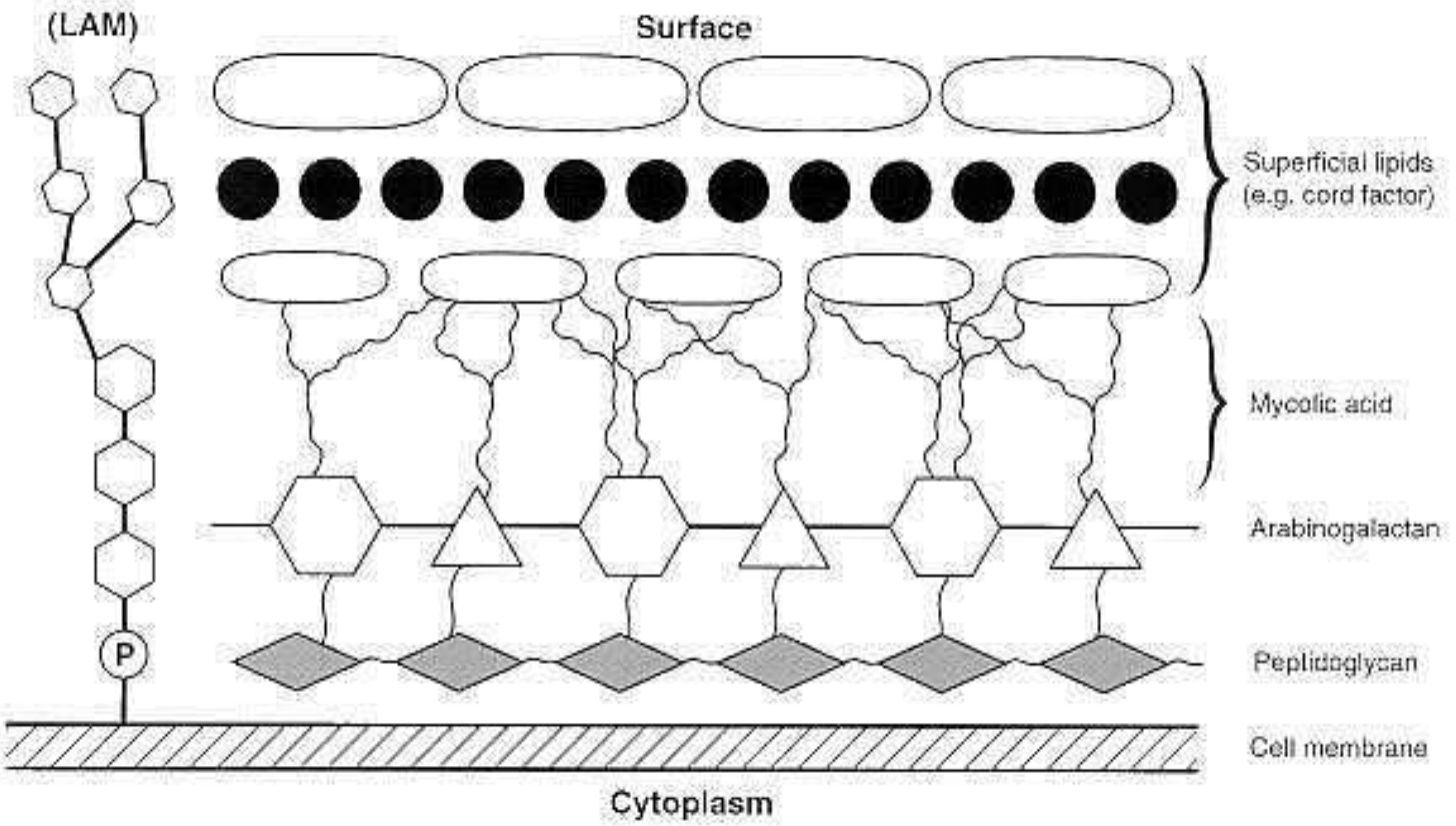
actinolysate

sensitization

Mycobacteria: main characteristics

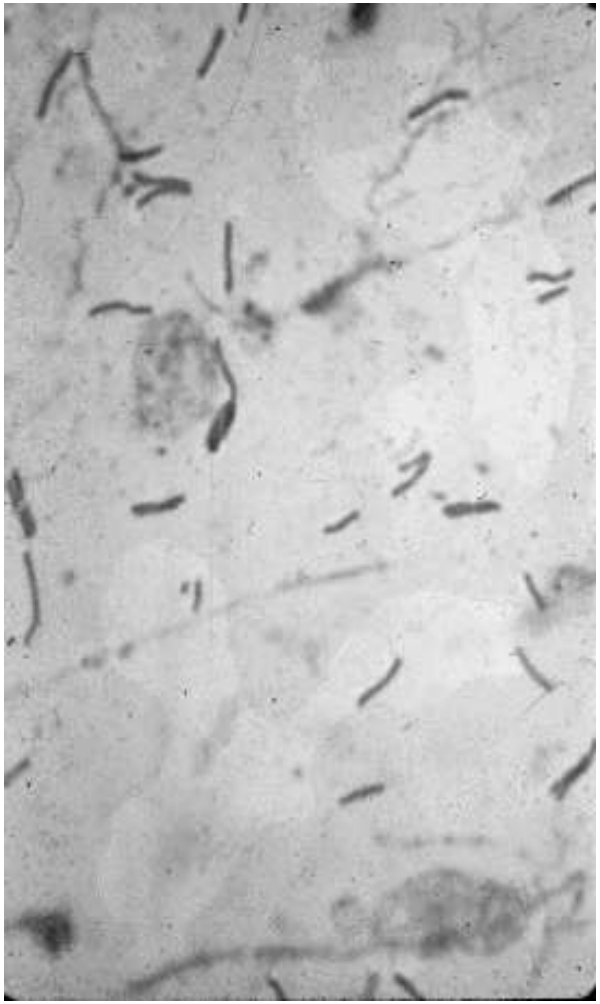
- Rods:
  - gram-positive bacilli (rods)
  - thin, straight and long
  - alcohol-, alkaline- and acid - fast (when use the Ziehl-Neelsen technique of stain they get red colour)
- Grow:
  - very slowly (about 3 months: the doubling time of tubercle bacilli is 12 hrs or more)
  - require enriched complex organic media containing egg yolk, glycerin and malachite green (Lowenstein-Jensen medium)
  - produce R-shaped colonies (resembling cauliflower)
  - colonies are pigmented (yellow) and waxy because of the high concentrations of lipids, fatty acids and waxes in the bacterial cell wall
- Pathogenicity:
  - the bacteria are distributed systemically within macrophages and survives intracellularly
  - inhibit phagosome - lysosome fusion and resistant to lysosomal enzymes

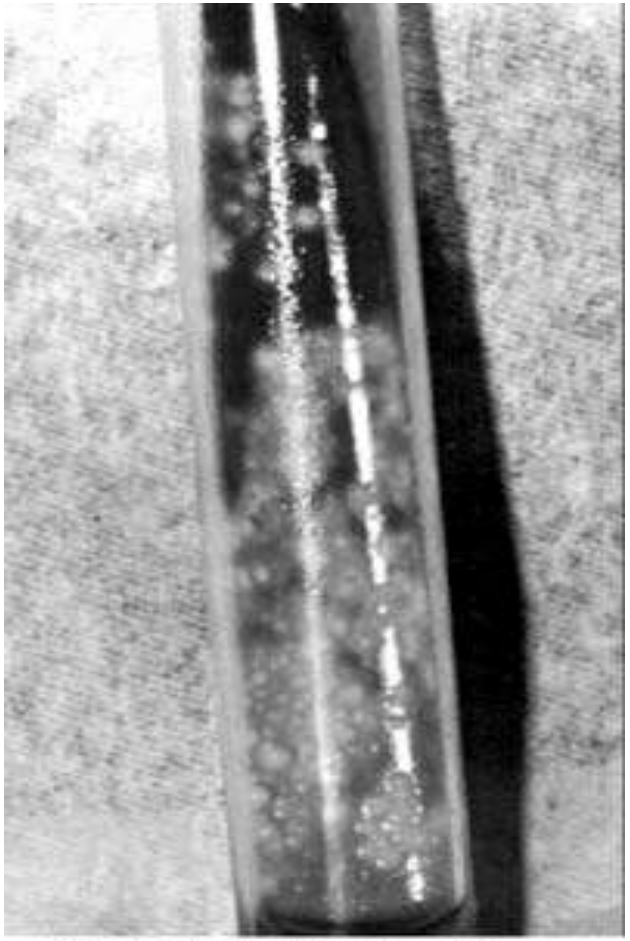
CELL WALL COMPOSITION IN MYCOBACTERIA



LAM – LIPOARABINOMANNAN

Mycobacterium bacilli : morphology of the bacteria and colonies produced by them when grown on Lowenstein-Jensen medium





Kolonije Mycobacterium tuberculosis na čvrstoj Lowenstein-Jensen podlozi.

Tubercle bacilli: virulence for humans

1. Mycobacterium tuberculosis (92%)
2. Mycobacterium bovis (5%)
3. Mycobacterium africanum (3%)

Tuberculosis: epidemiology and pathogenesis

sources of the infection:

- humans excreting bacilli with sputum (transmission by droplet nuclei)

↓

- humans excreting bacilli with feces and urea

ways of spreading of the disease:

↓

- air-born
- alimentary and contact

primary tubercle complex is presented by:

- lesions in the infected organs (more frequently in lungs)
- regional lymphadenitis
- DTH (as a result of sensitization)

Tuberculosis: pathogenesis and pathology

During the first contact with host bacilli are phagocytised by macrophages

↓

transport of bacilli by phagocytes to lymph nodes

↓

primary granuloma (tubercle) is formed (usually in lungs and lymph nodes) but any organ may be effected (when dissemination of phagocytised bacilli occurs) as a result of DTH response

↓

calcification of tubercle

(nonsterile immunity development)

↓

healing by fibrosis

↓

reactivation

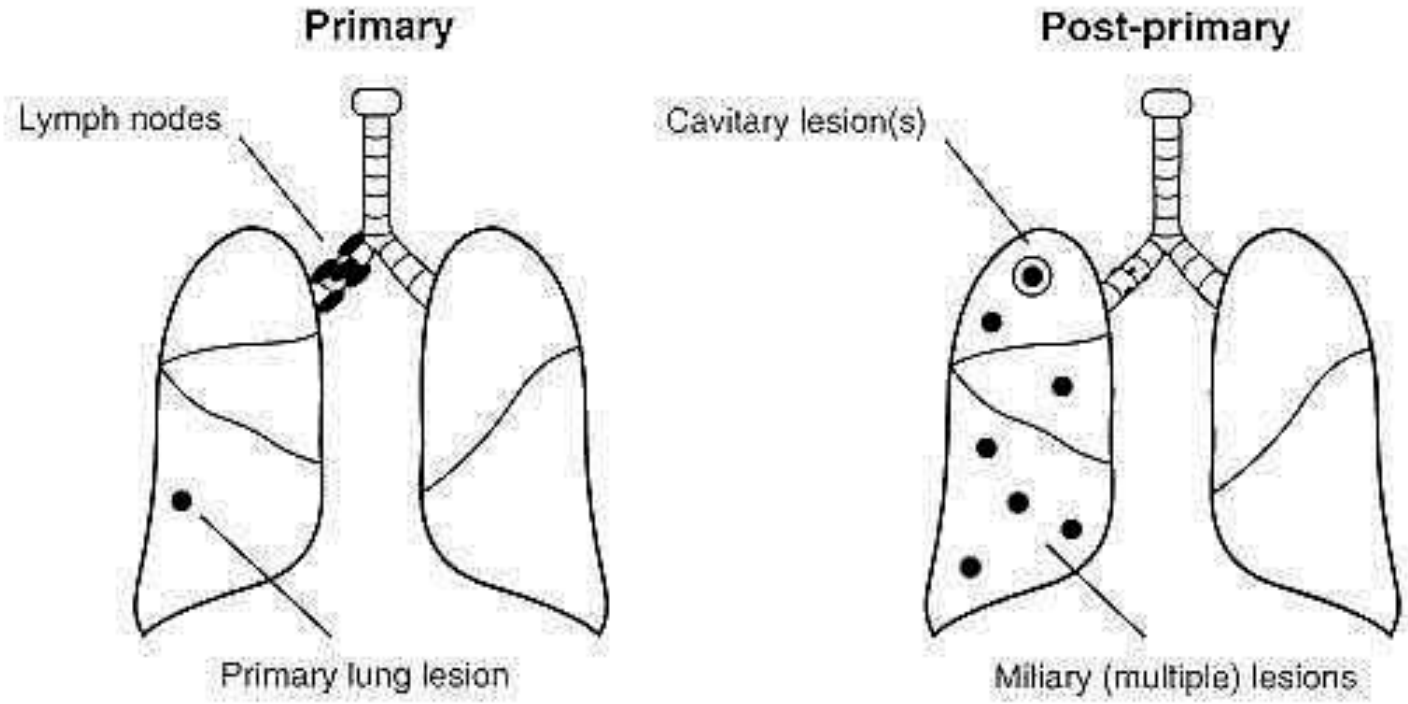
reactivated (secondary tuberculosis) in individuals with lowered immunity

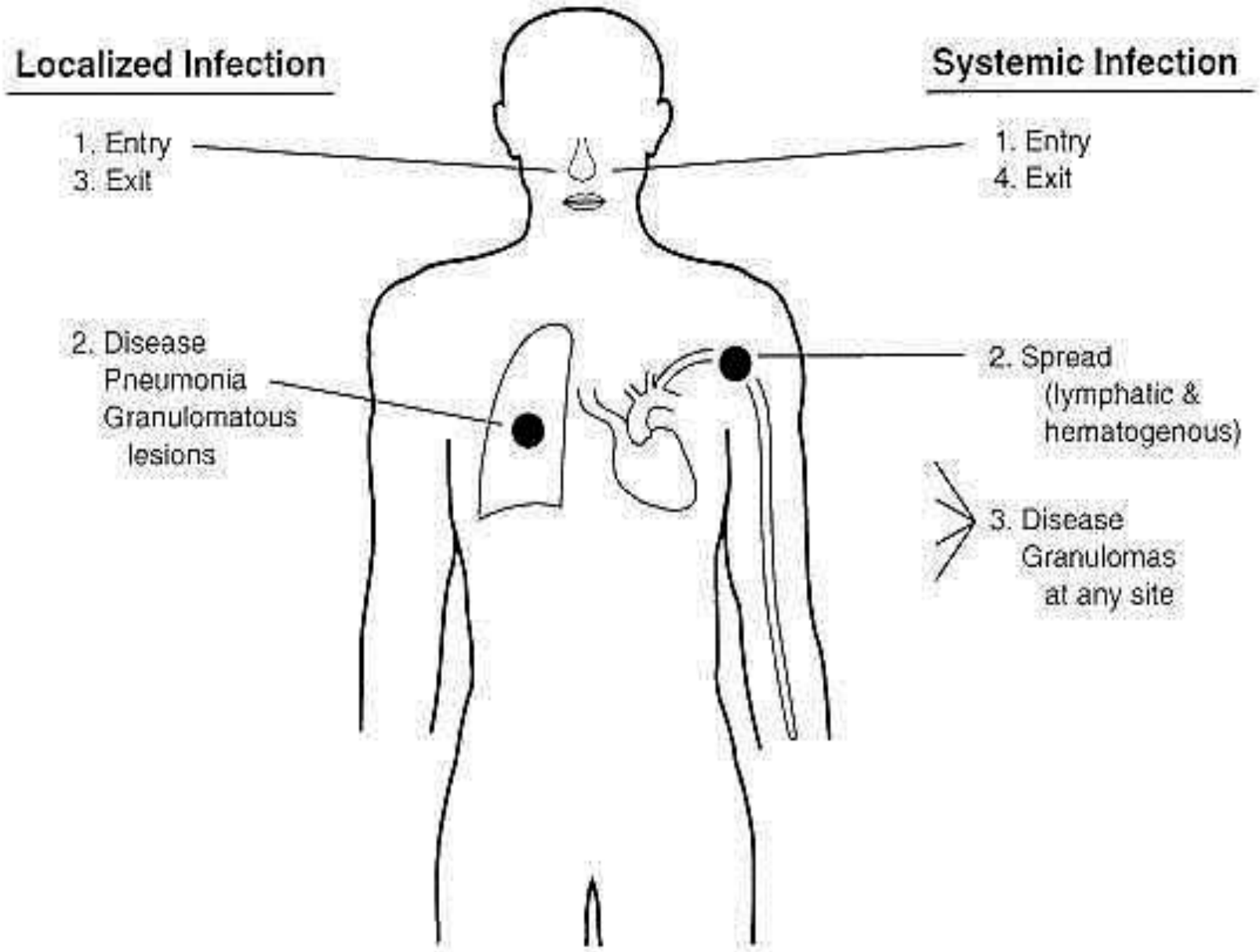
Reactivated tuberculosis

(may occur even 20 years or longer after primary infection)

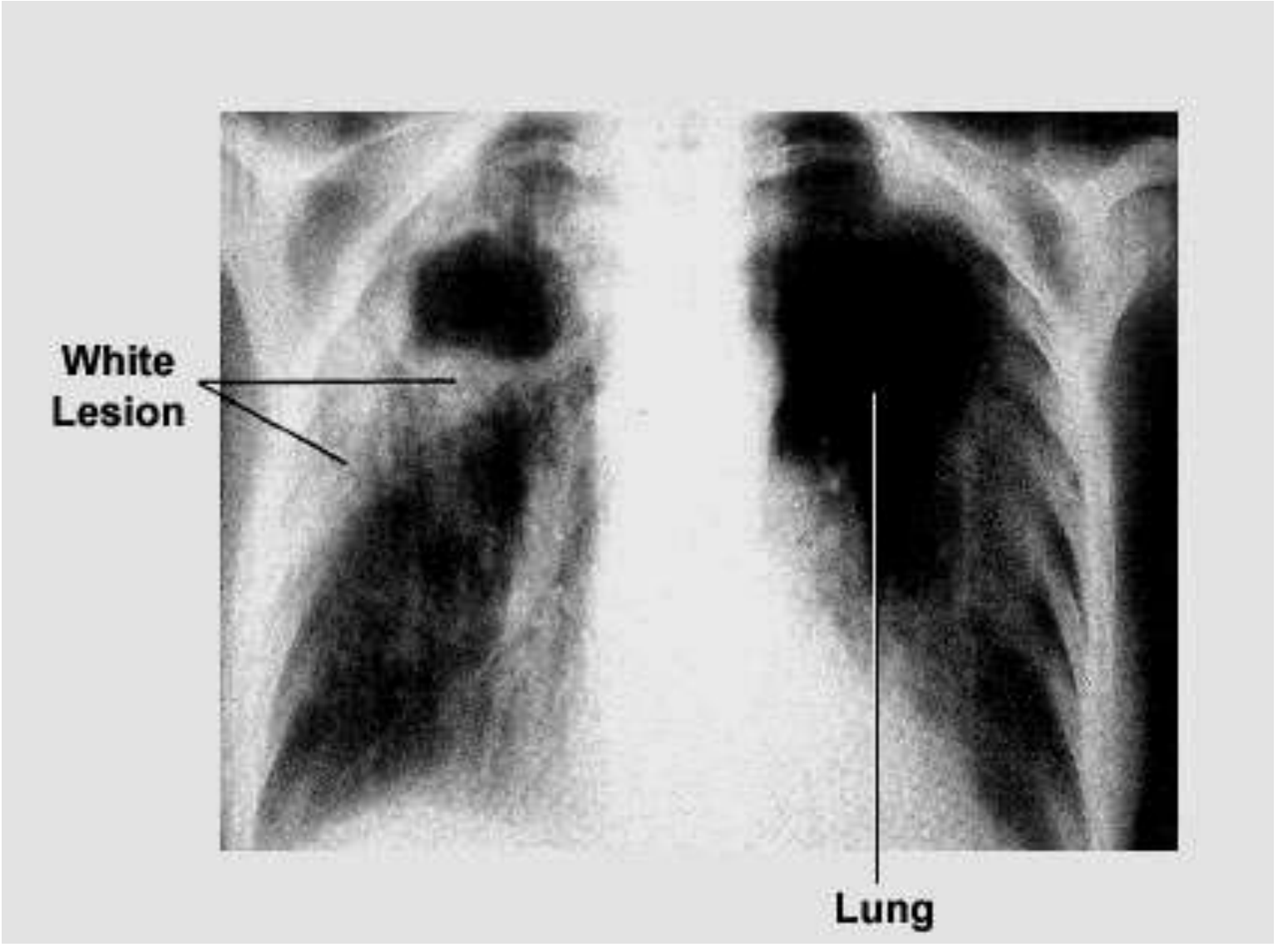
↓

numerous tubercles with large areas of caseous necrosis containing M. tuberculosis appear

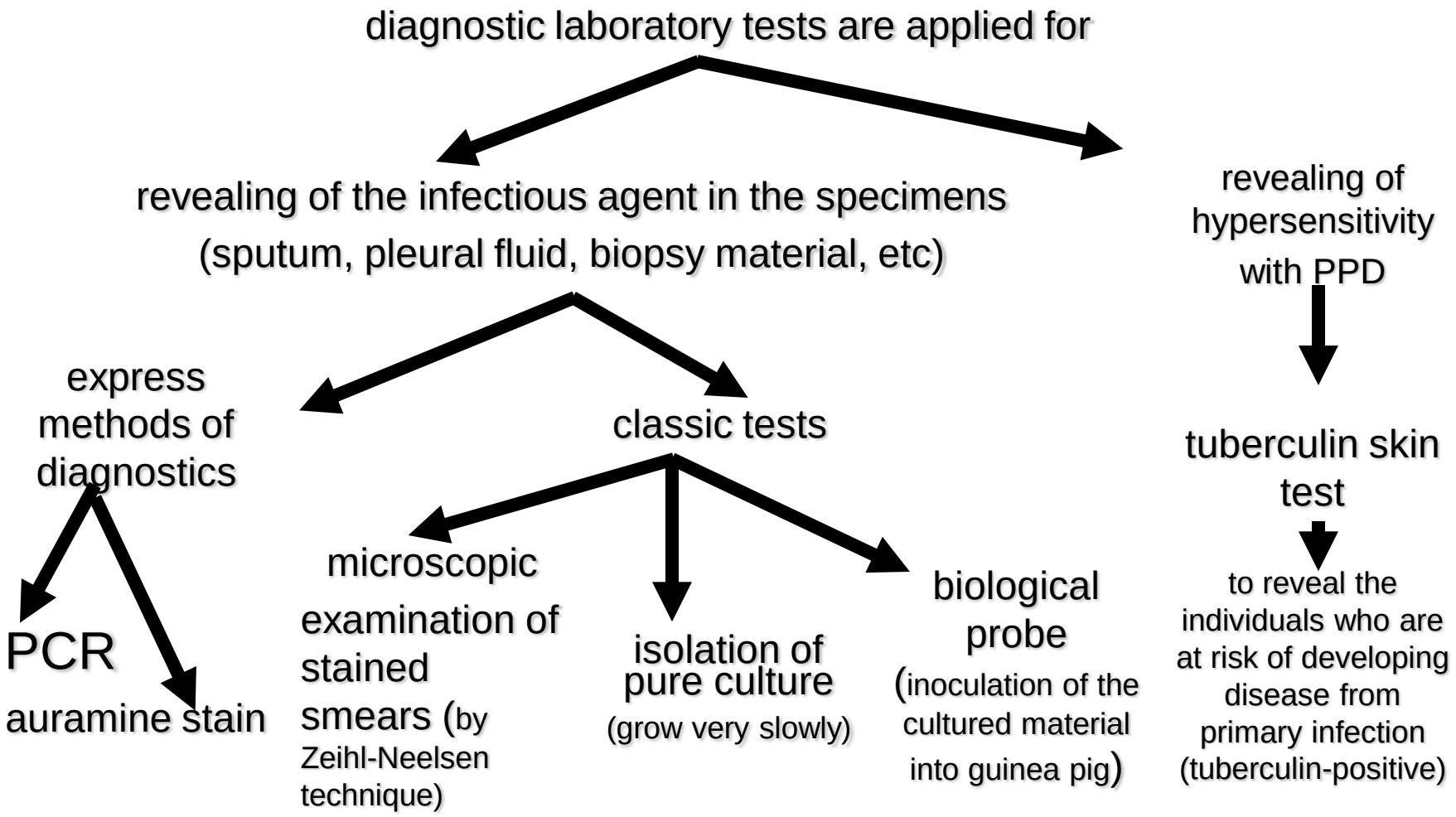

Primary
Lymph nodes
Primary lung lesion
Post-primary
Cavitary lesion(s)
Miliary (multiple) lesions


Localized Infection
1. Entry
3. Exit
2. Disease
Pneumonia
Granulomatous
lesions
Systemic Infection
1. Entry
4. Exit
2. Spread
(lymphatic &
hematogenous)
3. Disease
Granulomas
at any site

Tubercles in lungs



Tuberculosis: laboratory diagnostics



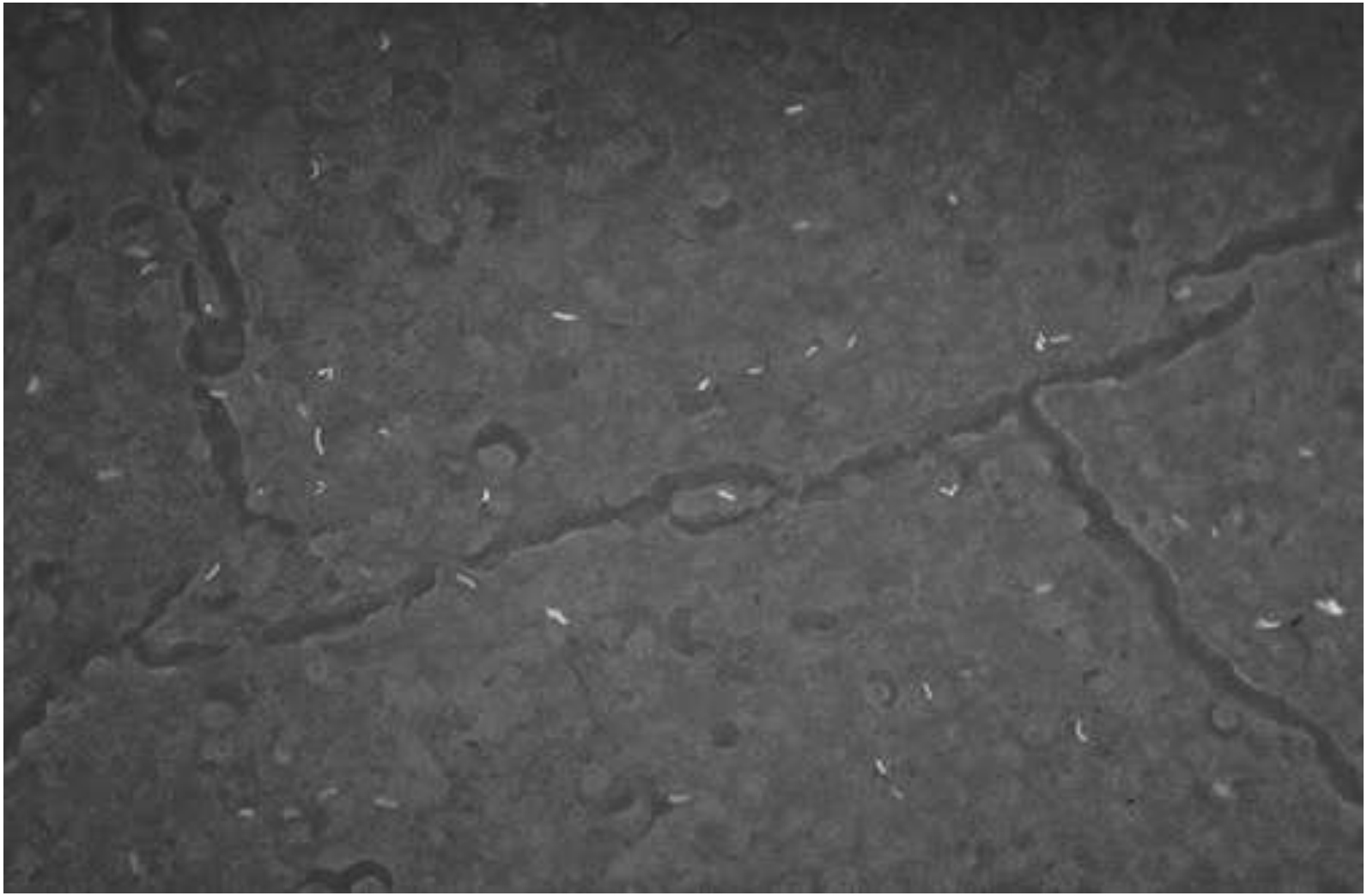
Tuberculosis: specific prophylaxis and treatment

Vaccination

- vaccine BCG (*Bacillus de Calmette et Guerin*, an attenuated strain of *M. bovis*)
- BCG-M (for children with low immune response)

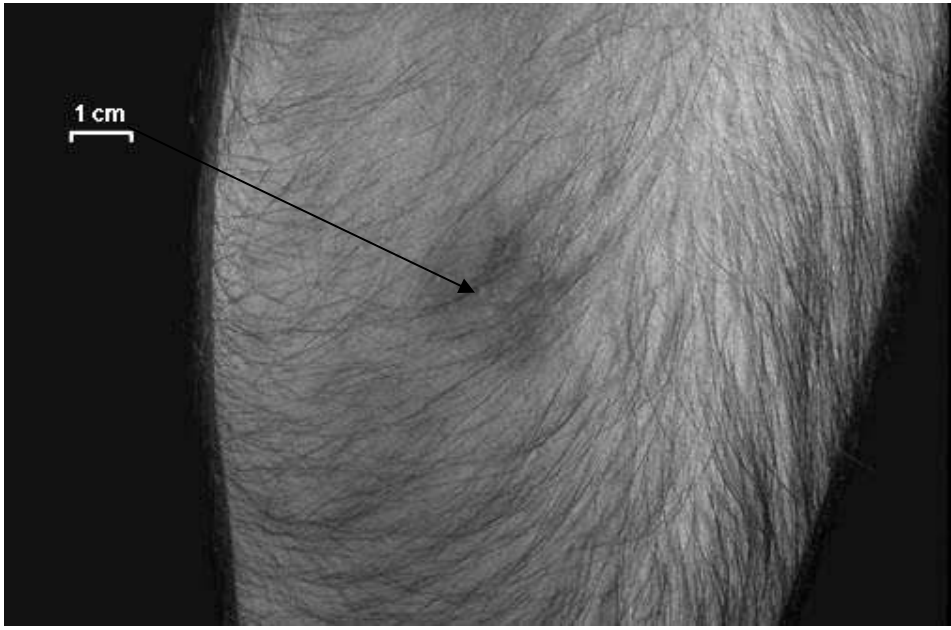
Treatment

- prolonged (sometimes for years)
- antituberculosis drugs are used in present: isoniazid, rifampin, etc

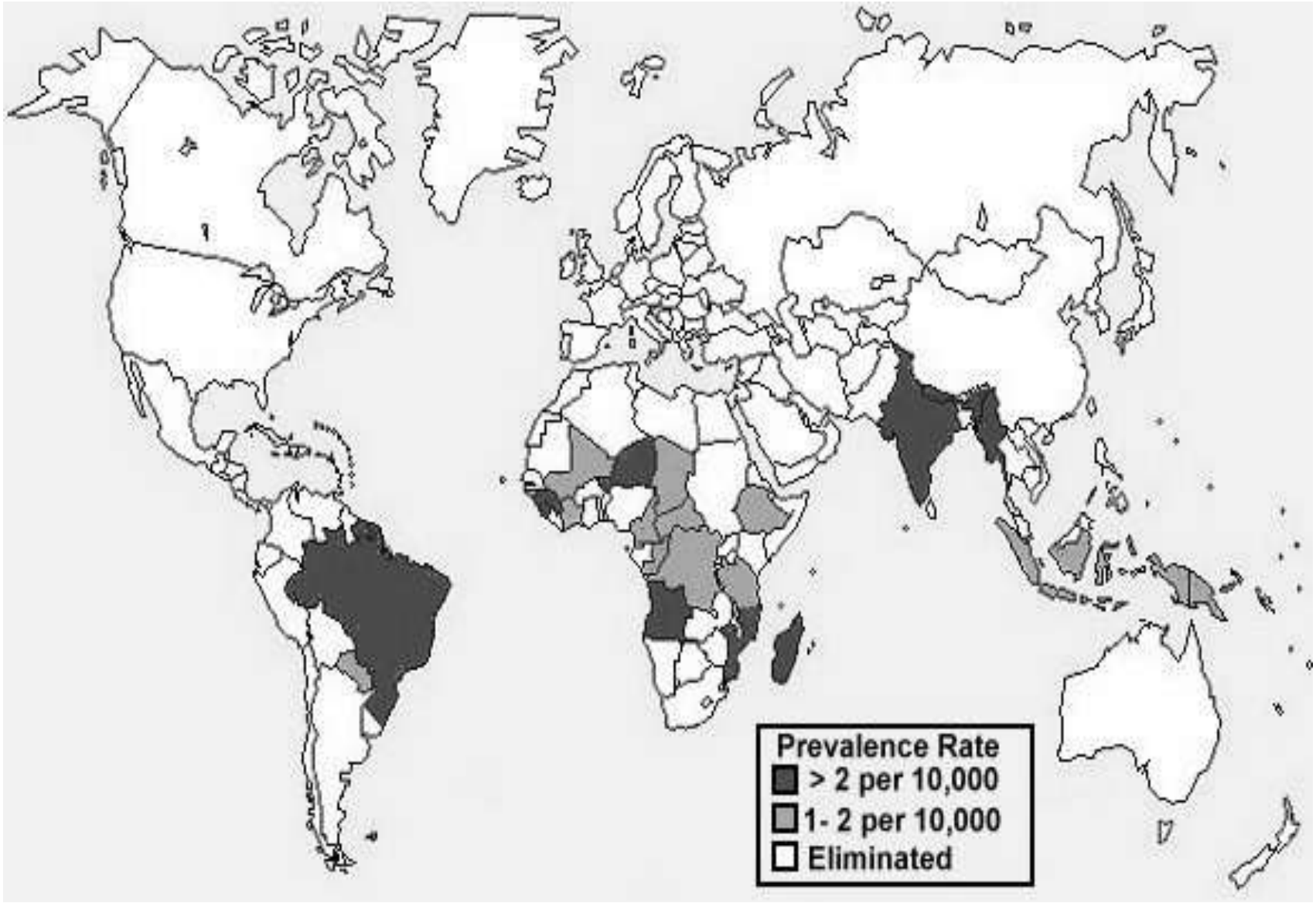


Mycobacterium bacilli – auramine stain

Tuberculin skin test



Mycobacterium leprae


Prevalence Rate
> 2 per 10,000
1- 2 per 10,000
Eliminated

Mycobacterium leprae: main characteristics

- *M. leprae* is the causative agent of leprosy (Hansen's Disease) - a chronic disease;
- the bacterium infects the skin (prefers to grow at low temperature) and has a strong affinity for nerves;

Morphology of *M. leprae* is similar to other mycobacteria but it possesses some specific features:

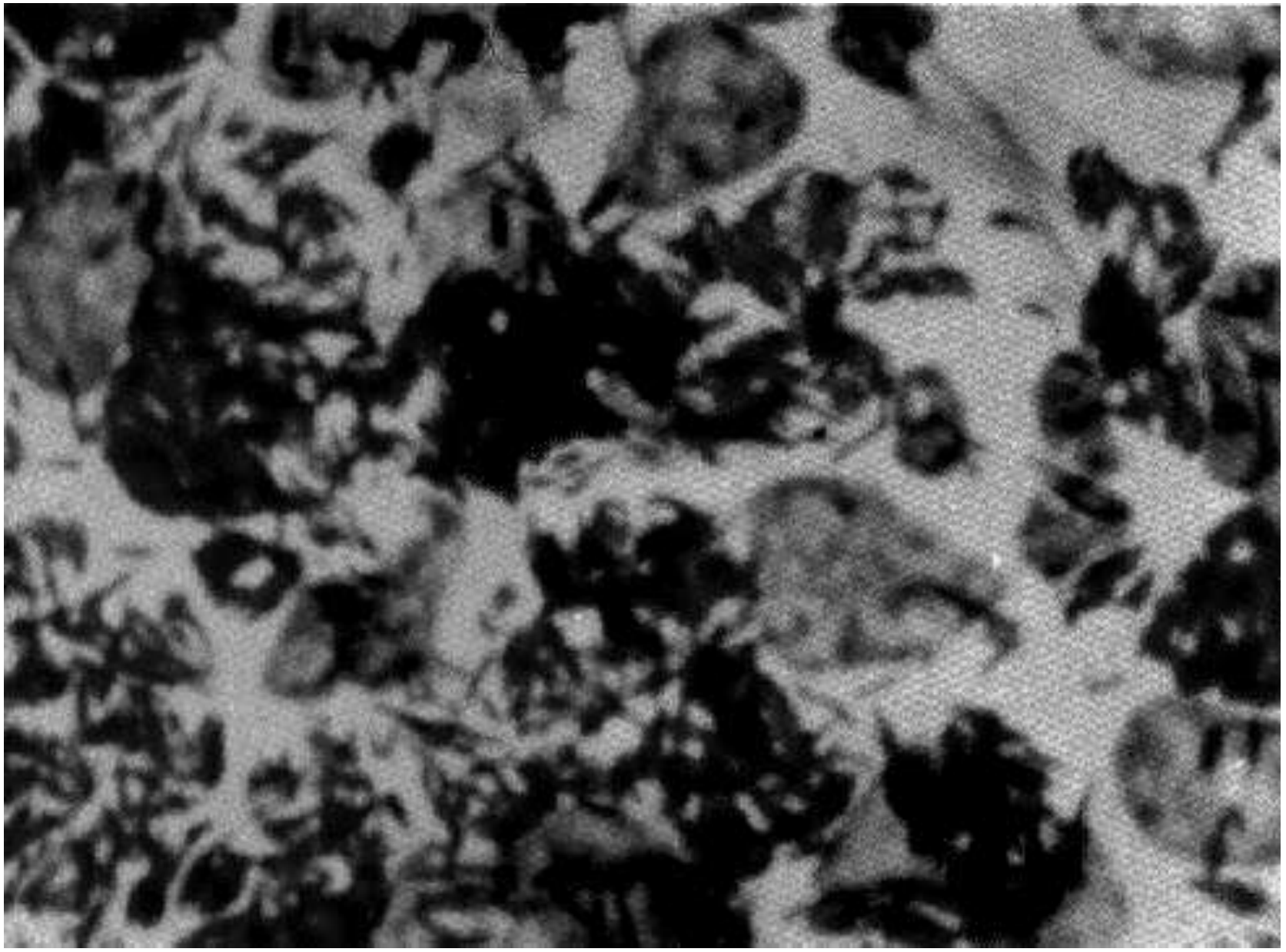
- the cell wall contains unique phenolic glycolipids;
- it cannot be cultivated *in vitro;*
- it grows well in the armadillo (which has a low body temperature) that could be used for accumulation of the pathogen,
- it multiplies very slowly *in vivo* (12-day doubling time).

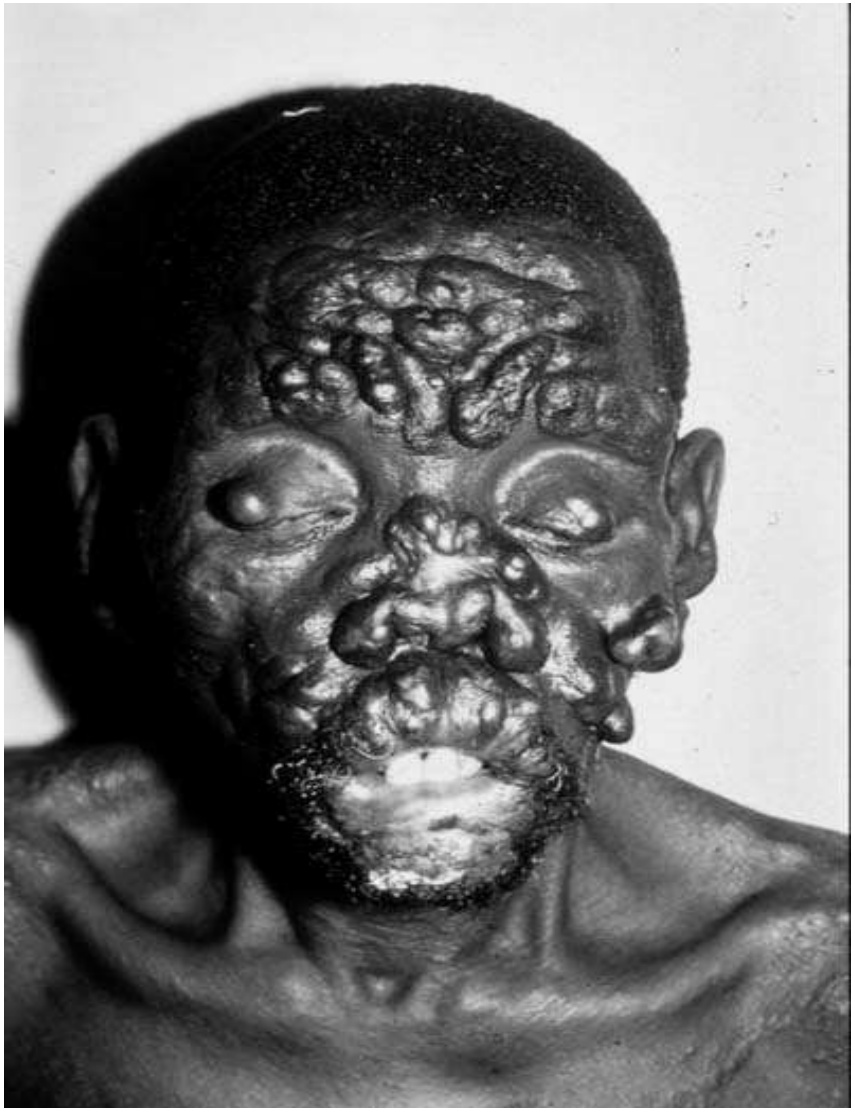
Mycobacterium leprae: epidemiology, pathogenesis and pathology

Transmission requires prolonged contact and occurs directly through intact skin, mucous membranes, or penetrating wounds.

The spectrum of leprosy are two main forms:

- ✓ lepromatous (disseminated, with loss of specific cell-mediated immunity),
- ✓ tuberculoid (localized, with strong cell-mediated immunity).
- The disease affects peripheral nerves, skin and mucous membranes.
- Skin lesions, areas of anesthesia, and enlarged nerves are the principal signs of leprosy.





The face of a patient with active, neglected nodulous lepromatous leprosy. With treatment, all nodules could be reversed.

Most patients with leprosy can be cured with multi-drug therapy in just six months.

Mycobacterium leprae : diagnostics and treatment

- Diagnosis is based on acid-fast stain of skin biopsies and cytologic examination of affected skin and response to the lepromin skin test.
- Treatment (including prophylaxis in close contacts) with multi-drug therapy (dapsone, rifampin, and clofazimine) is performed on an outpatient basis for 3 to 5 years.

Listeria: main characteristics

Listeria monocytogenes (induces monocytosis) – the pathogen that produces **listeriosis** in human

Rods:

- gram-positive
- polymorphic (usually short but sometimes long and even filamentous)
- motile

Grow:

- on complex nutritive media and the growth is enhanced by the presence of blood, ascitic fluid and glucose
- colonies produce odour of sour milk
- they are facultative intracellular parasites

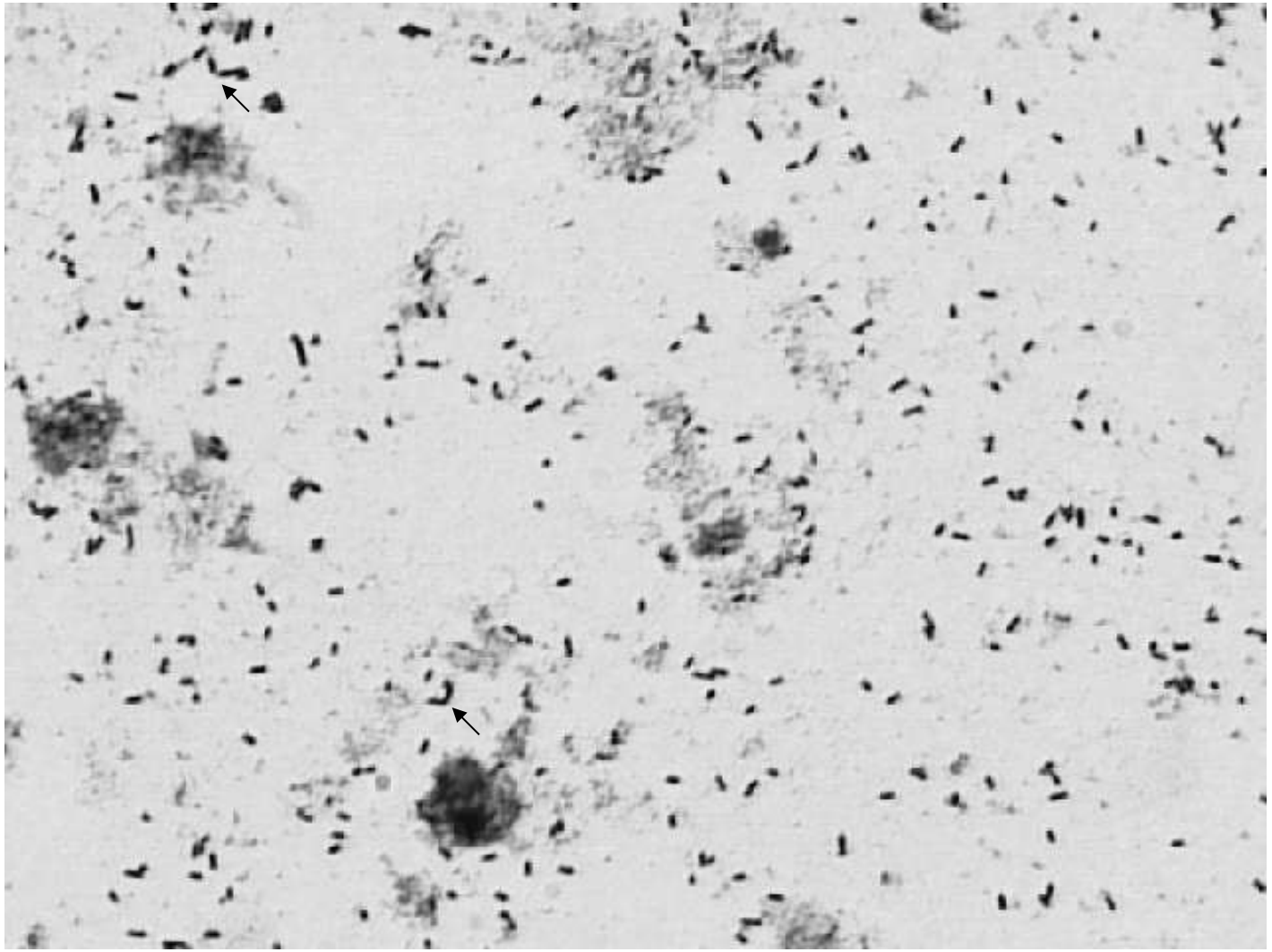
Infect:

- macrophages and parenchymal cells,
- escapes from the host phagosome and undergoes rapid division in the cytoplasm

At greatest risk for the disease are:

- the fetus
- neonates
- cancer patients and immune-compromised persons.

Listeria monocytogenes



Listeria monocytogenes: pathogenesis and pathology

Listeria monocytogenes (sources of the infection: domestic or wild animals)

↓

the pathogen produces:

- septicemia in immune-compromised people (alcoholics)
- acquired transplacetally causes abscesses and granulomas in multiple organs and very frequently results in abortion
- neonatal meningitis and sepsis (neonates get infected when passing birth canal of infected woman)
- lesions in parenchymatous organs
- may cause meningoencephalitis

Listeria monocytogenes: laboratory diagnostics

- Isolation of pure culture from blood, lesions or cerebrospinal fluid on blood agar and agar containing spinal fluid.
- Listeriosis is indicated when monocytosis (increased number of monocytes in blood) is observed.

Anaerobic bacteria

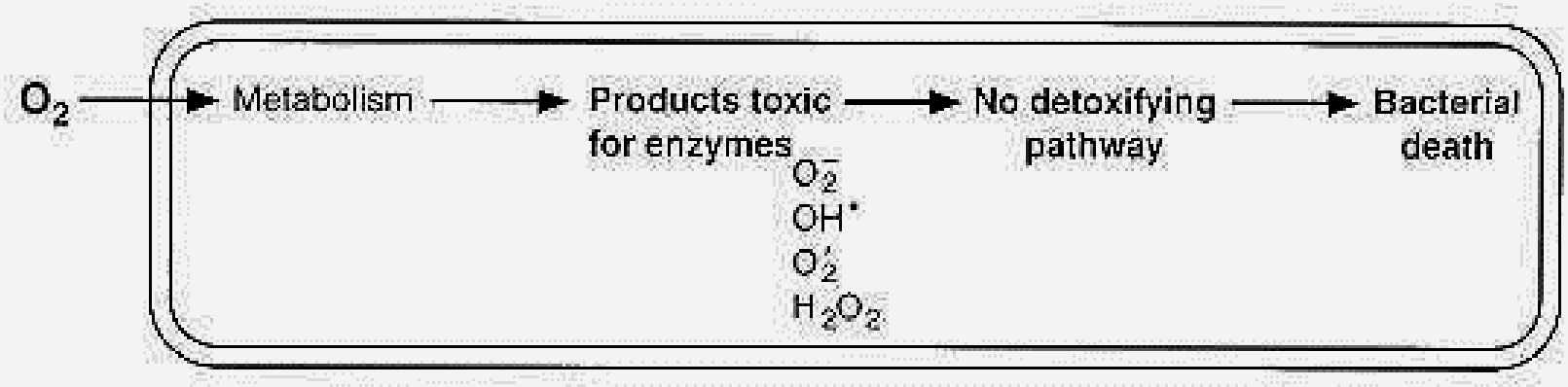
Theme N23

Anaerobic bacteria: main characteristics

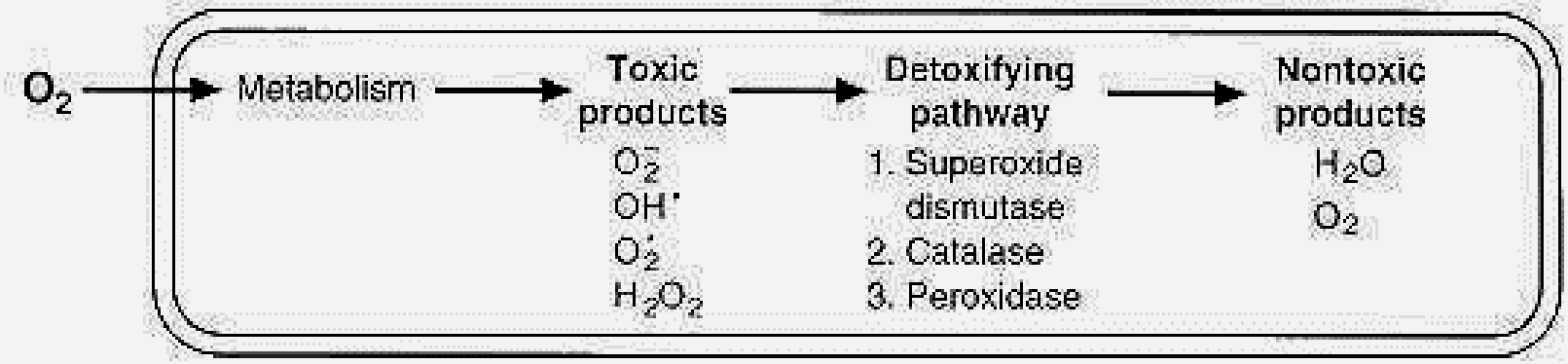
Grow only under anaerobic conditions (without oxygen)

- Obligate anaerobes are bacteria that cannot survive in the presence oxygen.
- During metabolism, bacteria can produce toxic products from oxygen including O_2' (superoxide radicals) and H_2O_2 (hydrogen peroxide).
- Strict anaerobes lack the enzymes superoxide dismutase and catalase that detoxify these products.
- Strict anaerobes can grow only in injured devitalized tissue where limitation of the blood (and oxygen) supply occurs.

Anaerobic Bacteria



Aerobic or Facultative Bacteria

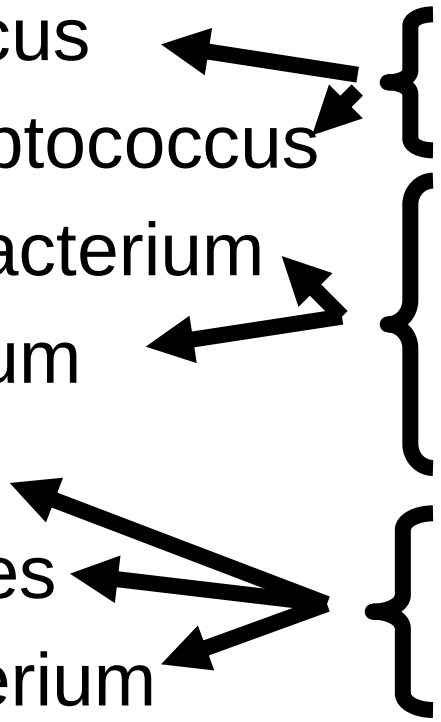


Anaerobic bacteria: classification

Gram-positive			Gram-negative	
cocci	rods		cocci	rods
	spore +	spore –	Veilonella	Bacteroides Fusobacte-rium
Pepto-coccus Pepto-strep-toco-ccus	Clost-ridium	Propio-niba-cteri-um Eubac-terium		

Asporogenous anaerobic bacteria: their role in human disease

- Peptococcus
- Peptostreptococcus
- Propionibacterium
- Eubacterium
- Veilonella
- Bacteroides
- Fusobacterium



Produce inflammation of internal organs

Produce abscesses in immune-compromise patients

Produce abscesses involving mixtures of bacteria - representatives of microbial associations

Clostridia: main characteristics

Rods:

- gram-positive
- anaerobic
- form endospores (the spores are wider than the diameter of the rods in which they are formed)
- produce protein toxins

Grow:

- on complex nutritive media
- produce R-shaped colonies (when cultivated in deep tubes they produce colonies looking like bits of fluff or lentils)
- most species are motile and possess peritrichous flagella (excluding C. perfringens)
- C. perfringens produces capsule

Classification of pathogenic clostridia

Clostridia which cause wound infections	Clostridia which cause enteric infections
C. perfringens A C. novyi (oedematiens) C. histolyticum C. septicum C. sporogenes C. sordellii C. tetani	C. perfringens A, C, D C. difficile C. botulinum

The clostridia of gas gangrene: main characteristics

- The 3 most common species of clostridia to be found in gas gangrene are

Cl. perfringens

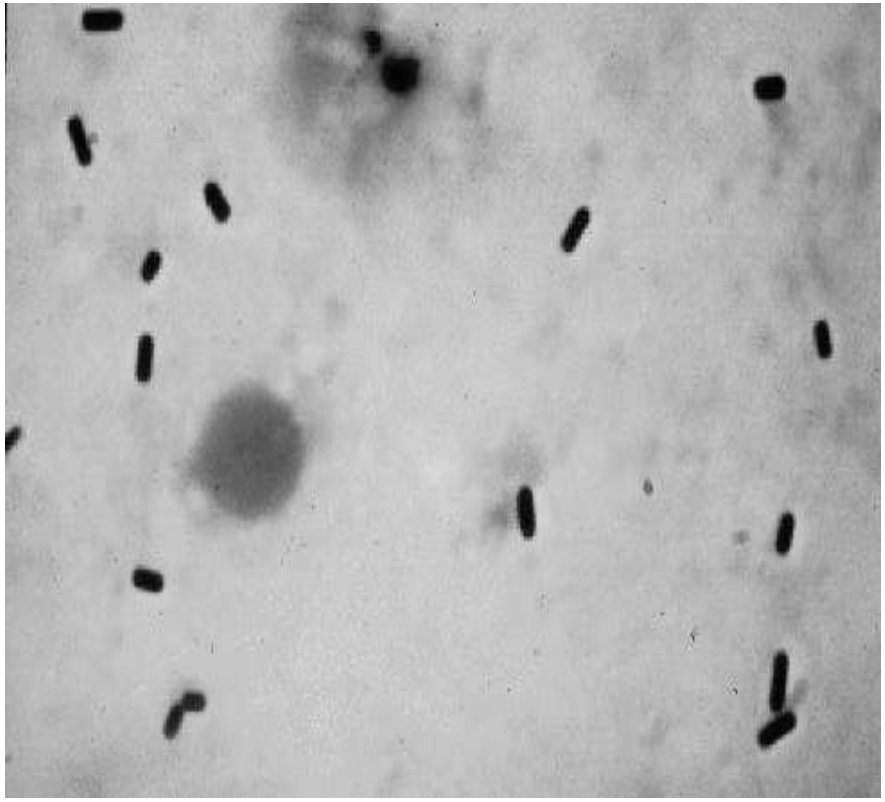
Cl.novyi (oedematiens)

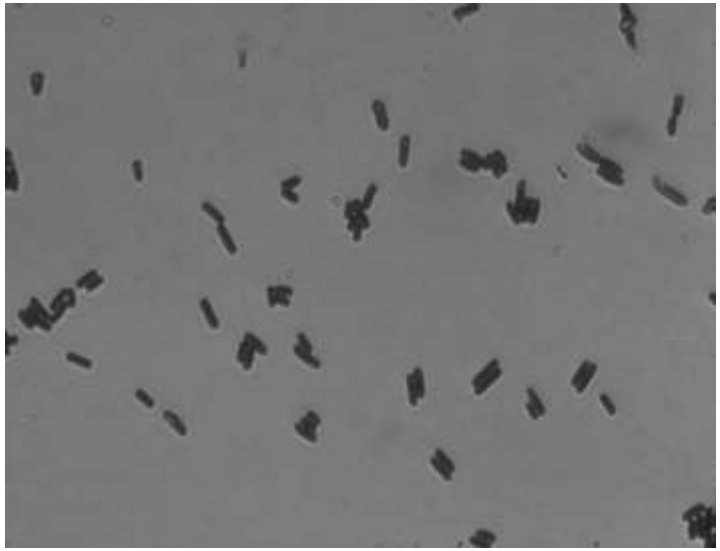
Cl.septicum

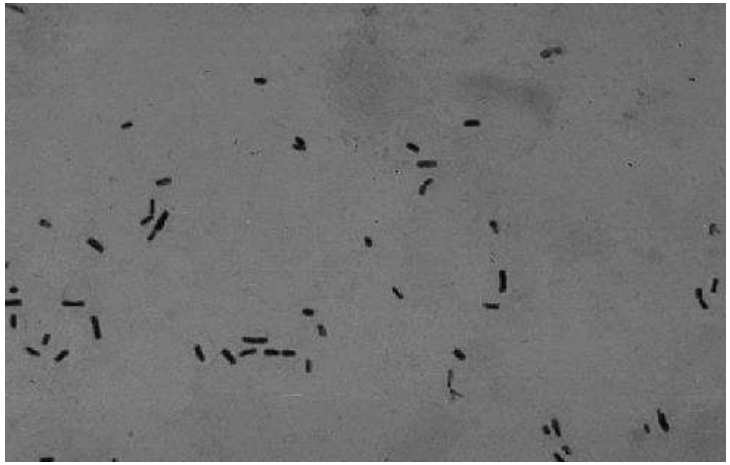
Cl. perfringens

- Forms the spores placed centrally or subterminally in the cells
- In tissue forms capsules
- Grows in anaerobic conditions on blood agar media and in milk (a clot torn by gas in 24 hrs – Cl.perfringens)
- Colonies are large with entire margins
- Biochemically active, ferments glucose, maltose, sucrose, lactose, produces gas
- These bacteria are found in the environment (particularly soil) but also intestine of man and animals.
- Contaminates necrotic poor oxygenated tissue.

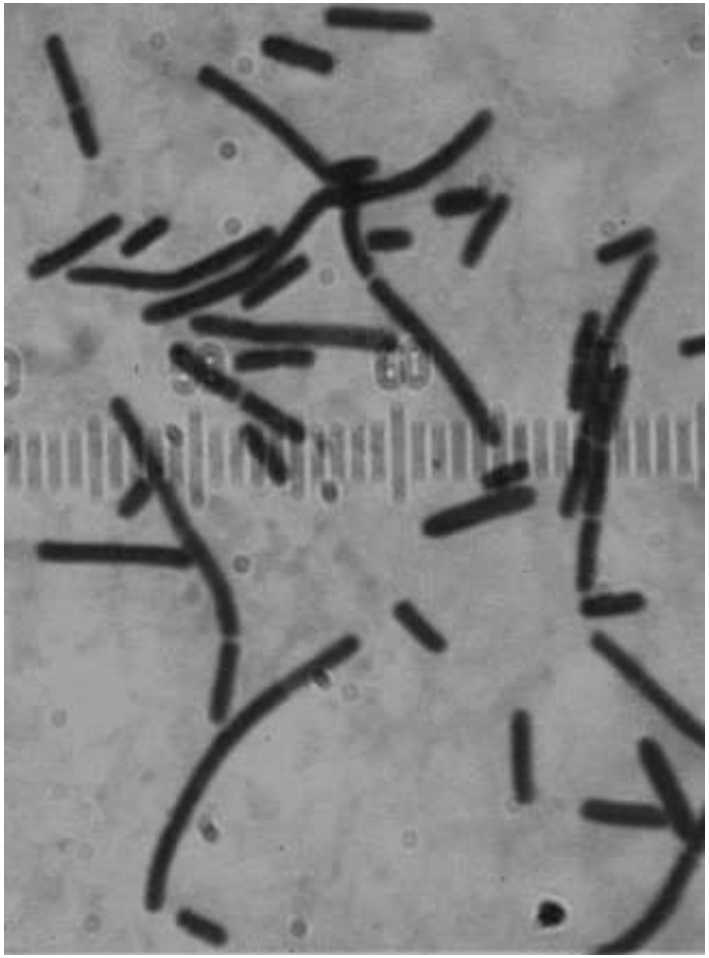
Cl.perfringens

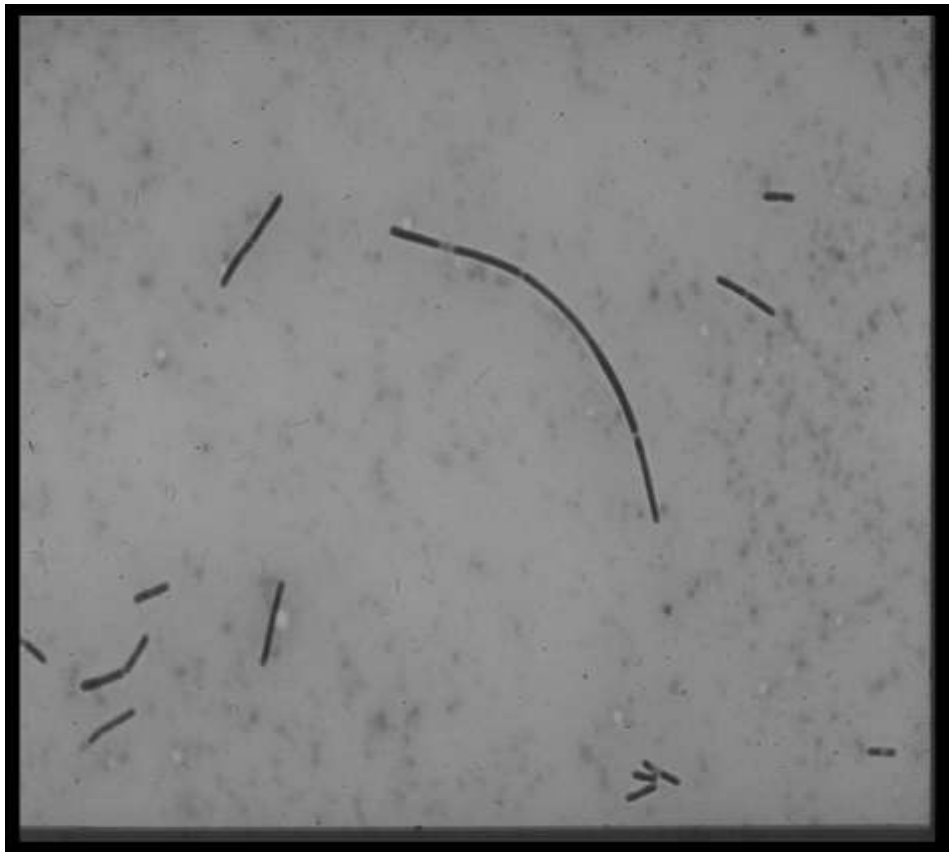






Cl.septicum

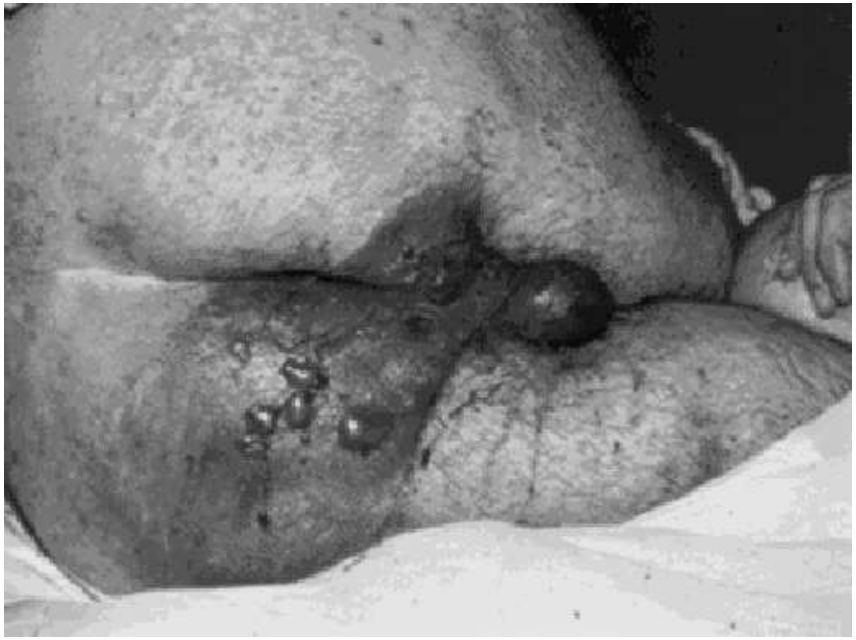




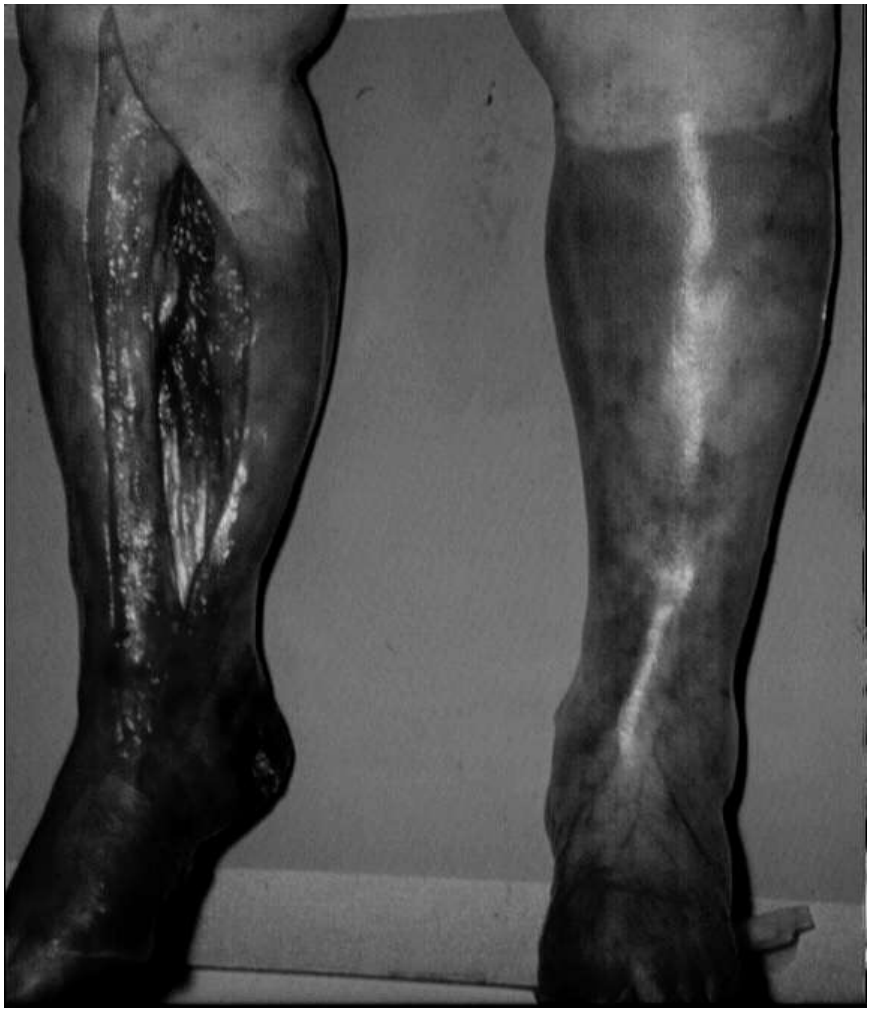
The clostridia of gas gangrene: factors of pathogenicity

- Secrete large variety of ***toxins*** and ***enzymes*** that result in a spreading infection: necrotising and hemolytic toxins.
- Some toxins are enzymes: hyaluronidase, DNase, lecithinase (splits lecithin – an important constituent of cell membrane), collagenase (digests collagen of subcutaneous tissue and muscle).
- Some strains of Cl.perfringens produce powerful enterotoxin which induces profuse diarrhea.

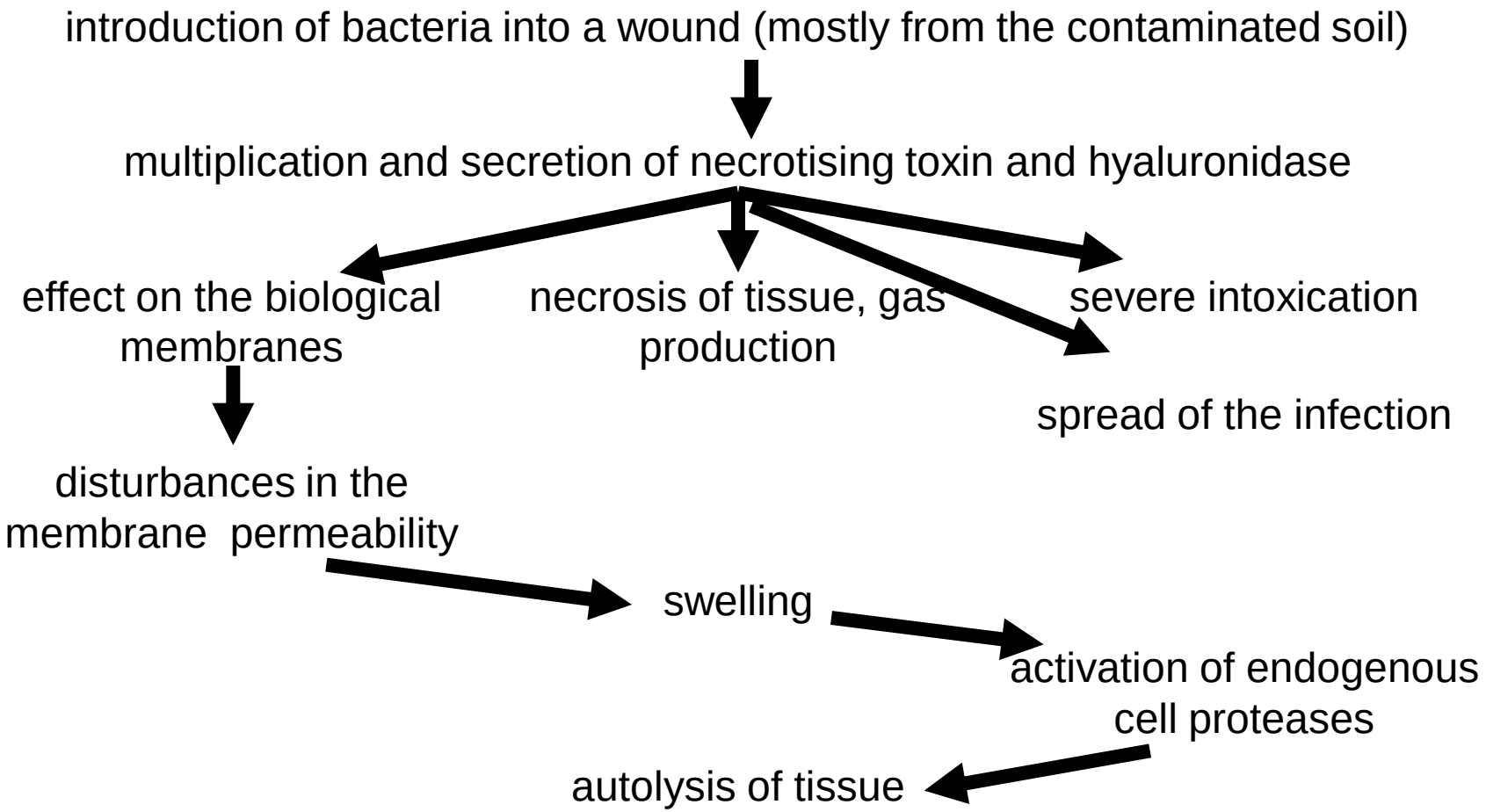
Clinical manifestations of gas gangrene

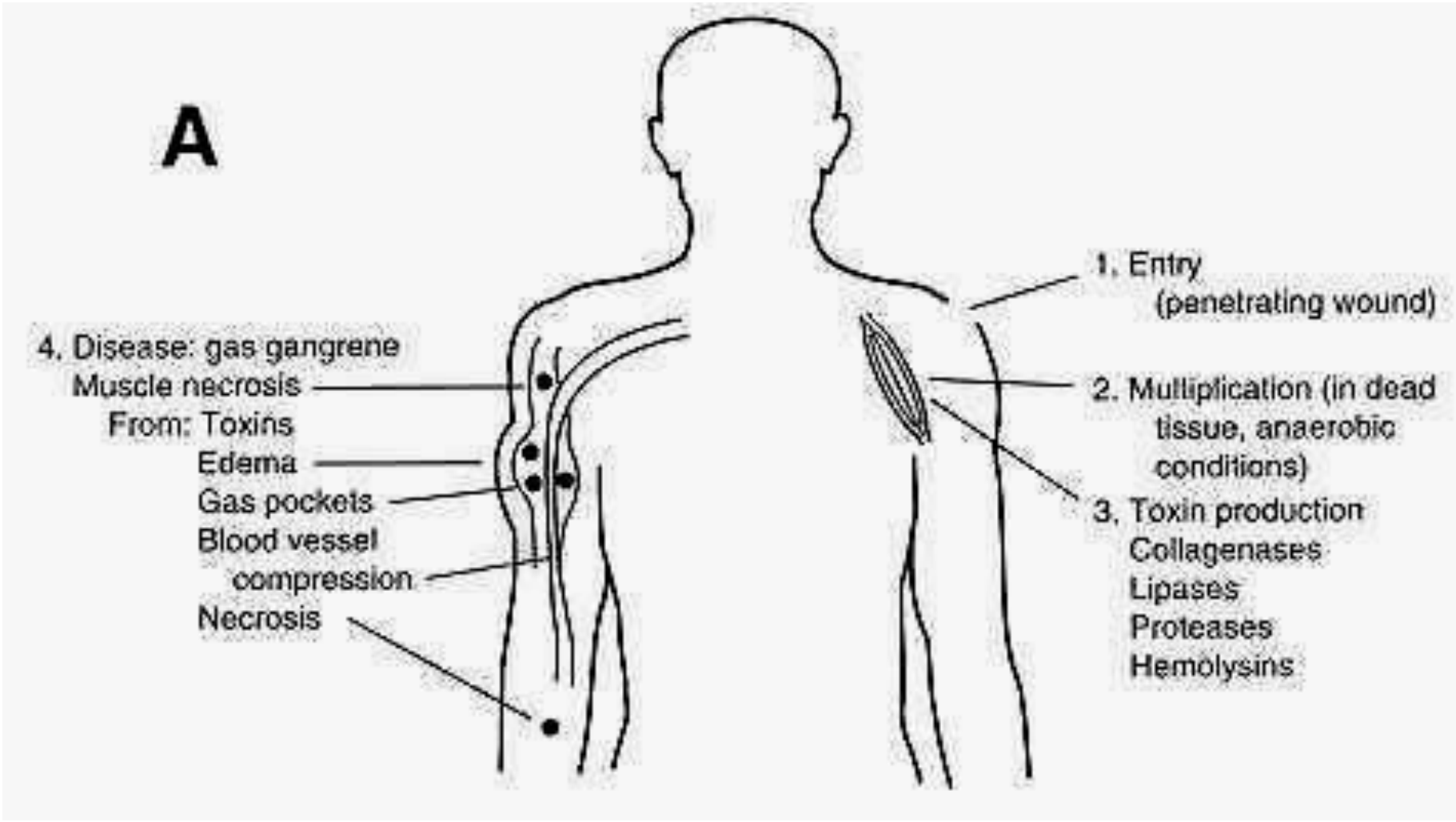


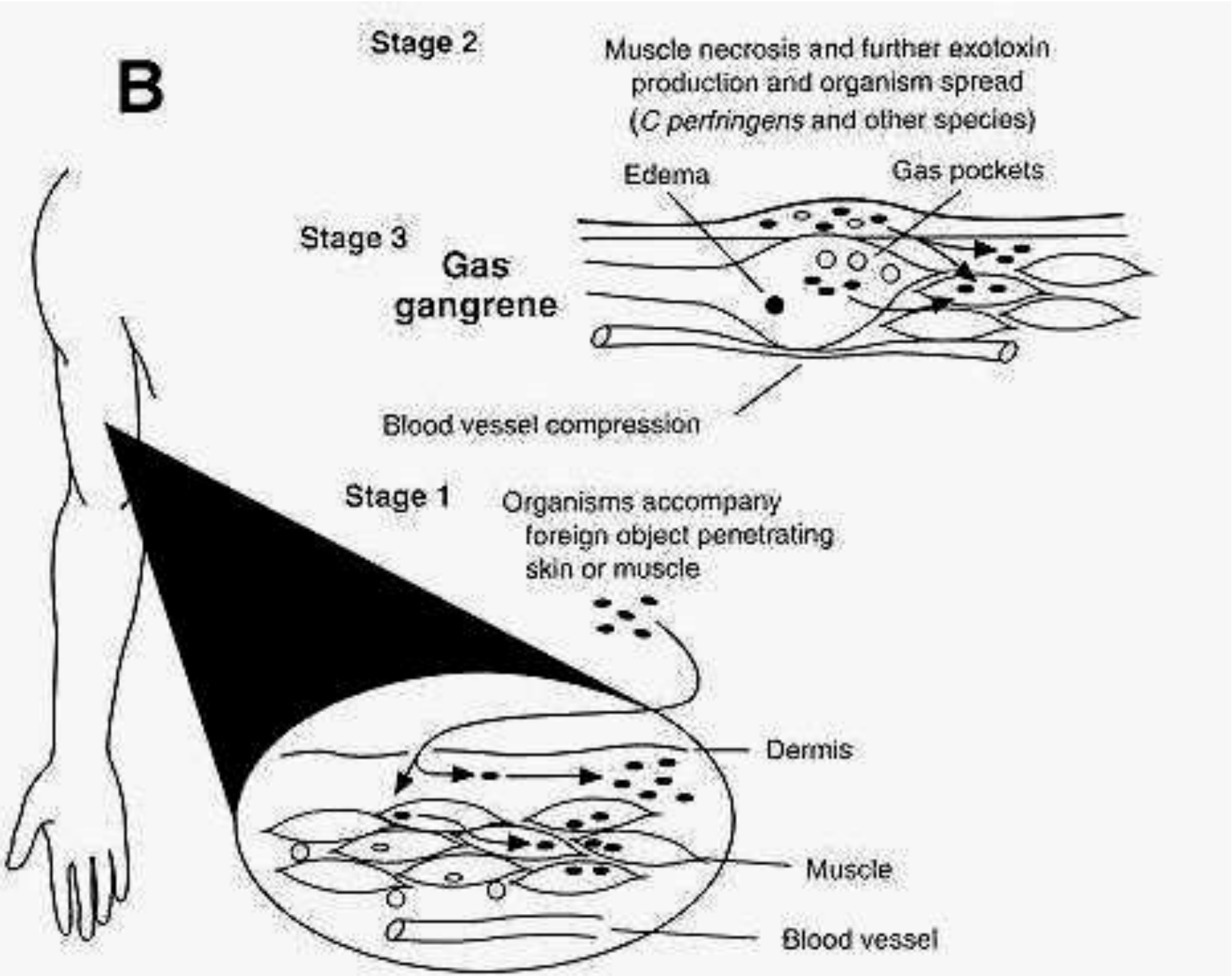
Gas gangrene of leg



Gas gangrene (anaerobic wound infection): pathogenesis and pathology




A
1. Entry
(penetrating wound)
2. Multiplication (in dead tissue, anaerobic conditions)
3. Toxin production
Collagenases
Lipases
Proteases
Hemolysins
4. Disease: gas gangrene
Muscle necrosis
From: Toxins
Edema
Gas pockets
Blood vessel compression
Necrosis


B
Stage 2
Muscle necrosis and further exotoxin production and organism spread (C perfringens and other species)
Edema
Gas pockets
Stage 3
Gas gangrene
Blood vessel compression
Stage 1
Organisms accompany foreign object penetrating skin or muscle
Dermis
Muscle
Blood vessel

Anaerobic wound infection: laboratory diagnostics

specimens (exudate from wound, pus, wound tissue)

⇓

isolation of pure culture

⇓

identification by biochemical reactions (sugars and milk utilisation)

⇓

revealing of toxin production

(testing of its neutralisation by specific antitoxin with use of laboratory animals)

- Modern gas chromatographic techniques could be used for identification of fermentation products: short chain fatty acids.

Anaerobic wound infection: immune prophylaxis and immune therapy

Immune prophylaxis

- Use of anatoxins (toxoids) for active immunisation
  - perfringens
  - oedematiens

Immune therapy

- Use of antitoxins
  - antiperfringens
  - antioedematiens
  - antisepticum

Anaerobic wound infection: treatment

- The most important aspect of treatment is prompt and extensive surgical debridement of the involved area and excision of all devitalised tissue.
- Administration of antimicrobial drugs (penicillin).
- Nowadays, treatment (including anti-toxin, antibiotic therapy, debridement) is extremely effective and amputation and death is rare if the treatment has been started in time.

Clostridium tetani: main characteristics

- Strictly anaerobic rods, produces spherical terminal spores which give the cells a drumstick appearance
- Could be easily isolated from the soil and also can be found in the intestinal tract of humans and animals
- Cause hemolysis on blood agar where the cultured cells form fine filaments of growth
- Metabolically not very active, nonproteolytic but produces gelatinase

Cl. tetani - *drumstick appearance*

Clostridium tetani: factors of pathogenicity

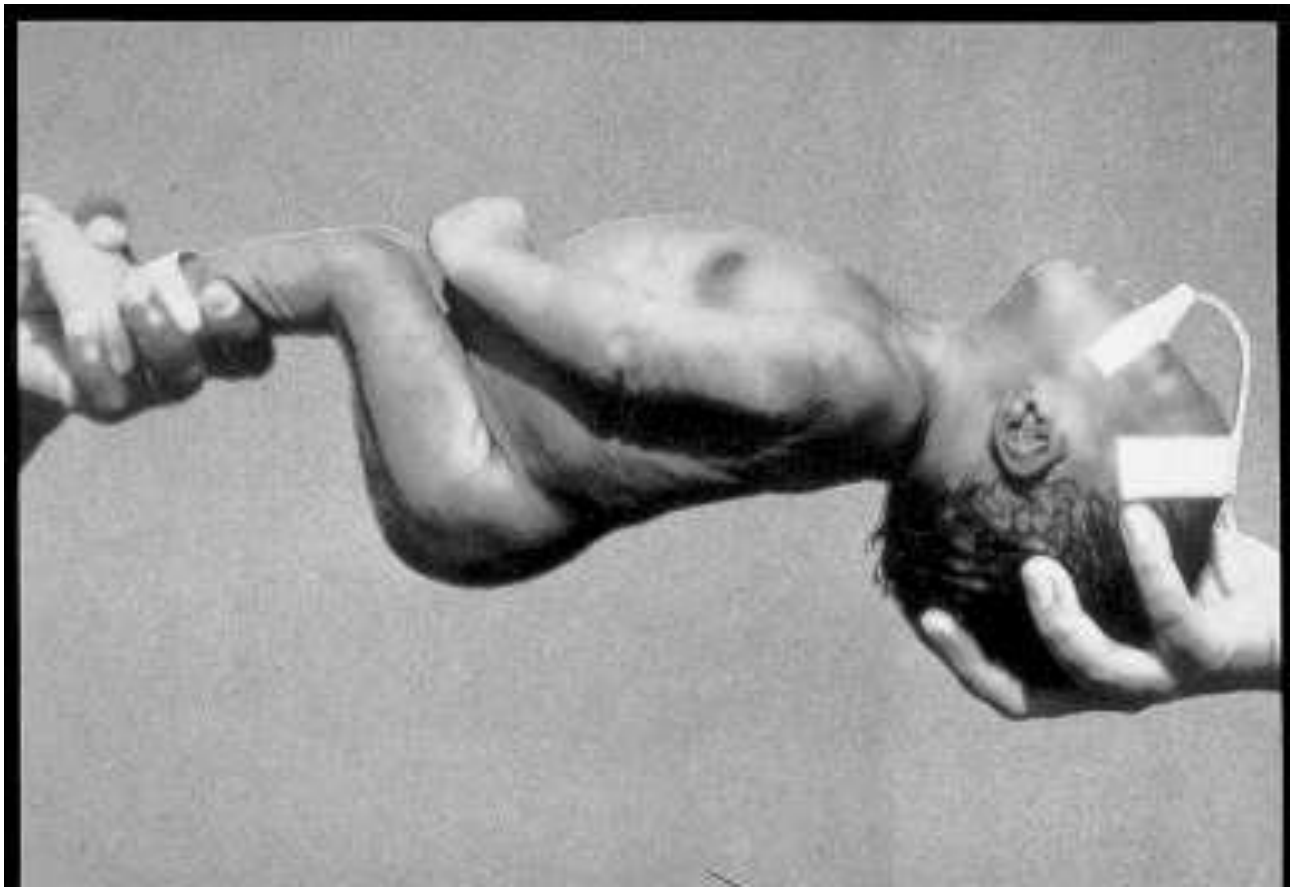
- ***Cl. tetani is the etiologic agent of tetanus***

- **Cl. tetani is not an invasive bacteria, the infection remains localised in the area of devitalised tissue (the volume of infected tissue could be small).**

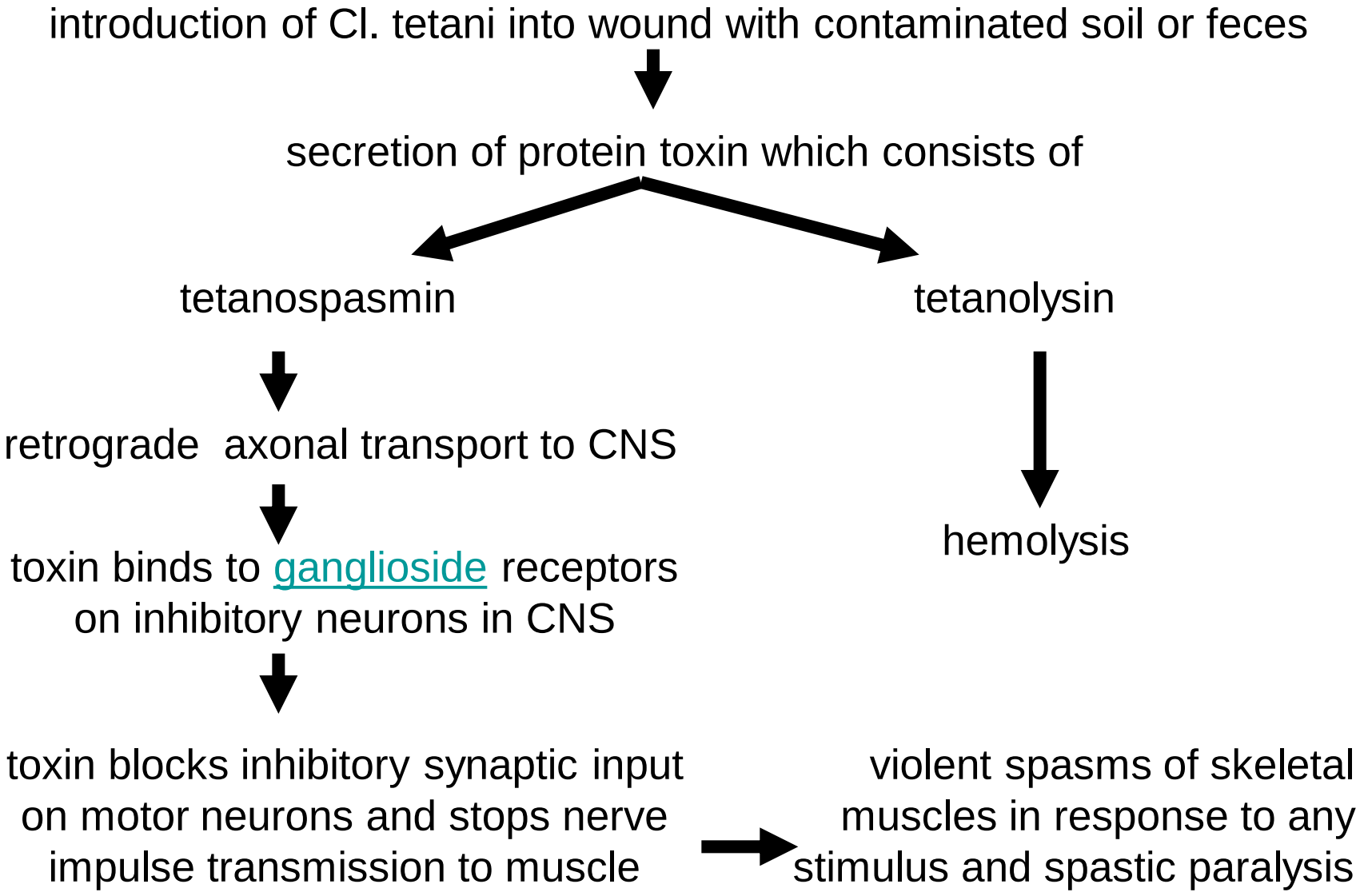
- **The disease is a result of toxemia. Cl. tetani produces neurotoxic exotoxin *tetanospasmin* and hemolytic *tetanolysin* (it is of minor importance in pathogenesis of tetanus).**

- **Tetanus kills most of the babies who get infection when newly cut umbilical cord is getting contaminated by Cl.tetani.**

The baby who has neonatal tetanus



Tetanus: pathogenesis and pathology



Severe case of tetanus

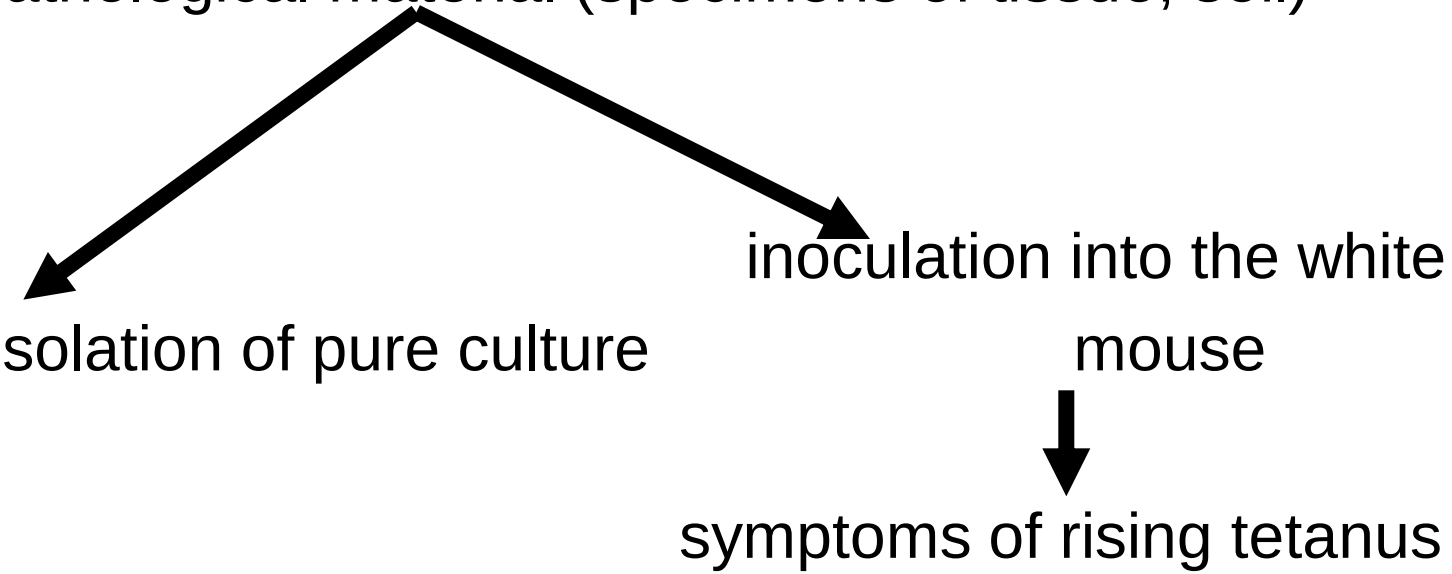
Tetanus: laboratory diagnostics

•Diagnostics rests on the clinical picture in clinical cases. But laboratory diagnostics is important in the cases:

• of the necessity to check possible contamination of medical materials (bandages, etc)

•epidemiological measures for control of the contamination of the soil

•pathological material (specimens of tissue, soil)



Tetanus: immune prophylaxis and immune therapy

Immune prophylaxis

- tetanus anatoxin (toxoid)
  - DTP – toxoids of diphtheria and tetanus combined with pertussis bacterial antigen (suitable for young children)
  - Td – toxoids of diphtheria and tetanus (suitable for adults)

Immune therapy:

use of antitoxin to neutralise the toxin *but only before it becomes fixed onto nervous tissue* (it is preferable to use human tetanus immunoglobulin + anatoxin).
Tests for hypersensitivity to the foreign serum protein must be done.

Tetanus: treatment

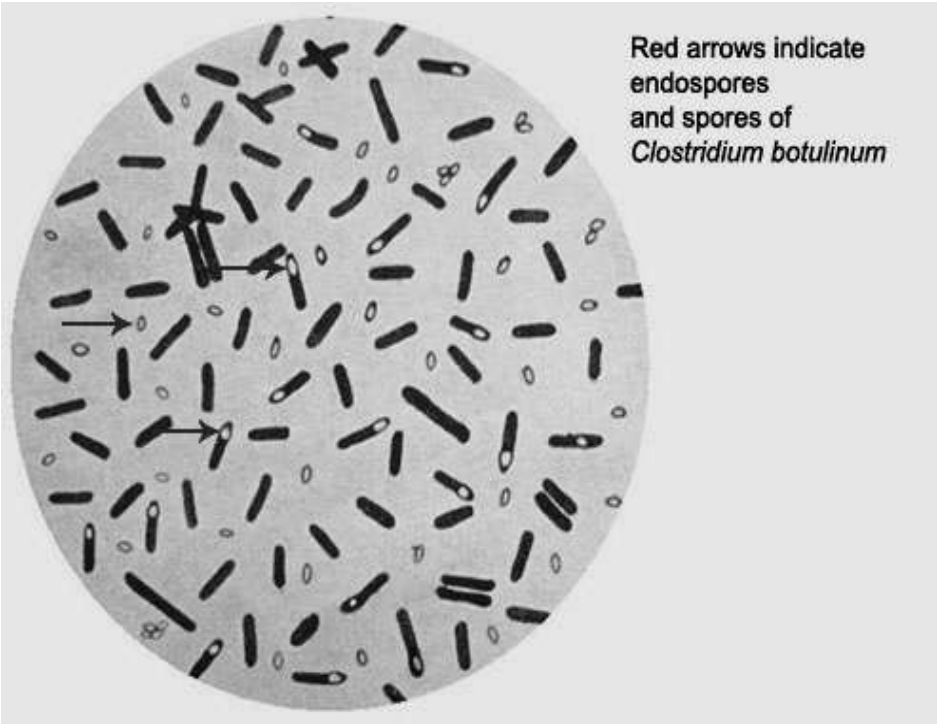
- Surgical debridement is vitally important because it removes necrotic tissue that is essential for proliferation of the bacteria.
- Penicillin strongly inhibits the growth of Cl.tetani and stops further toxin production.

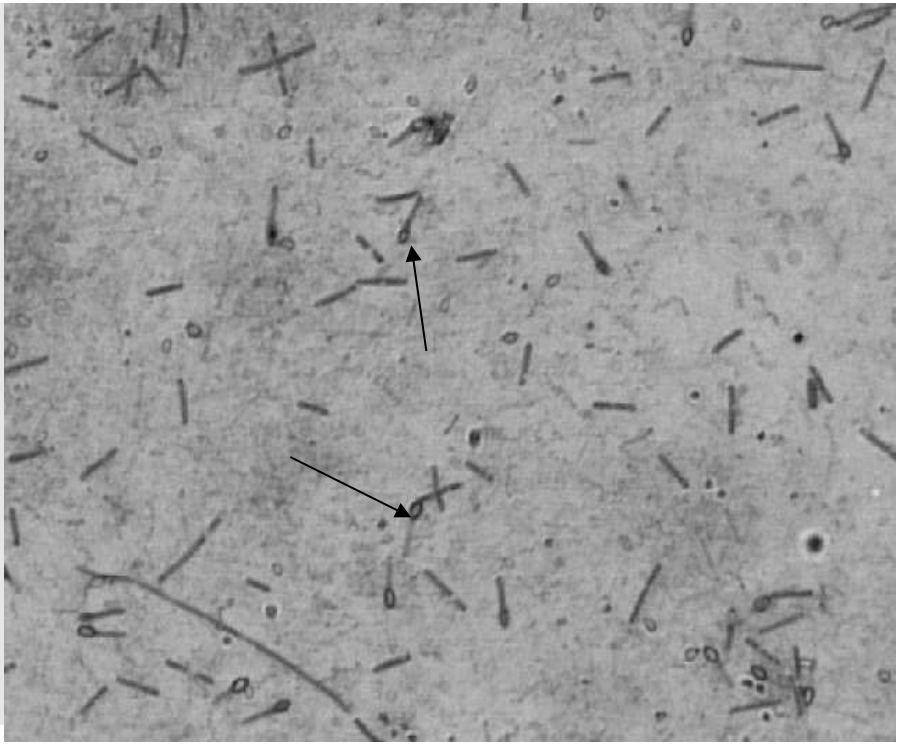
*But the treatment is satisfactory **before** the toxin becomes fixed onto nervous tissue.*

Clostridium botulinum: main characteristics

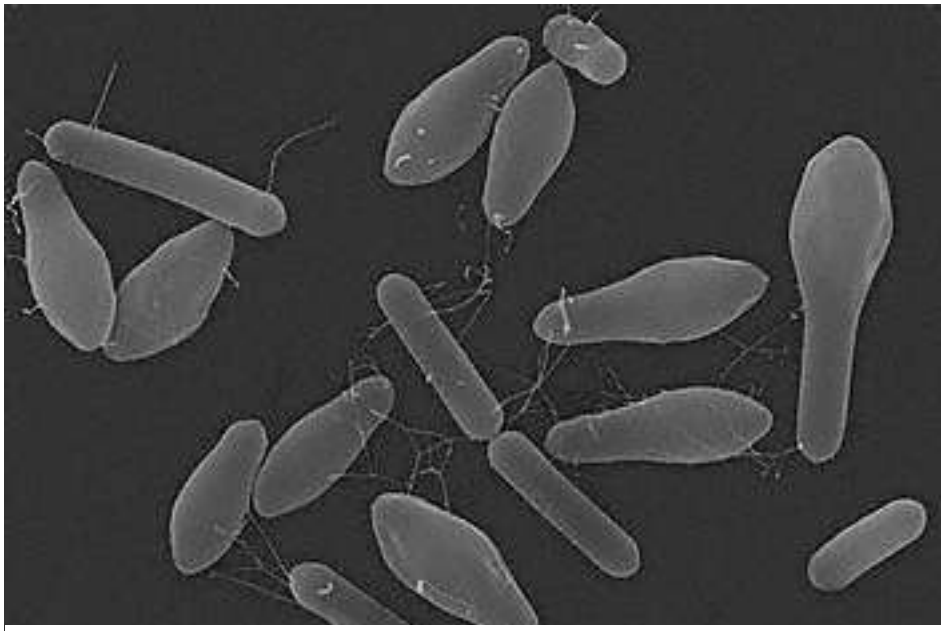
- Large and motile gram-positive rods. The spores are oval and subterminal and give the cells a tennis-rocket appearance.
- Colonies are translucent and can be circular or irregular shaped.
- Ferments glucose and maltose.
- The microorganism is worldwide in distribution, it is found in soil contaminated by animal feces.
- Cl.botulinum produces ***botulism***.
- Clinical form of disease is food poisoning: ingestion of the neurotoxin in food (smoked, vacuum-packed or canned);
- In infants it is known like floppy baby syndrome (result of ingestion of spores followed by germination and toxin production).

Cl. botulinum - a tennis-rocket appearance





Cl. botulinum



Clostridium botulinum: factors of pathogenicity

- ***Produces the most highly toxic substances known botulinum toxin: 1 mg contains more than 20 million mouse lethal dose. The lethal dose for human is less than 1µg.***
- The different types of Cl.botulinum show some degree of host specificity: types A, B and E cause botulism in humans.
- Botulinum toxin binds to receptors on peripheral nerves.
- The toxin acts by blocking acetyl-choline release at synapses and neuro-muscular junctions.
- Flaccid paralysis is the result of its action.
- Death from respiratory and/or cardiac failure is often result of botulism.

Botulism: pathogenesis and pathology

getting of spores or toxin into digestive tract (from contaminated food)

⇓

binding of the toxin with the cells at the surface of mucous membranes in intestine

⇓

getting of the toxin into the blood-stream (toxinemia)

⇓

destruction of peripheral nerves (spinal α-motor neurons)

⇓

blocking acetylcholine release or production at synapses and neuro-muscular junctions

⇓

flaccid paralysis results (ocular paresis, pharyngeal paresis)

⇓

paralysis of diaphragm and other muscles

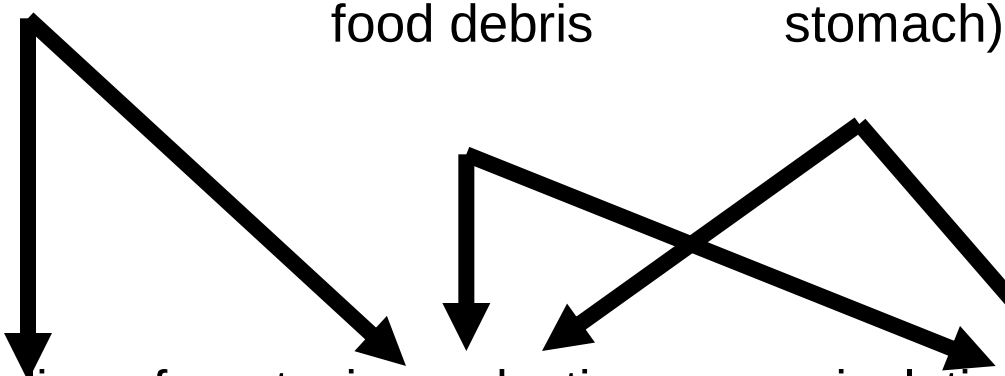
⇓

death results from respiratory paralysis or cardiac arrest

Botulism: laboratory diagnostics

SPECIMENS

blood | stomach washing fluids, food debris | Biopsy material (liver, intestine, stomach) | feces



revealing of antitoxic Ig

- RIHA (agglutination of red cells coated with specific antiserum)

toxin production revealing

- NR: injection into mice
- ELISA, RIHA

isolation of pure culture

(using the usual for isolation of clostridia algorithm)

Botulism: immune prophylaxis and immune therapy

Immune prophylaxis

- toxoids (specific for every biological variant of clostridia)

Immune therapy

antitoxins (specific sera)

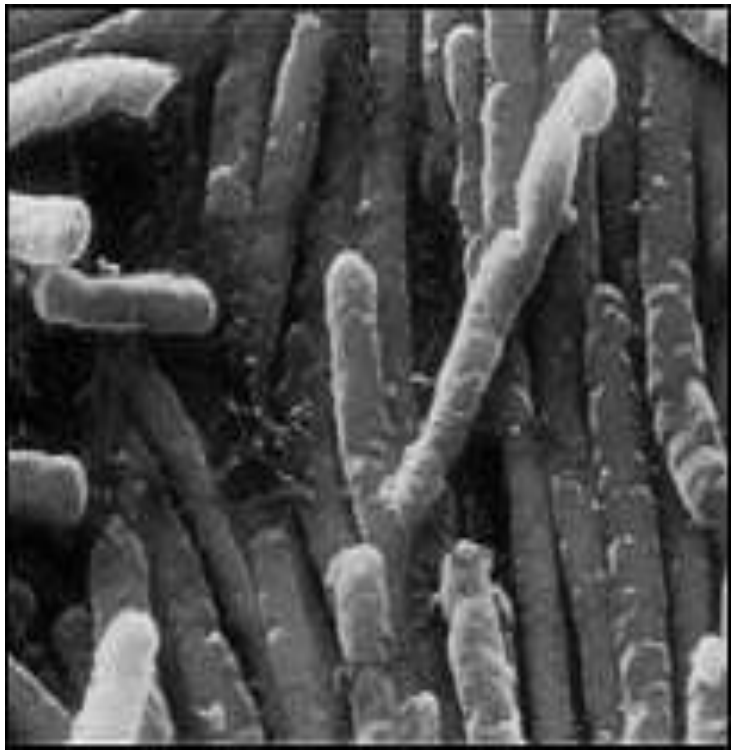
- polyvalent
- monovalent

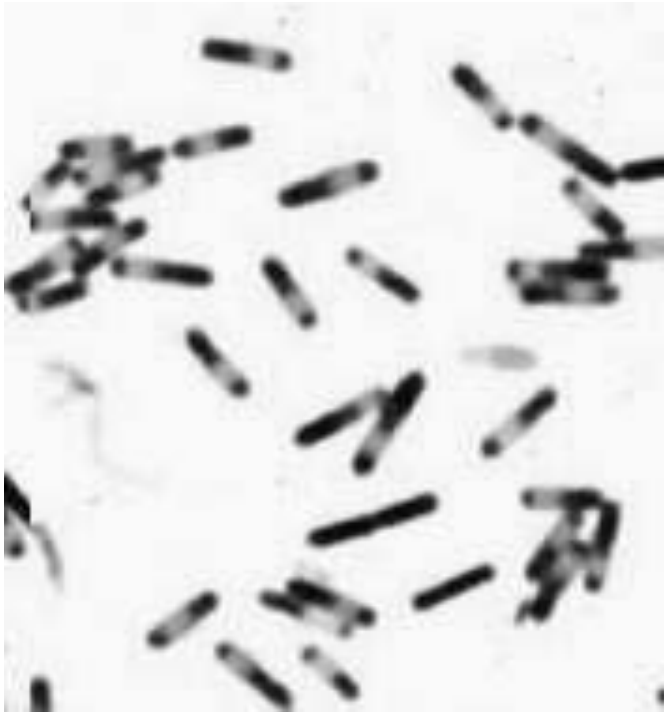
Botulism: treatment

- The administration of antitoxin intravenously as early as possible.
- The measures directed on clearance the gastro-intestinal tract of the toxin.
- Guanidine hydrochloride is given a an adjunct, and respiration is maintained artificially.
- Supportive treatment of infants is based on helping them breath and on tube feeding.
- Adults may also require a respirator and possibly a tracheotomy.

Clostridium difficile

this bug can be fatal in older people





C. difficile: the role in human disease

belongs to normal intestinal flora

⇓

therapy with use of antibiotics resulted in appearance of antibiotic resistant strains and their overgrowth

⇓

produces enterotoxin which cause cytopathic effect on intestinal epithelial cells

⇓

hospital infection

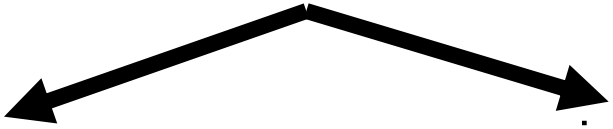
⇓

antibiotic-induced pseudomembranous colitis

- *C. difficile is a common hospital-acquired infection which usually causes diarrhoea but can lead to fevers or more serious infections.*
- *Nearly 500 cases of the super bug were recorded at hospitals in Cannock and Stafford in the past two years.*

Bacteria – infectious agent causing food poisoning infections

Food poisoning caused by bacteria



toxic infections

bacteria multiply in digestive tract

⇓

partial death of bacteria

⇓

getting of the cell debris into food

⇓

spoilage

- Proteus
- Bacillus cereus
- Vibrio parahaemolyticus

intoxication

secretion of protein toxins

⇓

food poisoning

- C. perfringens
- C. botulinum
- S. aureus (enteric toxin)

Corynebacterium. Bordetella. Haemophilus. Legionella

Theme N24

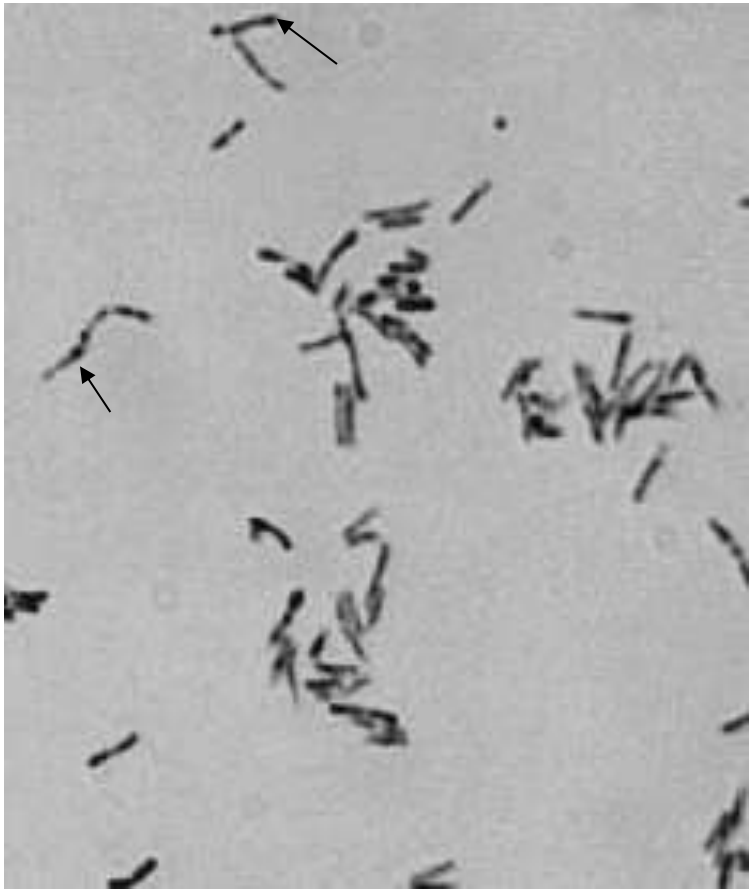
Corynebacteria:main characteristics

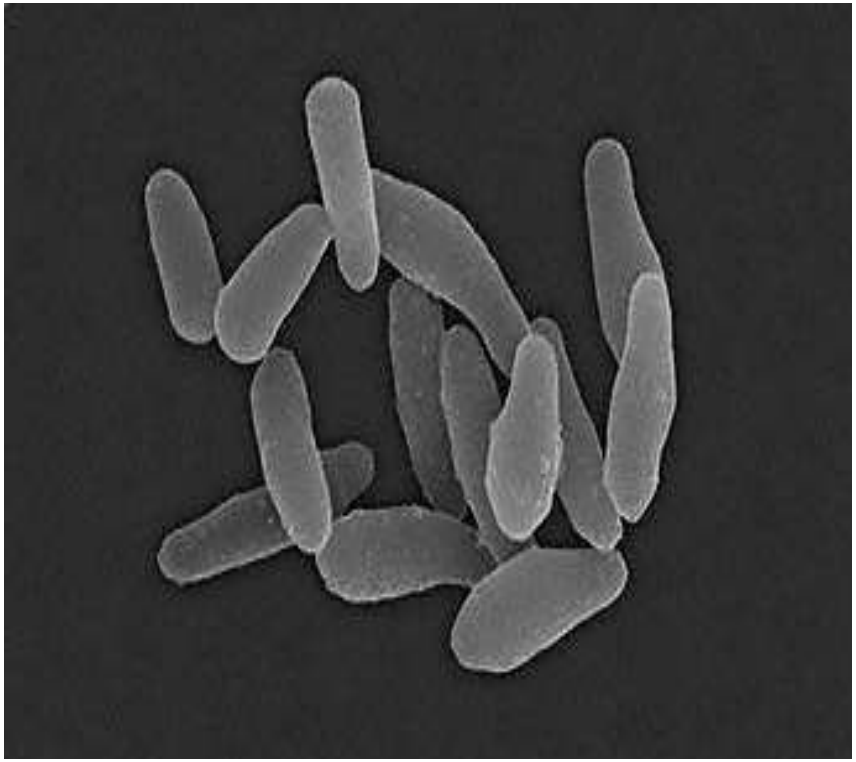
Corynebacterium diphtheriae:

- small, gram-positive rods;
- nonmotile;
- asporogenous;
- some rods have club-shaped ends and intracellular polyphosphate granules known as metachromatic bodies or Babes-Ernst bodies (could be revealed when the bacteria stained by Loeffler's or Neisser's techniques);
- the rods are often found in the smear in characteristic arrangements, resembling "Chinese letters" or palisades (they occur in pairs at acute angles to one another resembling letters V, Y, X);
- they grow on Loeffler's coagulated serum medium; the colonies are small, granular and grey with irregular edges: R-shaped (resembling daisies) – typical for virulent variant ***gravis;***
- characteristic black colonies are seen on tellurite agar (result of precipitation of tellurium on reduction by the bacteria);
- ***Corynebacterium diphtheriae causes diphtheria in humans.***

Corynebacterium diphtheriae.

- **Babes-Ernst bodies**





Corynebacteria: factors of pathogenicity

- Toxigenic C.diphtheriae produces a powerful protein **exotoxin** (can be lethal in a dose of 0.1μg/kg of weight) which causes arrest of protein synthesis in the cells and is responsible for the necrotic and neurotoxic effects.
- The gene for toxin synthesis is encoded on a bacteriophage (the tox gene). Corynebacteria, not infected with phage do not generally cause diphtheria.

Diphtheria: pathogenesis

C.diphtheriae spread by droplets or contact (wound or skin diphtheria) from ill human to susceptible person

↓

get into respiratory tract, grow on mucous membranes and produce toxin

↓

toxin causes destruction of epithelium and grayish “pseudomembrane” is formed

↙ nonremovable (when localised on the surface of multi-layered epithelium: in the tonsils, pharynx or larynx)

↘ removable (when localised on the surface of one-layered epithelium: lower respiratory tract) – leads to obstruction, swelling

↓

diphterial croup

↓

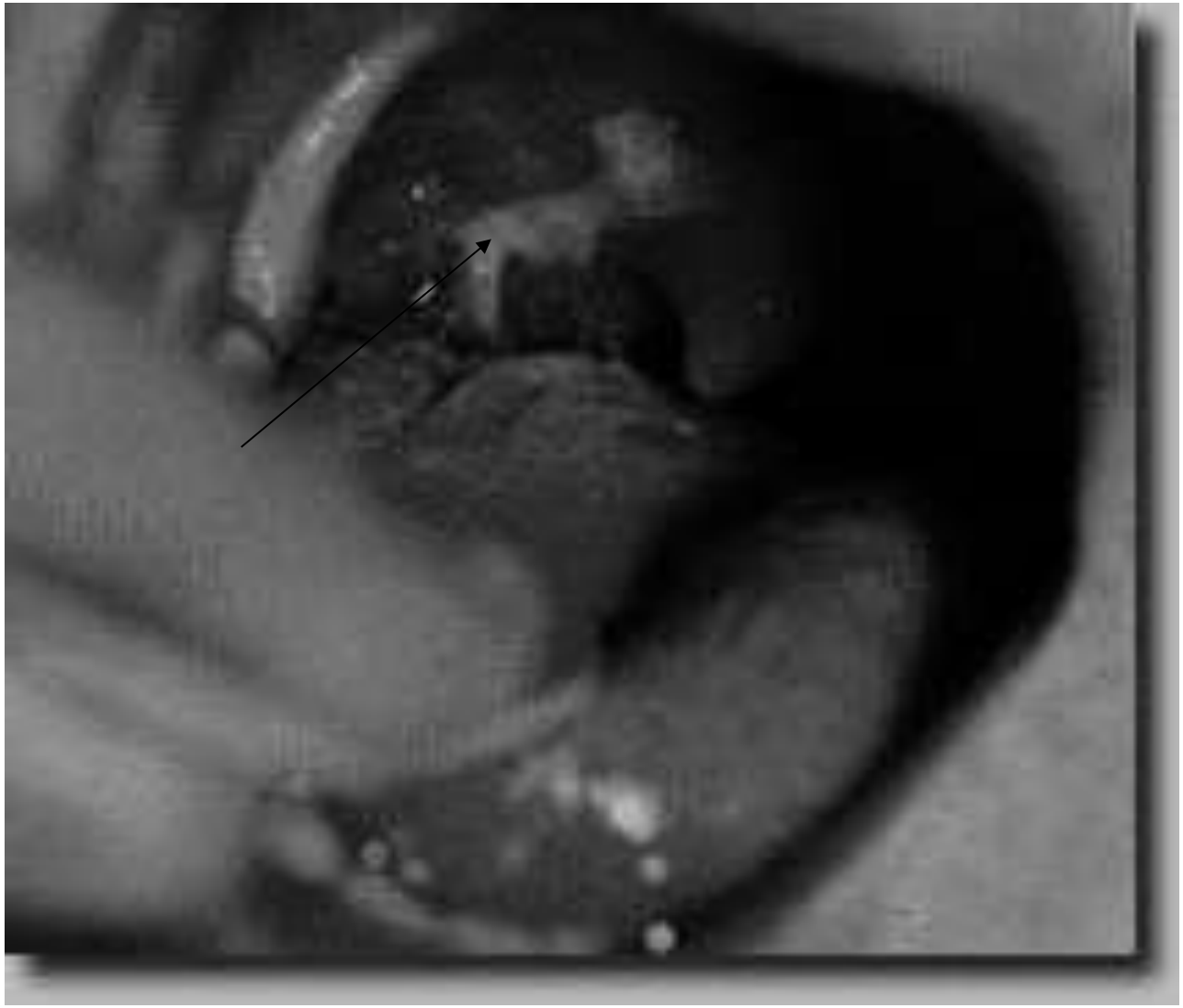
distant toxic damage as a result of toxinemia

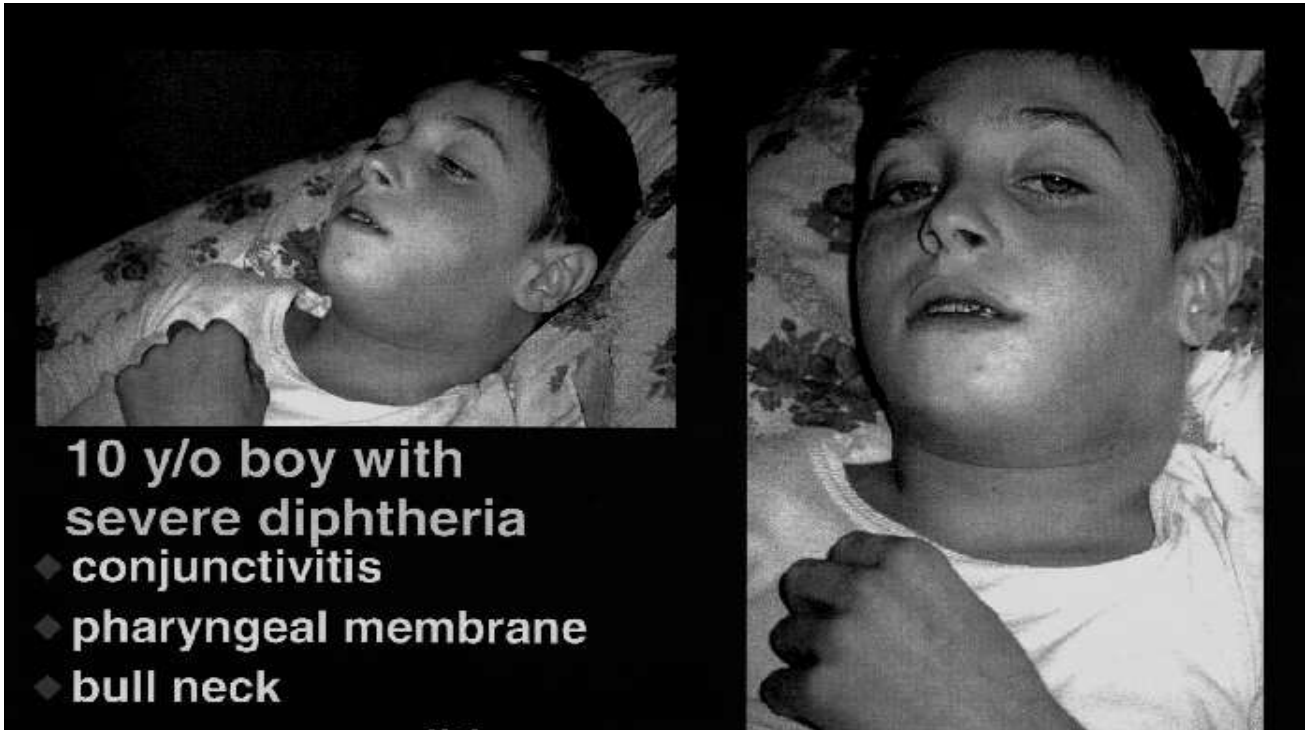
↓

•intoxication; <u>necrosis of heart muscle, kidneys and adrenals;</u> nerve damage: paralysis of the soft palate, etc

→ complications

Diphtherial “pseudomembrane”




10 y/o boy with
severe diphtheria
conjunctivitis
pharyngeal membrane
bull neck

Diphtheria: laboratory diagnostics

Swabs from the nose, throat (<3 hrs)

smear stained to reveal metachromatic granules

preliminary diagnostics

isolation of pure culture using special media

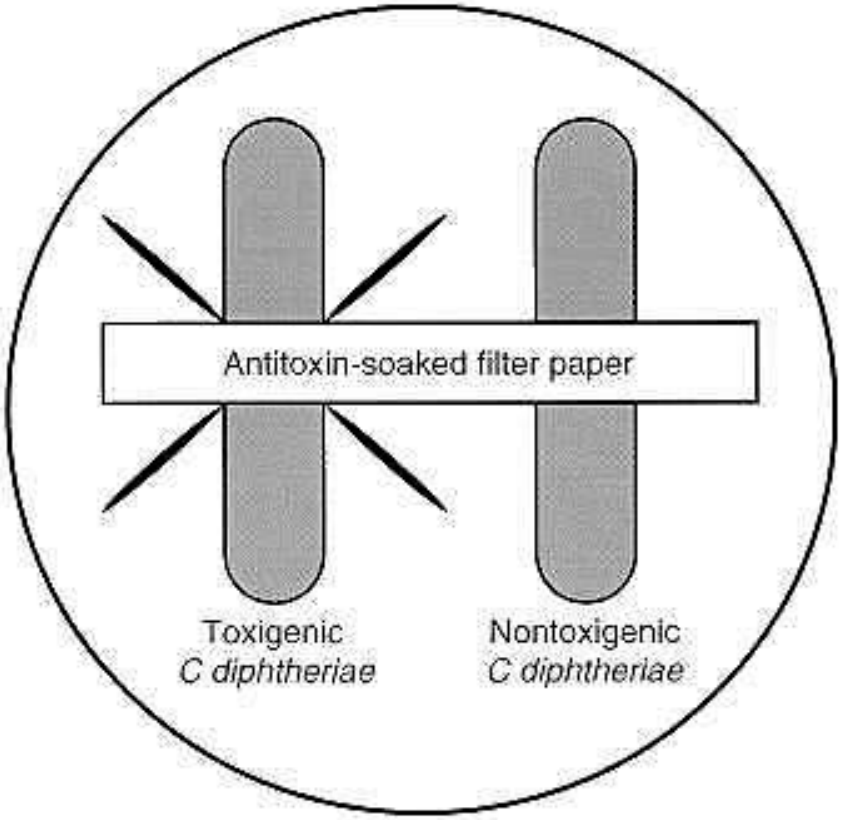
identification

- Growth characteristics
- Biochemical tests

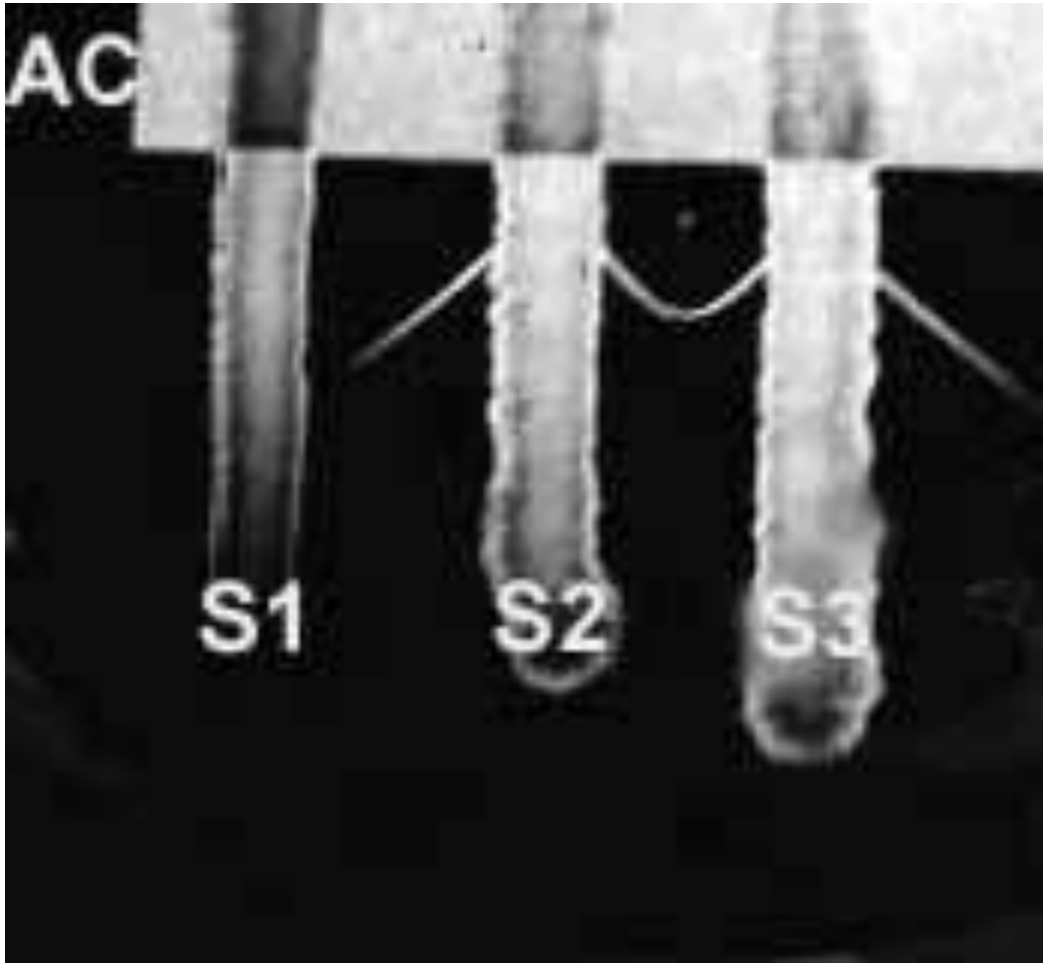
- in vivo test (guinea pig)
- in vitro (immunodiffusion test)

} “virulence” test

Double immunodiffusion (by Ouchterlony) for revealing of toxigeniciy of C.diphtheriae



Double immunodiffusion (by Ouchterlony) for revealing of toxigeniciy of C.diphtheriae



Diphtheria: immune prophylaxis and immune therapy

Immune prophylaxis

- Diphtheria anatoxin in conjunction with pertussis and tetanus (vaccine DTP) and vaccineTd provides immunisation for 10 years.

Immune therapy

- Antitoxin is administrated when there is a strong clinical suspicion of diphtheria. It inhibits the growth of bacilli.

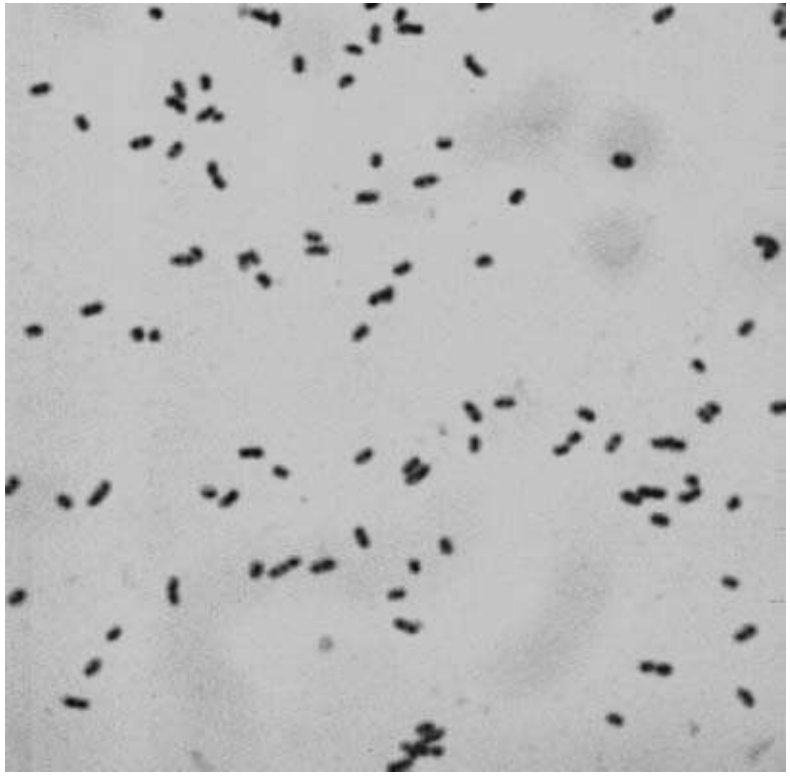
Diphtheria: treatment

- Treatment with antitoxin (see previous slide).
- Antimicrobial drugs (penicillin, erythromycin) inhibit the growth of diphtheria bacilli.

Bordetella: the main characteristics

- Gram-negative, non-motile, short ovoid rods - cocobacilli.
- The bacteria grow slowly on complex enriched media. Commonly employed is Bordet- Gengou's medium (potato-blood-glycerol agar), on which small, convex, smooth colonies with a pearl-like luster develop in 38-72 hrs.
- They are highly sensitive to unfavorable conditions and survive only brief period outside the human organism.
- They are not active metabolically.
- ***Bordetella pertussis*** produces **whooping cough**.
- ***Bordetella parapertussis*** produces a disease similar to whooping cough but the infection is often subclinical.
- Most of the patients with whooping cough are less than a year old although older children may also get the disease.
- The organism, contained in aerosol droplets, gains access via inhalation and colonizes the bronchial ciliary epithelial cells.

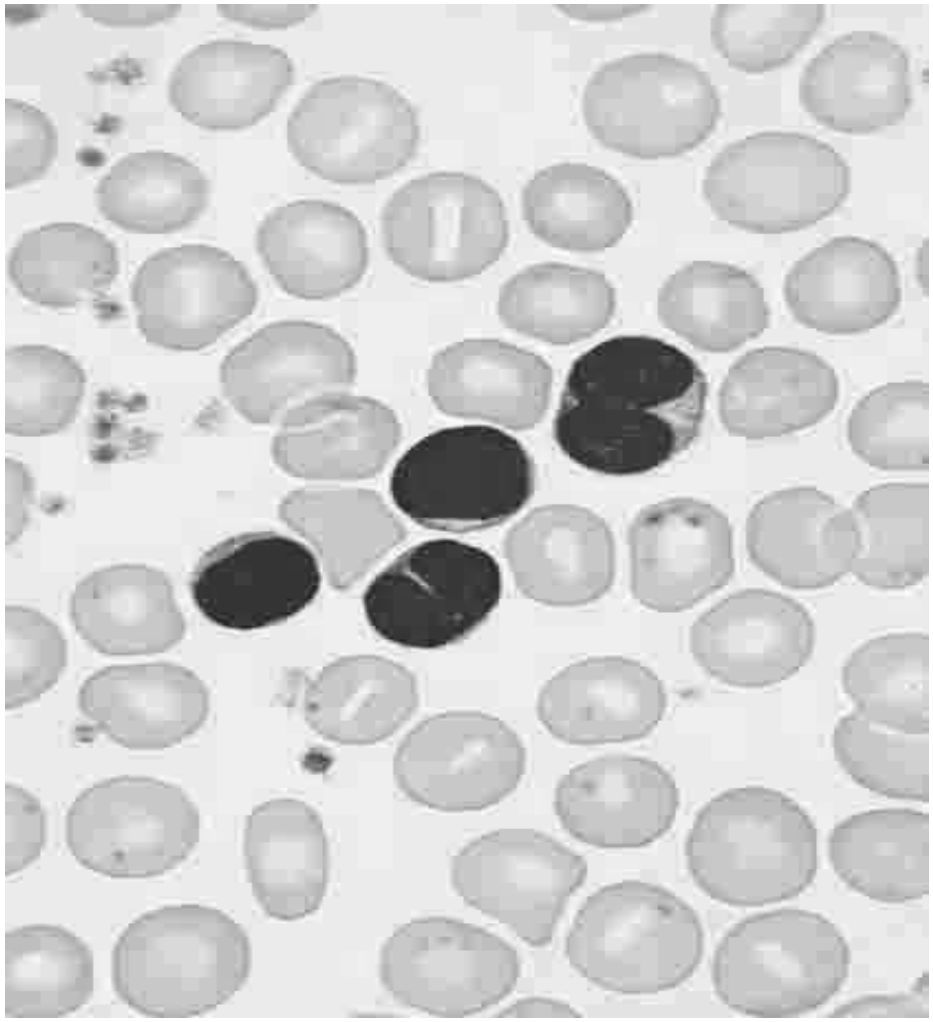
Bordetella - coccobacilli



Bordetella: factors of pathogenicity

- **Pertussis toxin** (pertussigen) which causes sensitisation that irritates surface cells, giving rise to catarrhal symptoms and causing marked lymphocytosis. It also contributes to binding of bacteria to ciliated epithelial cells of the respiratory tract.
- **Tracheal cytotoxin** interferes with ciliary movement, causes ciliated epithelial cell destruction. Later there may be necrosis of parts of epithelium and polymorphonuclear infiltration with peribronchial inflammation.
- **Dermonecrotic (heat-labile) toxin -** a very strong vaso-constrictor causes ischemia and extravasation of leukocytes and, in association with tracheal cytotoxin, causes necrosis of the tracheal tissue.
- **Filamentous haemagglutinins (agglutinogens)** – permit the adherence of the bacteria to the ciliated epithelium of the respiratory tract.

BLOOD LYMPHOCYTOSIS IN A PATIENT WITH PERTUSSIS



Whooping cough: pathogenesis

B.pertussis (the source of the infection is an infected human)

⇓

respiratory route is the main way of getting into the human organism

⇓

adhesion and multiplication on the surface of epithelium
in the trachea and bronchi (the blood is not invaded)

⇓

inflammation and swelling

⇓

liberation of the toxins

⇓

irritation of the surface cells, necrosis of part of the epithelium

⇓

obstruction of the smaller bronchioles by mucous plugs

⇓

cough characterised by explosive character and “whoop” upon inhalation

Whooping cough - the paroxysmal stage

Whooping cough: stages of the disease

- **The catarrhal stage** develops after an incubation period (about 2 weeks): mild coughing and sneezing. *The patient is highly infectious.*
- **The paroxysmal stage**: the cough develops its explosive character and characteristic “whoop” upon inhalation. Disease could lead to rapid exhaustion and may be associated with vomiting, cyanosis and convulsions.
- The **convalescent stage:** the cough episodes slowly decrease and there is gradual recovery over 3-16 weeks.

Whooping cough: laboratory diagnostics

The basic method

- Isolation and identification of pure culture:

  specimens are nasopharyngeal swabs or cough droplets expelled onto a “cough plate” held in front of the mouth of patient during a paroxysm.

  The bacteria are growing on Bordet- Gengou’s medium and produce 1-mm droplet colonies surrounded by a zone of hemolysis.

  Final identification - by slide agglutination with specific antiserum or direct immunofluorescent staining of a smear made of nasopharyngeal swabs.

Retrospective diagnosis

(it is necessary to remember that the rise of in agglutinating or precipitating antibodies does not occur until the third week of illness)

- Serologic tests
  - RA
  - RIHA
  - CF reaction

Whooping cough: specific prophylaxis

1. *Active immunization: DTP vaccine (*toxoids of diphtheria and tetanus combined with a killed whole pertussis bacteria) is normally administrated to every infant during the first year of life.
2. *Passive immunisation with hyperimmune globulin administrated to:*
   - infants exposed to whooping cough
   - persons without prior immunization

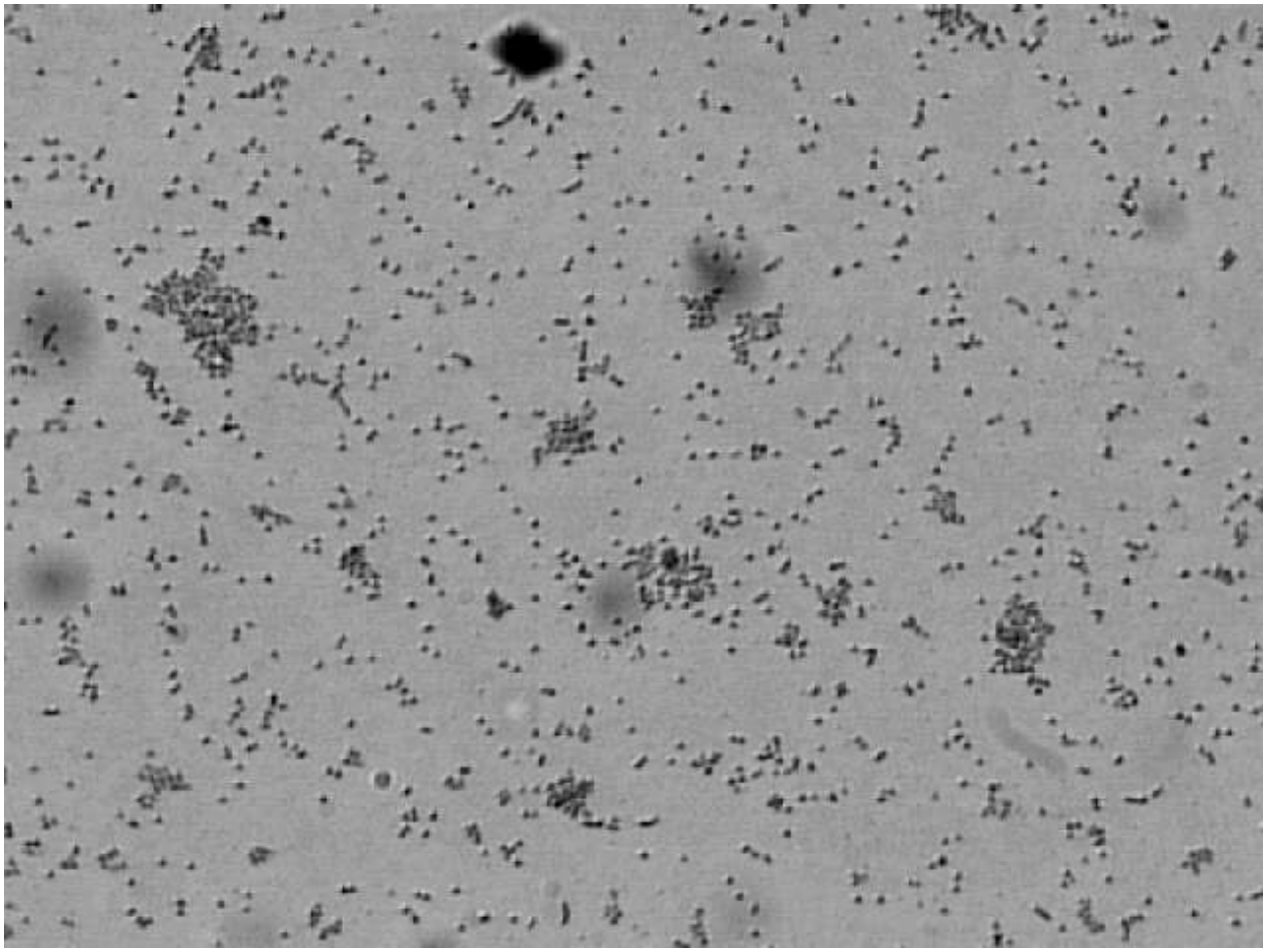
Whooping cough: treatment

- Administration of erythromycin or ampicillin during the catarrhal stage promotes the elimination of the organisms and may have prophylactic value.
- Oxygen inhalation (fresh air) may prevent anoxic damage to the brain.

Haemophilus influenzae: the main characteristics

- Short coccoid bacilli (in specimens from acute infections).
- The definite capsule is developed in pathogenic b serotypes.
- The bacteria are growing on “chocolate” agar (the medium containing heated blood) during 36-48 hrs. Colonies are small round and convex – S-shaped.

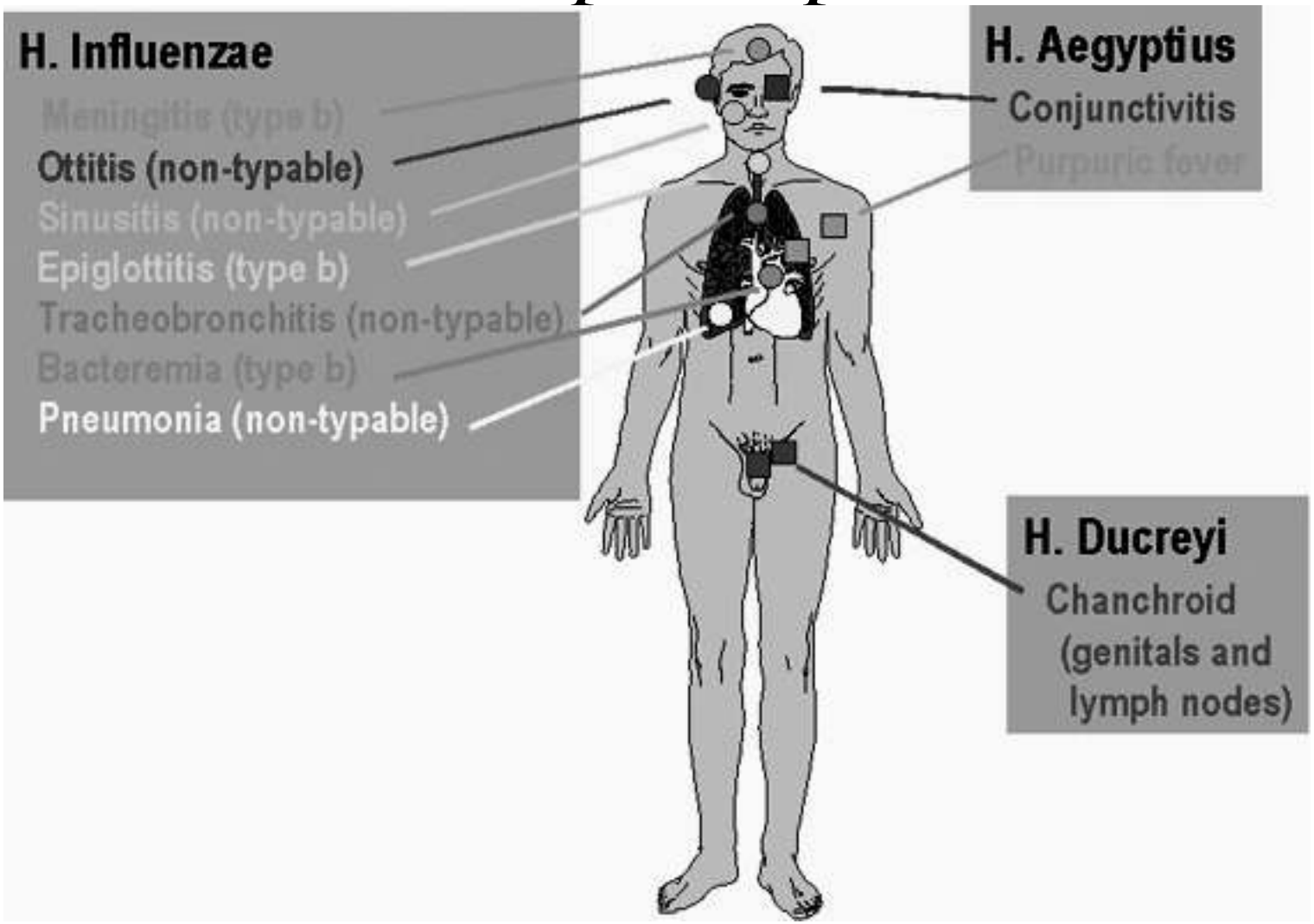
Haemophilus influenzae – coccoid bacilli



Haemophilus influenzae: the role in human disease

1. H.influenzae produces no exotoxins.
2. The encapsulated type b produces respiratory infections: sinusitis, laryngotracheitis, acute epiglottitis (swallen, red and edematous epiglotis) – serious disease in children.
3. In young children produces meningitis.
4. In adults produces pneumonia.

Clinical symptoms of infection produced by Haemophilus species



Haemophilus influenzae: laboratory diagnostics and prophylaxis

- Several serological test have been developed for identification of clinical specimens:

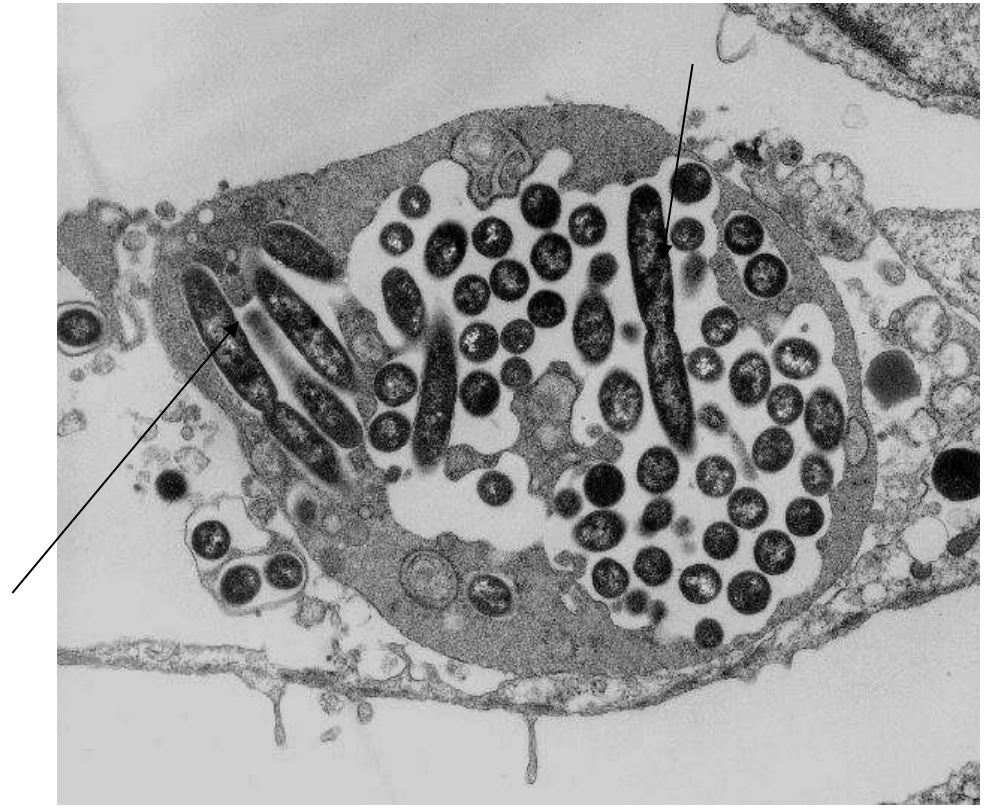
  - immunofluorescence with fluorescently - labeled antibodies,

  - immunoelectrophoresis.

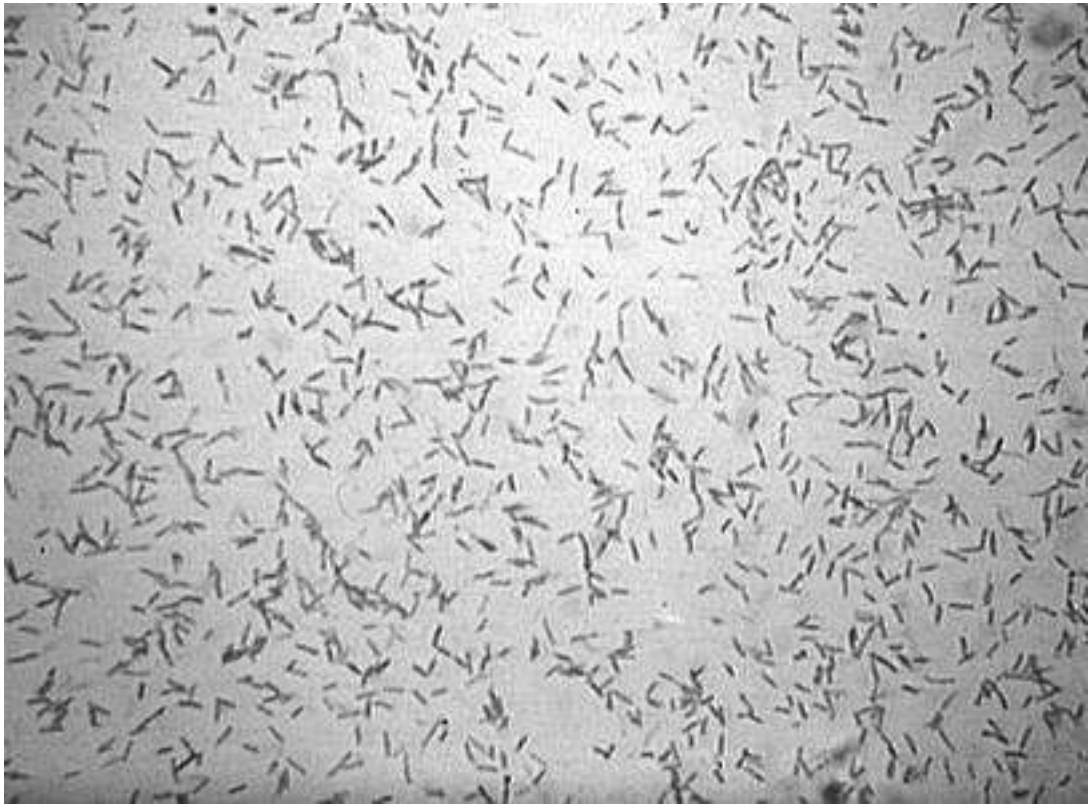
Legionella pneumophila: the main characteristics

- The bacteria are thin, gram-negative rods.
- They are motile with polar flagella.
- Legionellae have complex nutritive requirements: grow on the media with addition of cysteine and soluble iron (provided in the form of hemoglobin). The aerobic conditions are better for growth.
- During 3-5 days produce S-shaped pigmented colonies.
- It resides in the environment in pools of stagnant water and is found as an intracellular agent within protozoa.
- It often infects hot water towers and air conditioning systems
- In man, it also survives as a facultative intracellular pathogen.

Legionella pneumophila multiplying inside a cultured cell



Legionella pneumophila – thin rods



Legionnaires' disease

- *Legionnaires' disease (legionellosis)* is the name given to an outbreak of pneumonia with several fatalities that first was documented among American Legion conventioneers at Philadelphia in 1976. The etiologic agent was identified as a new genus and species *Legionella pneumophila*.
- Small epidemics of Legionnaire's disease in apparently healthy people can occur but the microorganism is more frequently observed in sick or elderly individuals whose immune responses may be compromised.

Legionella pneumophila: pathogenesis of Legionnaires' disease

Legionella pneumophila

(outbreaks of disease associated with air-conditioning system, showers, lawn-sprinkling systems and disrupted soil, fresh water reservoirs are also the sources of infection)

⇓

bacteria inhaled in aerosolised water and getting into respiratory tract (lungs) ⇒ produce patchy or interstitial infiltration which is present in lungs ⇒ focal necrosis occurs in alveoli and pleura (a severe progressive pneumonia develops) ⇒bacteremia (could occur)

⇓

endotoxin

⇓

can cause dysfunction of

- vascular system
- gastrointestinal tract
- CNS
- kidneys
- liver

Legionnaires' disease: laboratory diagnostics

Specimens
(lung aspirate, lung biopsy)

serum

immunoflurescent stain of smears

isolation of pure culture

(ELISA, immunofluorescent technique)

- ✓ complex solid media
- ✓ chick embryo
- ✓ inoculation into guinea pigs

PCR tests are commercially available

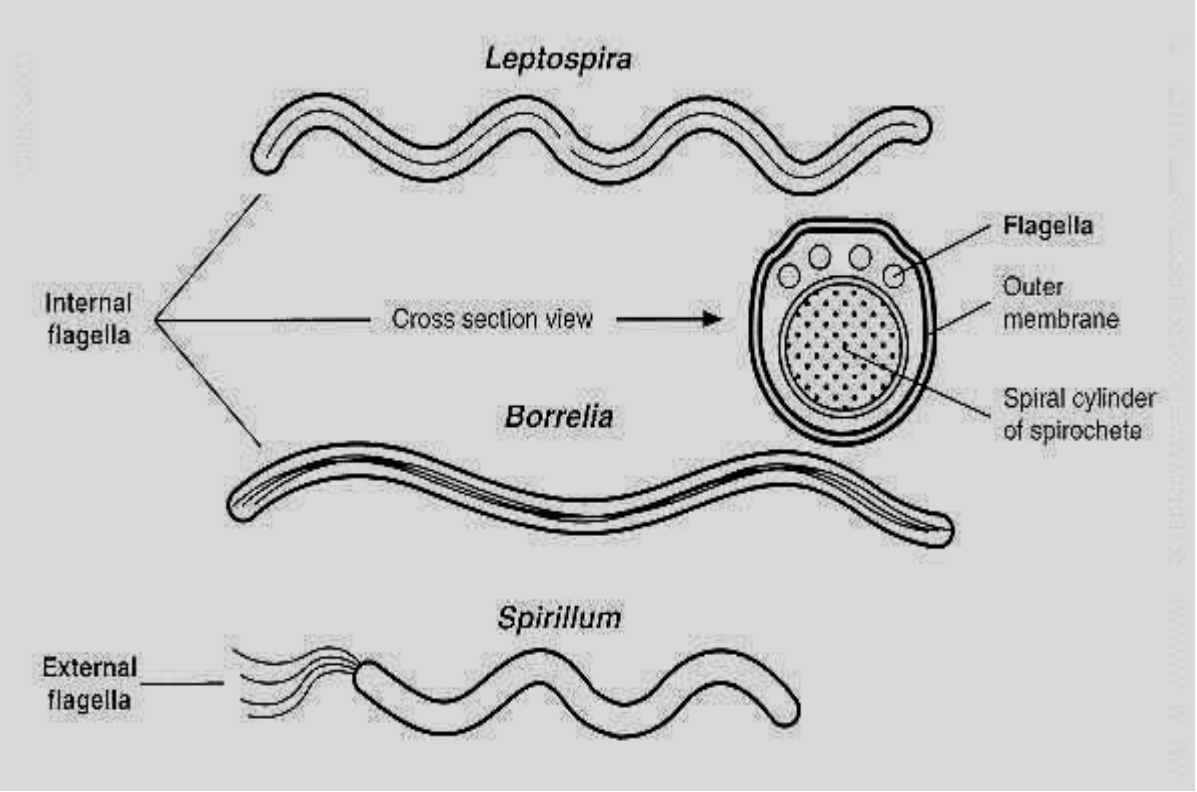
Legionella pneumophila:
Pontiac fever

- The same microorganism is responsible for *Pontiac fever* a different respiratory syndrome first documented in Pontiac, Michigan, in 1968.
- That is self-limited, nonfatal infection.
- The disease usually does not progress to pneumonia (full recovery within 2-5 days).
- About half of the people with this infection develop dry, nonproductive cough.

Treponema, Borrelia and Leptospira

Theme N25

Morphological comparison of spirochetes with axial filaments and Spirillum with external flagella



Spirochetes: classification

- Gracilicutes (division)
- Spirochaetales (order)
  - – Spirochaetaceae (family)

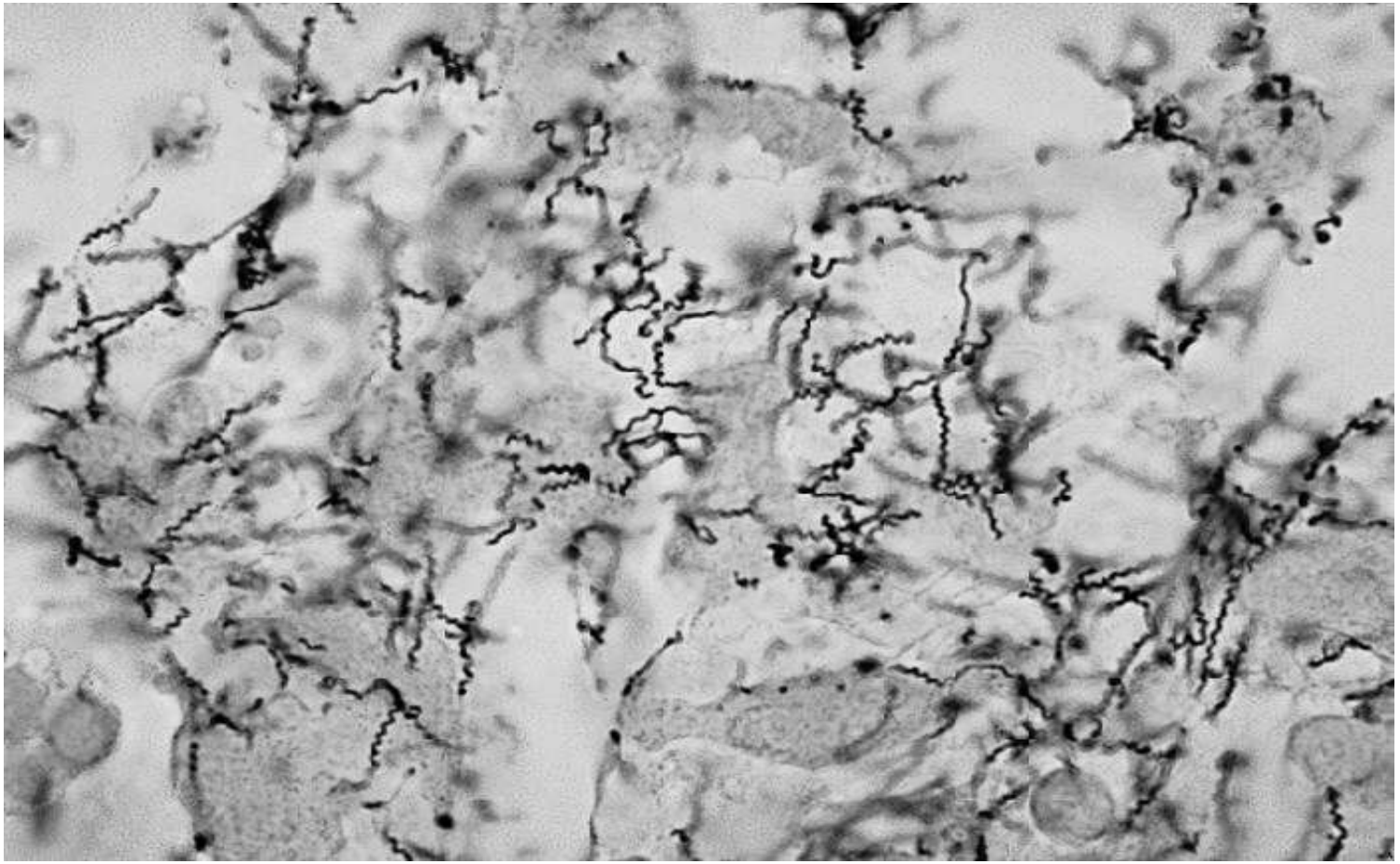
  Genera:
    - ✓Treponema
    - ✓Borrelia
  - – Leptospiraceae (family)

  Genus:
    - ✓Leptospira

Treponemes: main characteristics

- They are Gram negative, long, spiral and motile bacteria. The spiral coils (usually their number is 8 – 12) are regularly spaced.
- The spirals are so thin that they are not readily seen unless dark-field illumination or immunofluorescent stain is employed.
- They do not stain well with aniline dyes. When stained by Romanovsky-Giemsa technique the bacteria are stained light pink.
- Could be cultured on artificial media anaerobically but loss their pathogenicity.
- Axial filaments (a form of flagella) found between the peptidoglycan layer and outer membrane are the locomotory organelles.
- *Treponema pallidum produces syphilis (a common sexually-transmitted disease) – the infection that is limited to the human host.*

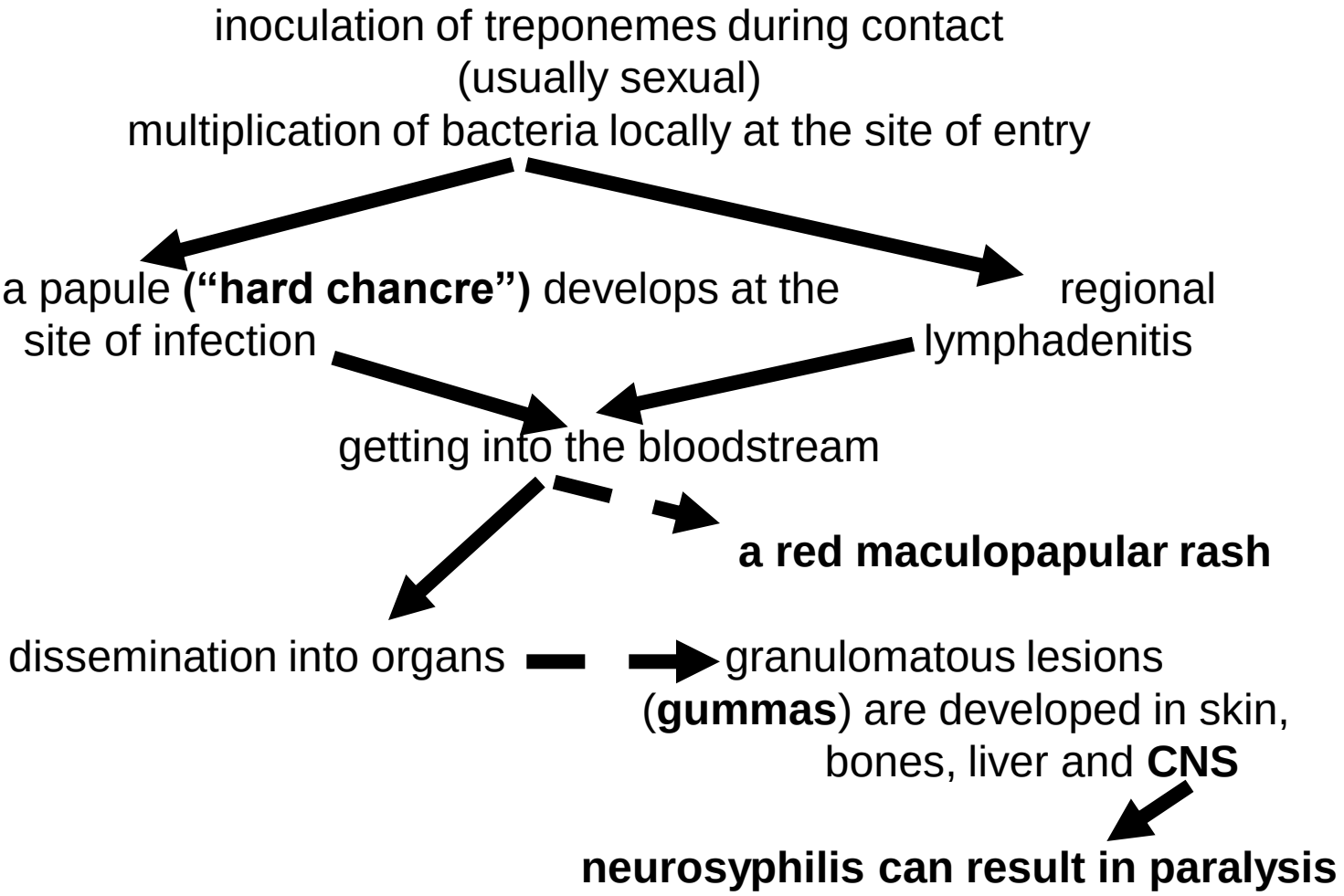
Treponema pallidum – in testis of experimentally infected rabbit (modified Steiner silver stain)



Treponemes: factors of pathogenicity

- resistance to phagocytosis
- high invasiveness
- presence of endotoxin
- presence of lipoproteins in the bacterial cell (they cause immunopathological reactions in human organism: development of gummas)

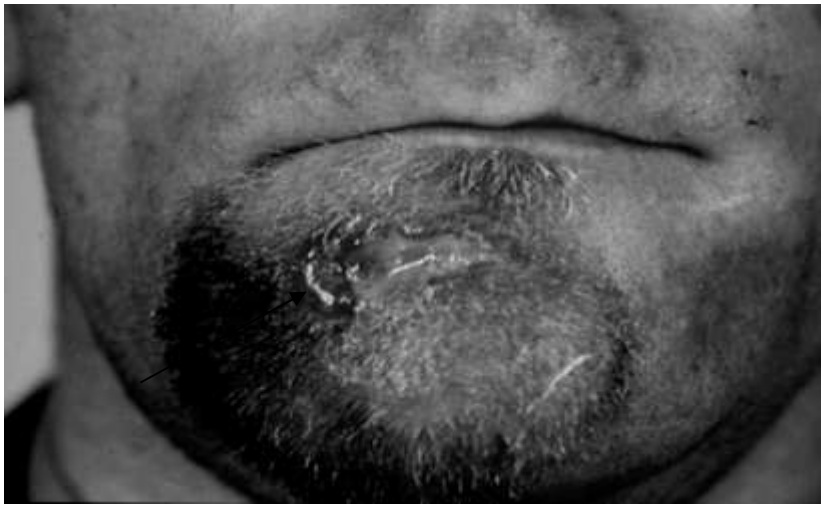
Syphilis: pathogenesis

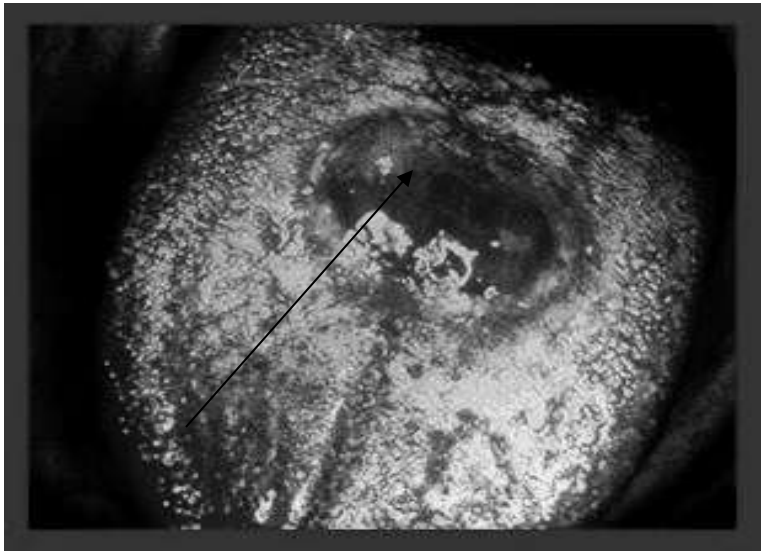


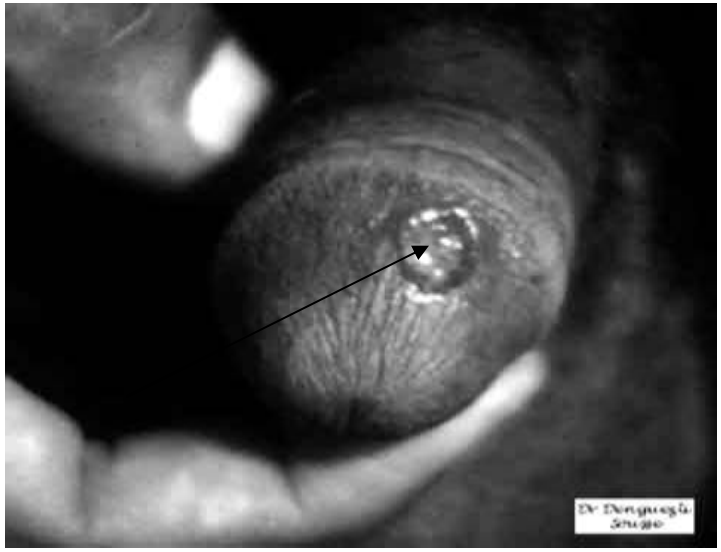
Syphilis: stages of the disease

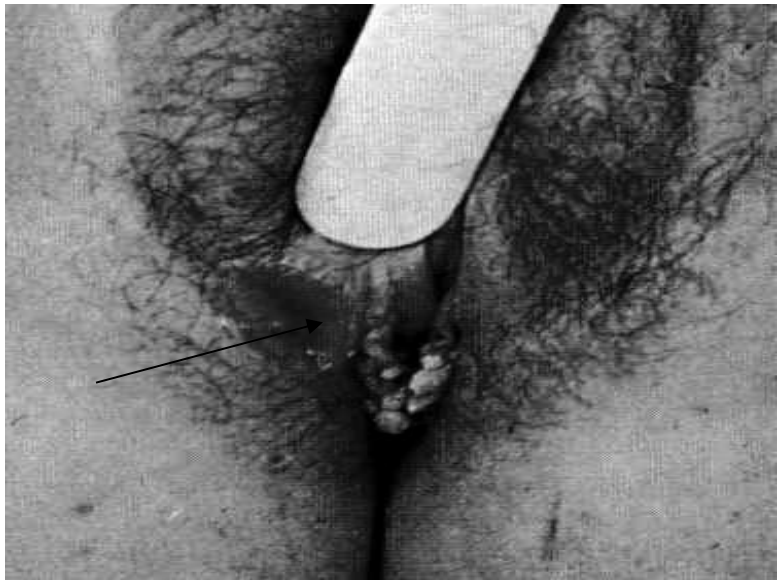
- **Primary syphilis**: the bacteria enter the body through tiny breaks in the skin and mucous membranes, reach regional lymph nodes; a chancre appears seen in genital areas or elsewhere within 10-60 days after initial contact (contains large numbers of treponemes and it is not painful).
- **Secondary syphilis**: occurs after the primary chancre heals (2-10 weeks later) as a result of getting bacteria into the bloodstream. The regional lymph nodes remain swollen. The red rush appears anywhere on the body as a result of dissemination.

Hard chancre – primary syphilis

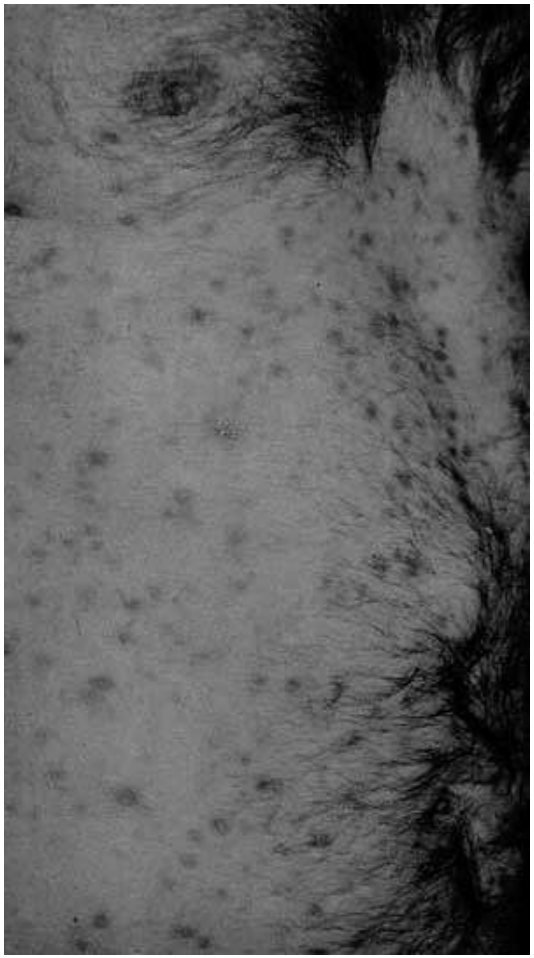


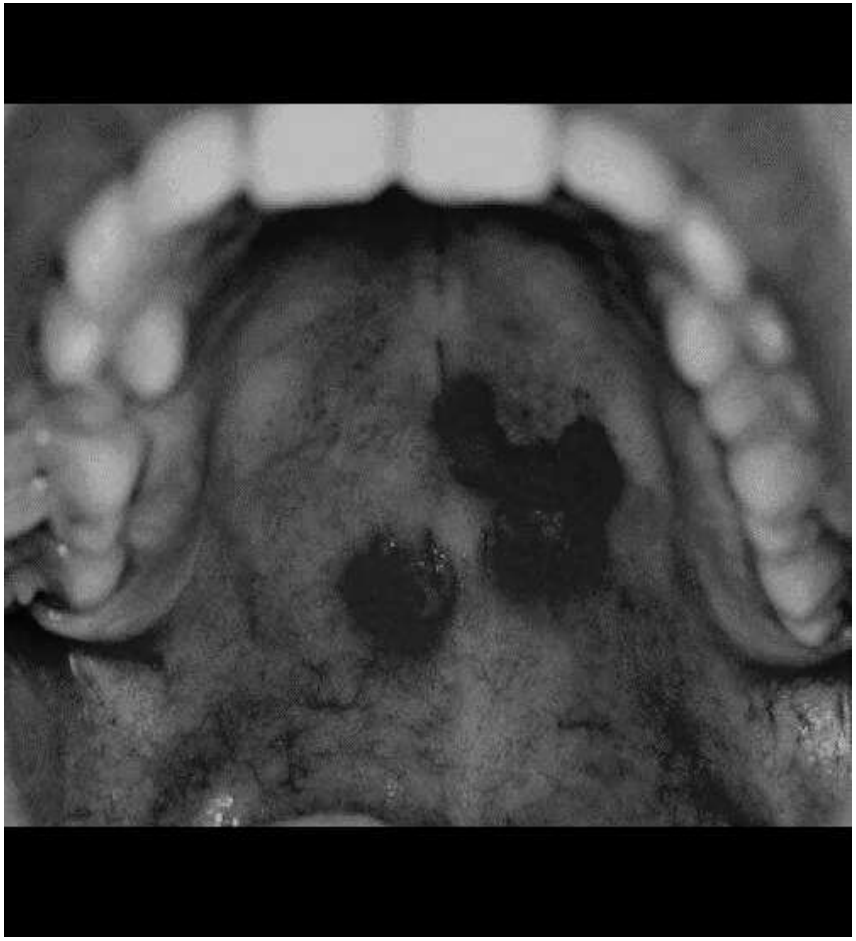






Secondary syphilis

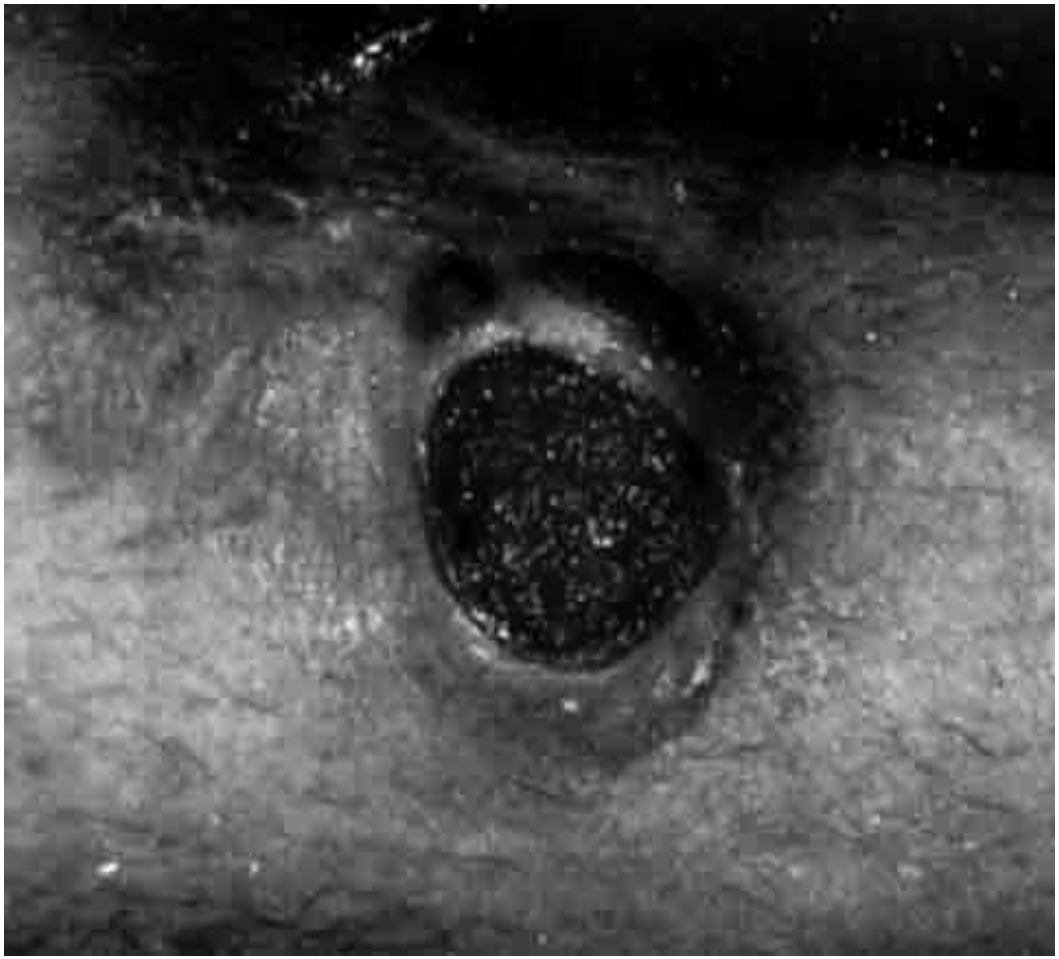




Syphilis: stages of the disease (continuation)

- **Tertiary syphilis** (if syphilis is untreated) – DTH is developed, a cellular immune response to T.pallidum: gummas can appear in any organ 5-40 years after initial infection.
- **Neurosyphilis** is the stage which characterised by CNS involvement and its serious damage (could develop in ill human without adequate treatment 8-15 years after initial infection)
- **Congenital syphilis** develops by transplacental passage of treponemes from an infected mother. Often the fetus dies and is aborted. Survivors appear to have maculopapular rash and liver damage. Later manifestations include severe bone damage, blindness, deafness and neurological problems.

Tertiary syphilis (gumma)



Syphilis: laboratory diagnostics

Stage	Microscopic investigations	Serological tests
Primary syphilis	+ (tissue fluid expressed from chancre, material got from puncture of nodulus)	+ (starting from the 4th week)
Secondary syphilis	+ (content from rush lesions)	+
Tertiary syphilis	–	+
Neurosyphilis	–	+ Ig: revealing in liquor

Syphilis: serological tests

Nonspecific serological tests

(help to detect Wassermann antibodies with use of bovine heart lipoprotein cardiolipin antigens):

- CF tests (Wassermann's tests)
- RP on glass slide (preliminary diagnosis)
- RIHA (express-diagnosis)

Specific serological tests

(with use of bacterial treponemal antigens):

- CF tests (Wassermann's tests)
- T.pallidum immobilisation test
- RIF with fluorescent treponemal antibodies
- ELISA

Syphilis: prophylaxis and treatment

- A person with active syphilis is resistant to superinfection with T.pallidum but when infection is eradicated the individual again becomes fully susceptible.
- *No vaccine has been produced for specific prophylaxis.*

Nonspecific prophylaxis includes control measures (prompt treatment of all discovered cases, sex hygiene, mechanic prophylaxis – condoms and chemoprophylaxis).

Antibiotic therapy (usually penicillin G) is usually highly effective.

Diseases related to syphilis: infectious agent and characteristics of the disease

- **Endemic syphilis** (*T.pallidum var. endemic*) produces bejel that occurs in Africa, Middle East, Southern Asia among children. It is transmitted by contact between persons (but not by sexual route). Highly infectious skin lesions appear, late visceral complications are rare.

- **Pinta** (*T.carateum*) occurs endemically in all age groups in Mexico, Central and South America, some areas of Pacific. The primary lesions occur on exposed areas, and some months later, flat, hyperpigmented lesions appear on the skin; depigmentation and hyperkeratosis take place years afterward.

Diseases related to syphilis: infectious agent and characteristics of the disease

- **Frambesia (yaws)** (*T.pertenue*) is epidemic particularly among children in many humid, hot tropical countries. The primary ulcerating papules occur usually on the arms and legs. Scar formation of skin lesions and bone destruction are common. It is transmitted by person-to-person contact.

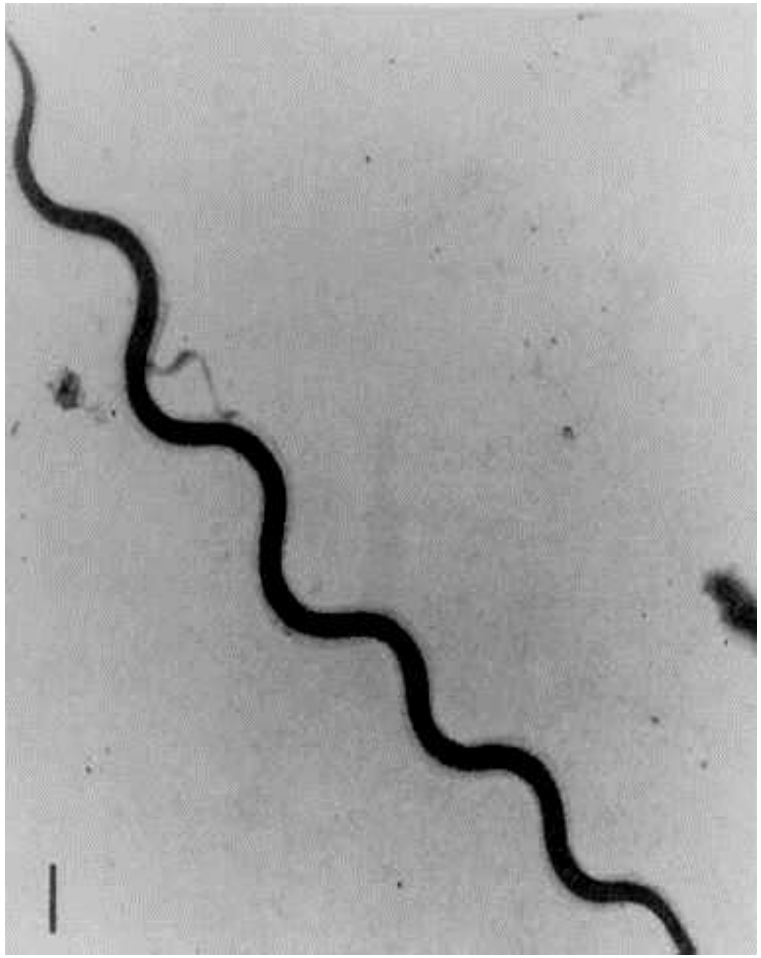
All the infections listed above could be treated by penicillin administration.

Yaws

Borrelia: main characteristics

- It is irregular spiral bacterium: the distance between turns varies.
- It stains readily with bacteriologic dyes as well as Romanovsky-Giemsa’s technique.
- Could be cultured in liquid media containing blood, serum or tissue extracts.
- These bacteria change the antigenic structure very quickly.

Borrelia – irregular spiral bacterium



Borrelia: factors of pathogenicity

- High variability of the antigenic structure.
- The immune response (production of antibodies) develops during the initial period of the disease and can end the attack.
- But antigenically distinct variants survive and cause a relapse.

Relapsing fever: characteristics of the disease

- ***Borrelia recurrentis*** produces *relapsing fever* not pathogenic for guinea pig and occurs worldwide.
- Infected rodents are the source of the infections, ticks can pass B.recurrrentis transovarily and infect other animal – endemic relapsing fever.
- The human disease is acquired by a tick bite but from human to human the infection is passed by lice (rubbing crushed lice into the bite wound).
- The disease derives its name from the fact that three to ten recurrences (attacks of disease) are common when the symptoms of the disease reappear.

Relapsing fever: pathogenesis

B.reccurrentis

↓

getting into the bite wound and then into the bloodstream

↓

bacteremia and the fever (abrupt rise of temperature)

↓

spirochetes are getting into spleen, liver, kidney
where necrotic foci and hemorrhagic lesions are formed

↓

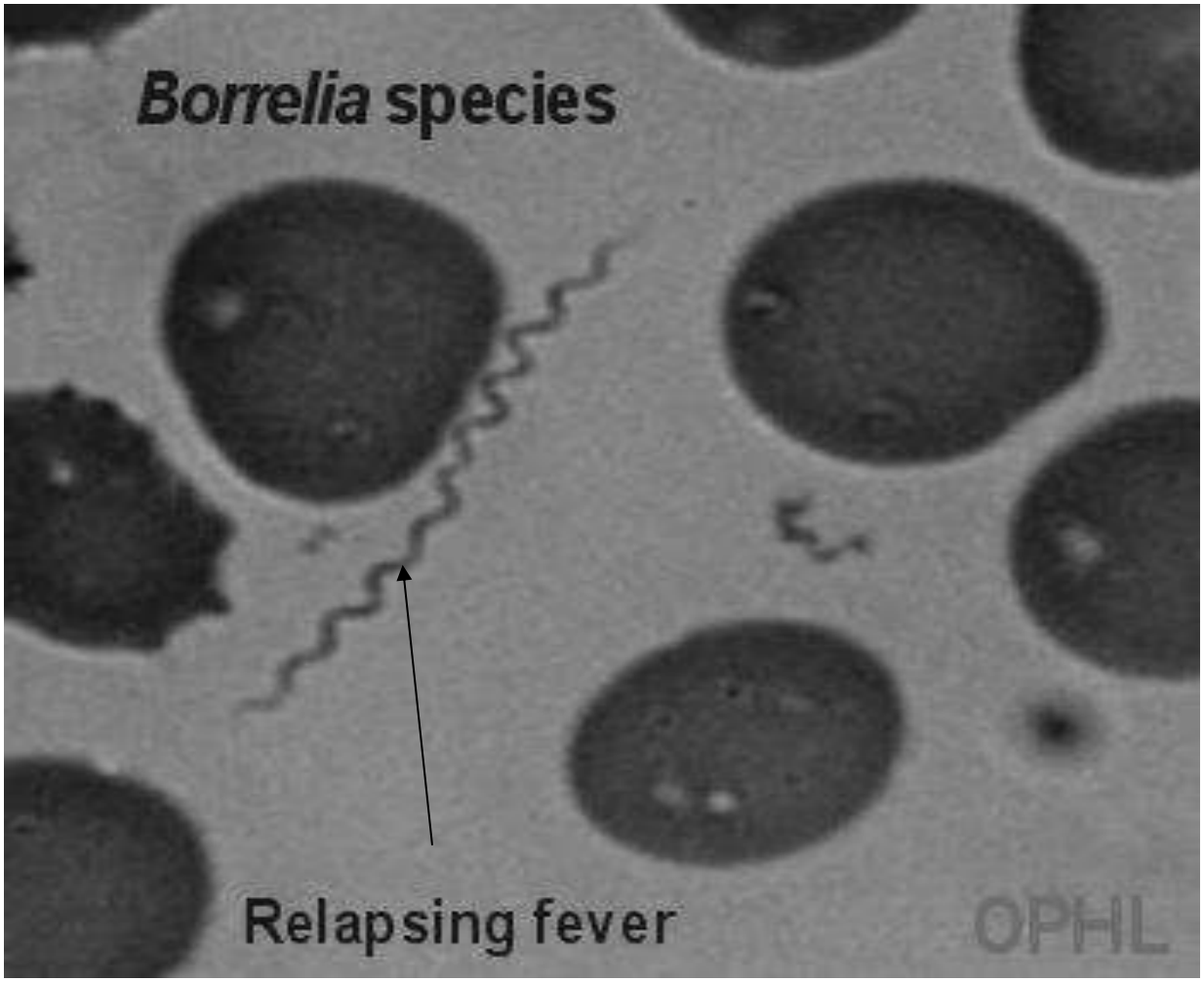
they can get into spinal fluid and brain

The fever persists for 3-5 days and then declines and after 4-10 days is followed by the next attack of fever

Relapsing fever: laboratory diagnostics

- The microorganism is extremely difficult to culture.
- Microscopic investigations of the blood smears stained by Romanovsky-Giemsa technique are mainly employed to detect B.recurrentis.
- Inoculation of the specimen into guinea pig to distinguish epidemic and endemic relapsing fever.

Borrelia recurrentis – *blood smear*



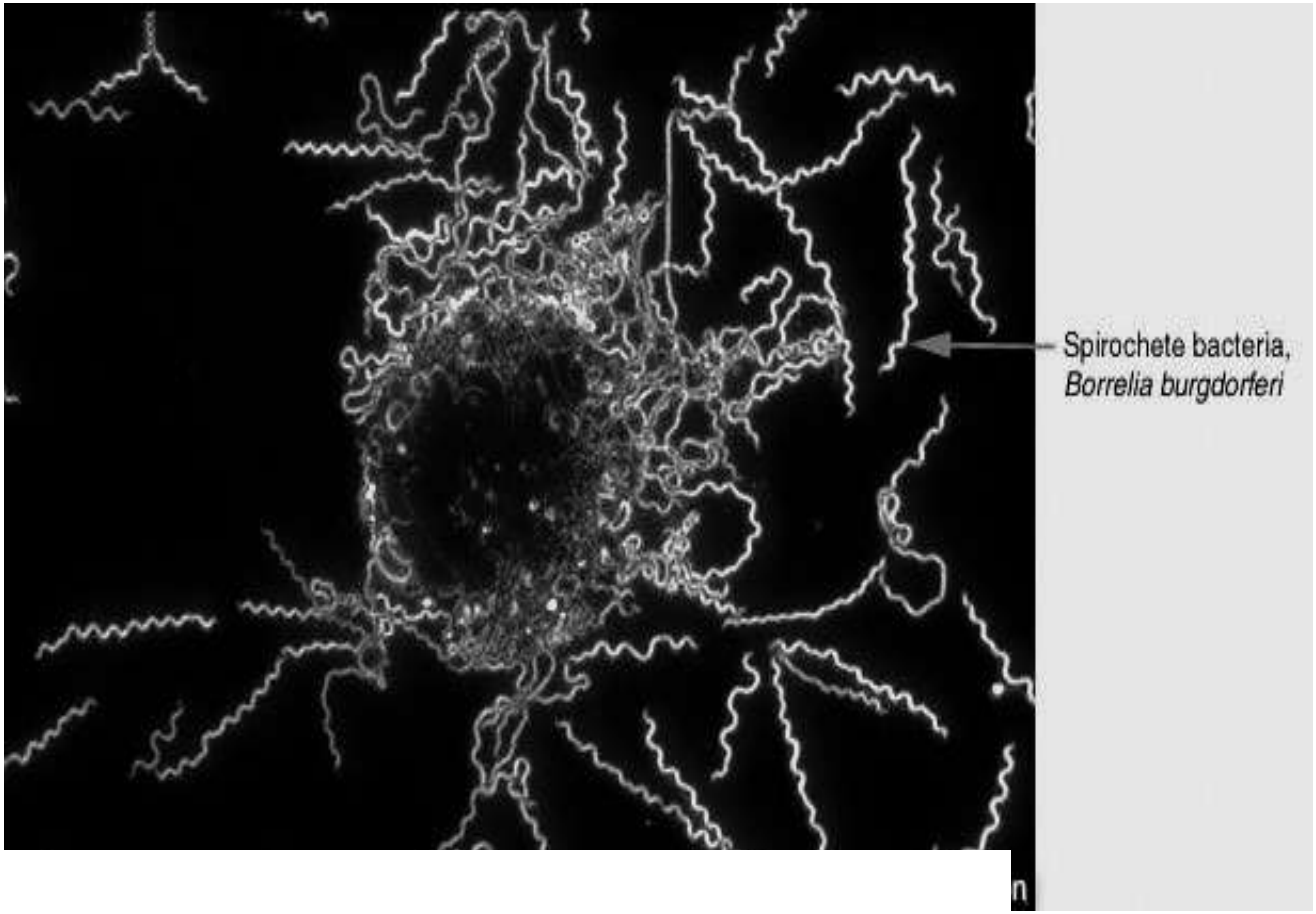
Relapsing fever: prophylaxis and treatment

- Currently, no vaccine is available for human use.
- Antibiotics (tetracycline or penicillin) could be effective in eradicating of borrelia from the body and they are capable to terminate individual attacks and to prevent relapses.

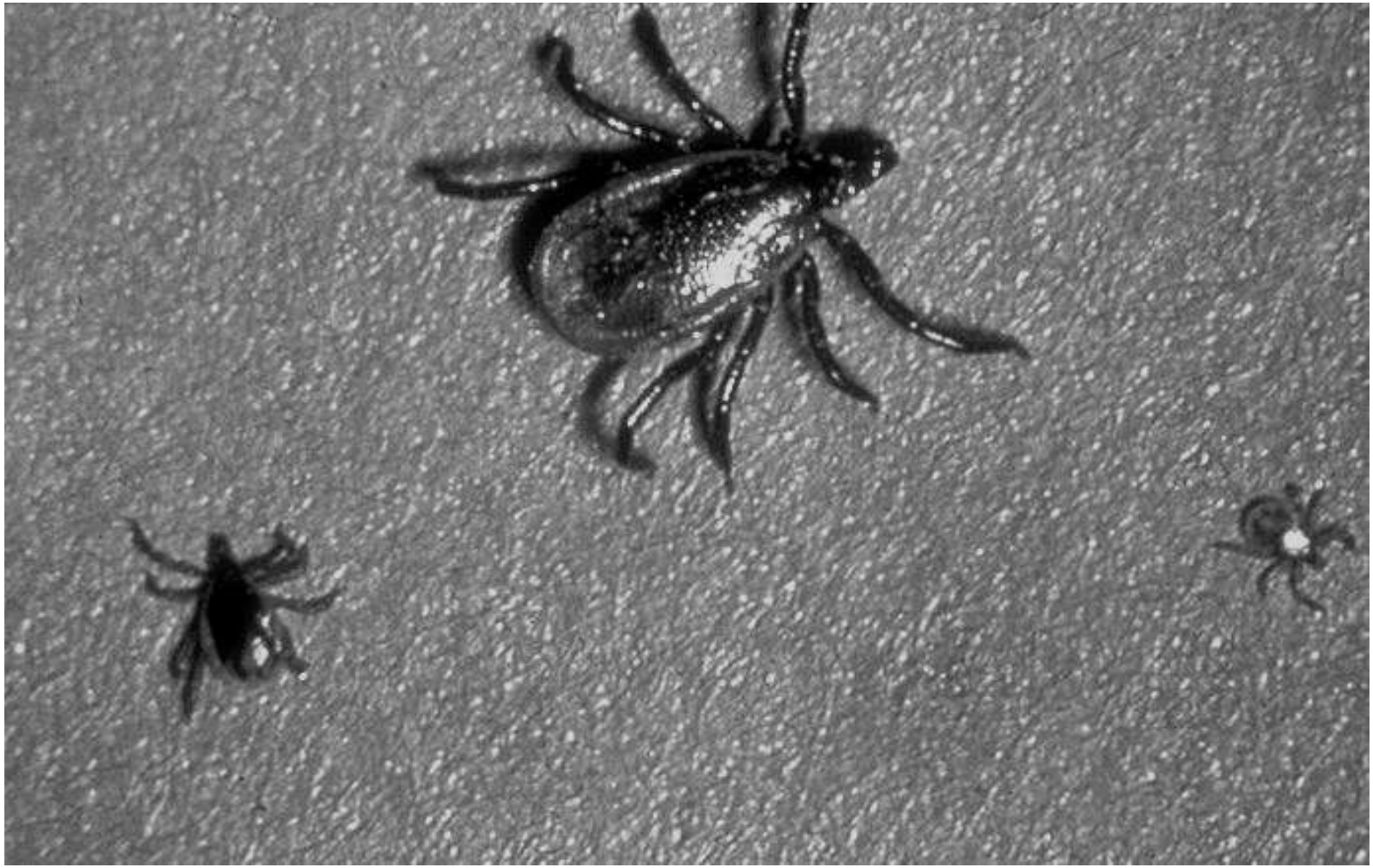
Lyme disease: characteristic of the disease

- A relatively newly recognized disease. It was clinically first described in 1975, and the role of a tick-borne spirochete was proven in 1983.
- The source of the infection are animals: mice and dear in endemic areas.
- ***Borrelia burgdorferi*** transmitted to human by a tick belonging to the genus Ixodes.
- The disease is typically seen between May and November (80% of cases – in June and July).

Borrelia burgdorferi - dark field image



Ixodes scapularis - tick vector for Lyme disease



Lyme disease: pathogenesis

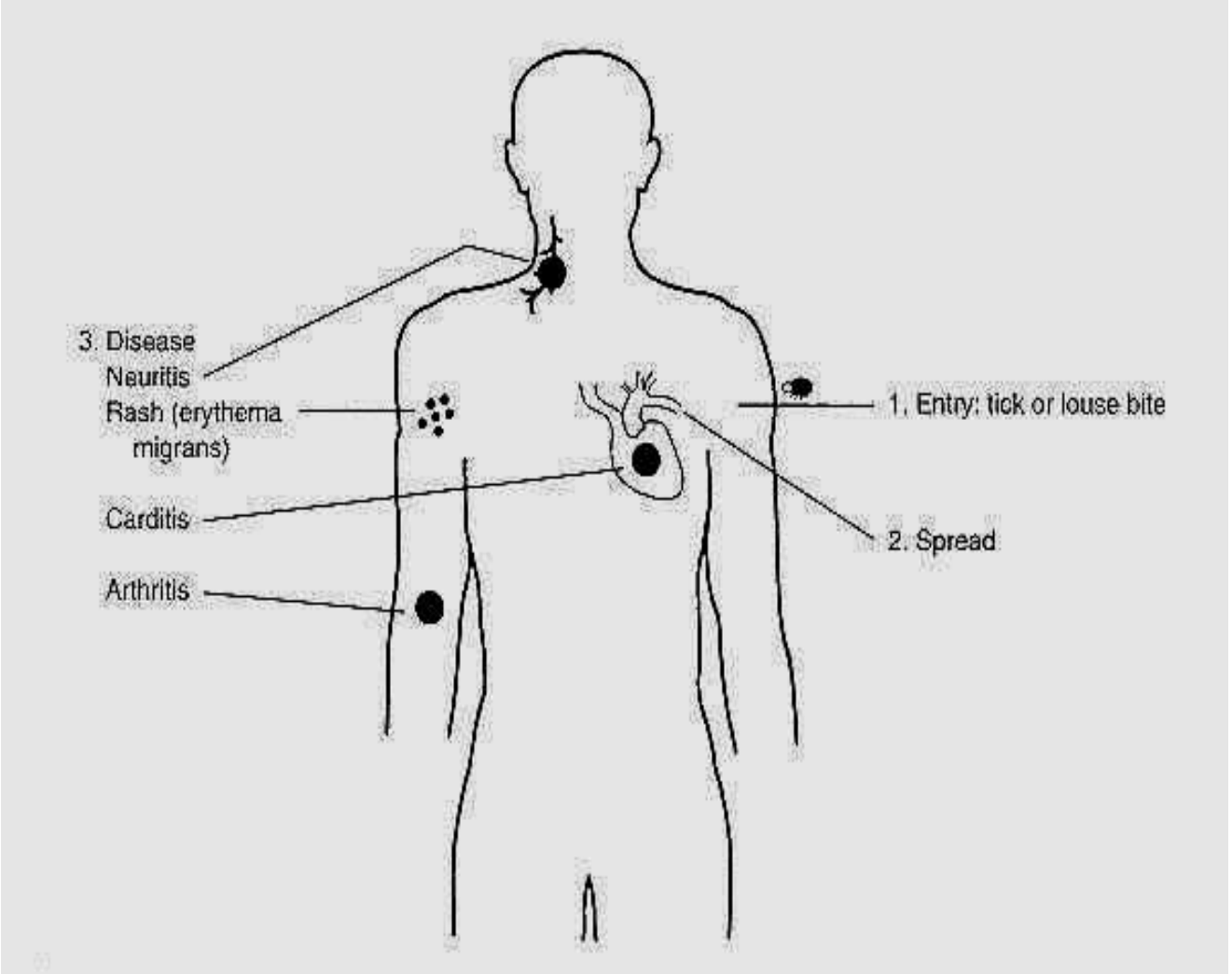
- The disease occurs in **three stages**:

  - **the first stage** – development of a distinctive expanding skin lesion (known as ***erythema chronicum migrans***) following with joint stiffness, headache, lymphadenopathy;

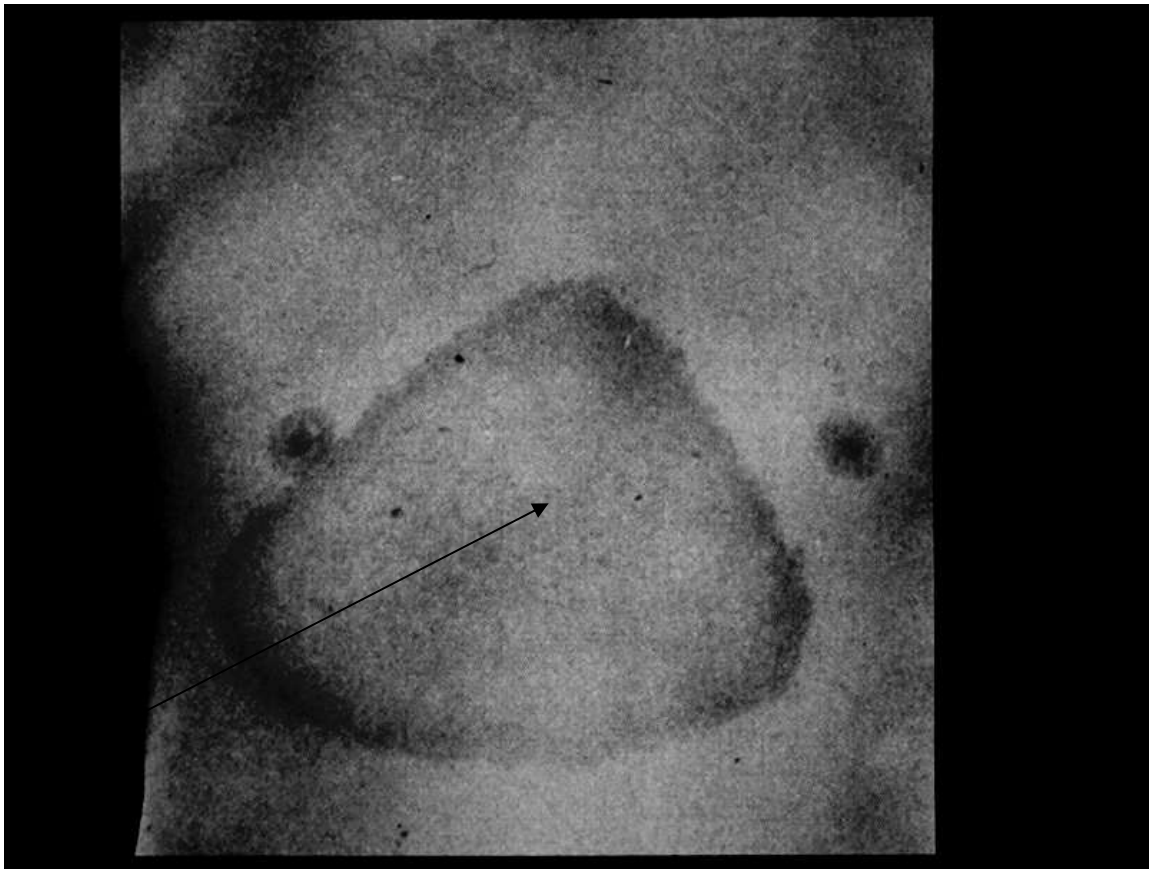
  - **the second stage** – development of multiple skin patches, arthritis, central and peripheral nervous system disorders;

  - **the third stage –** arthritis and neurological symptoms become chronic: symptoms of meningitis or meningoencephalitis are displayed as well as cardiac abnormalities.

Pathogenesis of Borrelia burgdorferi infection



Erythema chronicum migrans



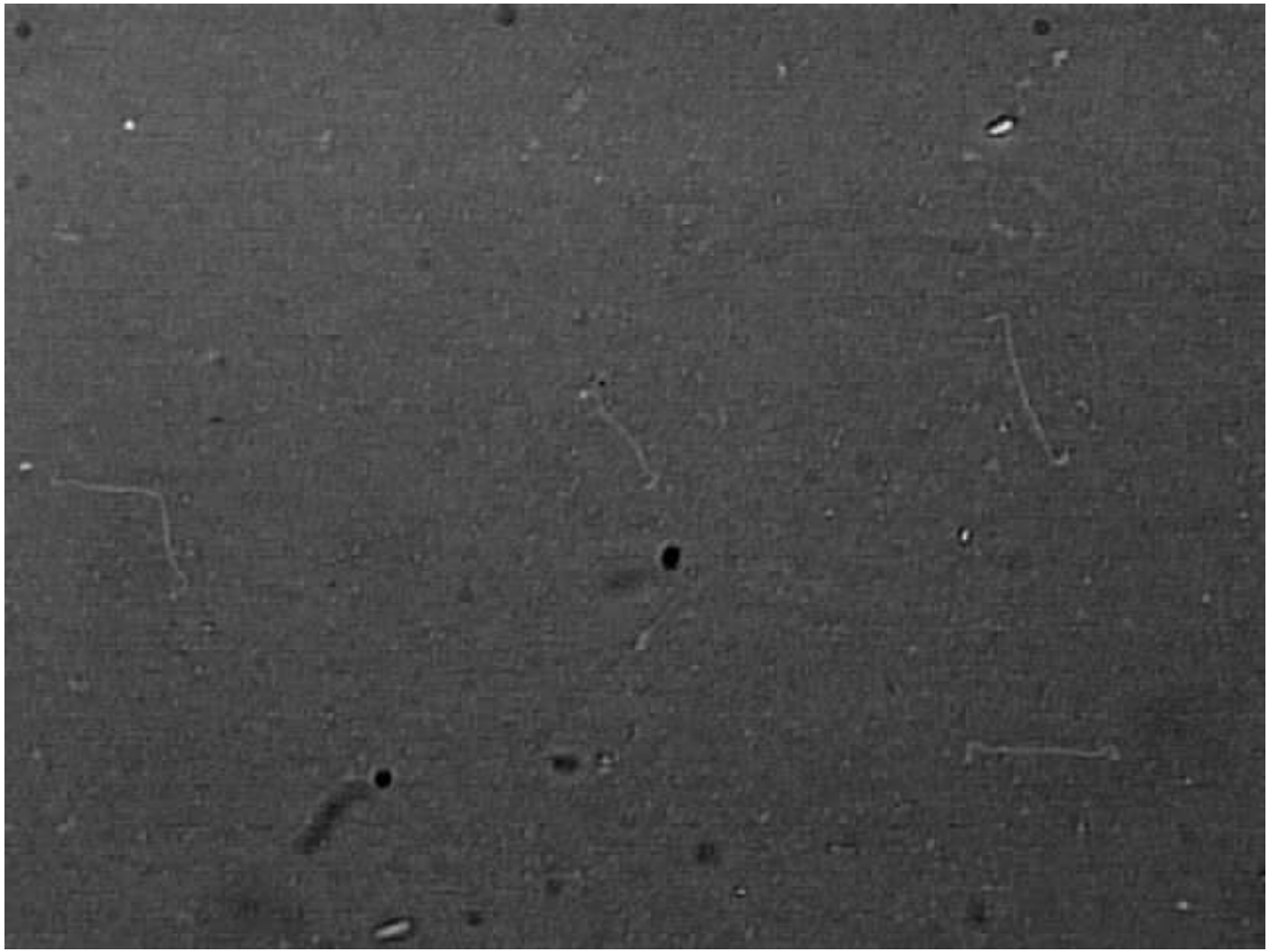
Lyme disease: diagnostics and treatment

- *Erythema chronicum migrans* is very important for diagnostics especially when followed by arthritis and neurological symptoms. Serological tests for revealing Ig in blood serum (immunoblotting technique) are also used for diagnosis.
- Lyme disease can be treated early with tetracycline. Later in the disease, large doses of antibiotics are necessary for prolonged periods.

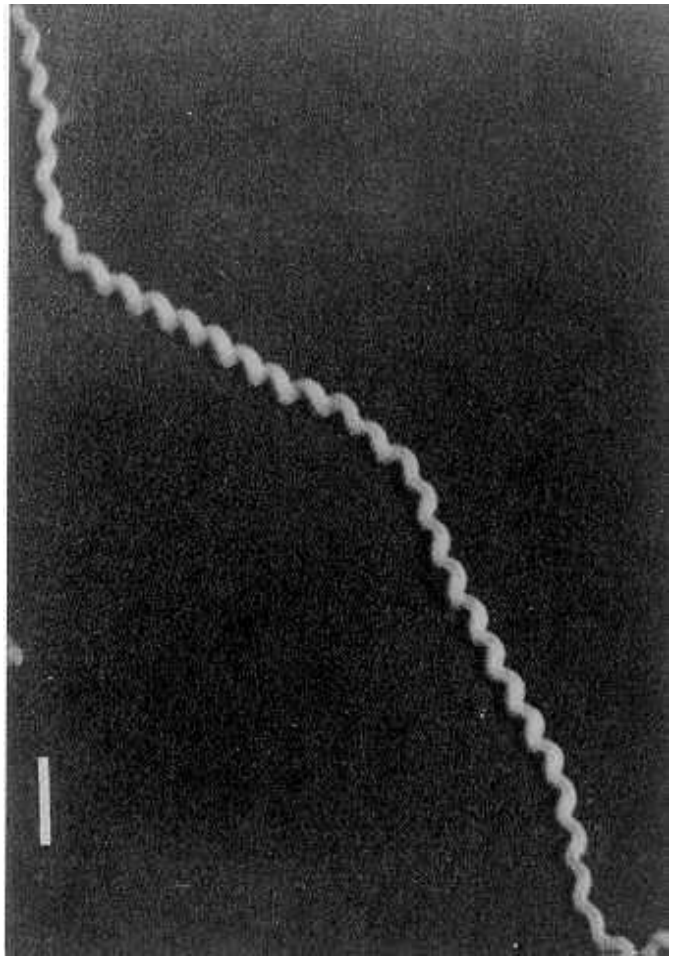
Leptospira: main characteristics

- Tightly coiled, thin, flexible spirochetes with very fine spirals and the ends bent, forming hook.
- Highly motile, the organ of motion is a thin axial filament.
- Could be investigated with use of dark-field microscopy.
- Leptospira bacteria grow in peptone broth containing rabbit serum and on the semisolid meat extract (Fletcher) media containing rabbit serum.
- Main pathogen is Leptospira interrogans (there are know more then 200 serological variants) that causes disease in human.
- They are able to survive for long period in water (stagnant water), particularly at an alkaline pH.
- Animals are the main source of infection.

Leptospira *– dark-field microscopy*



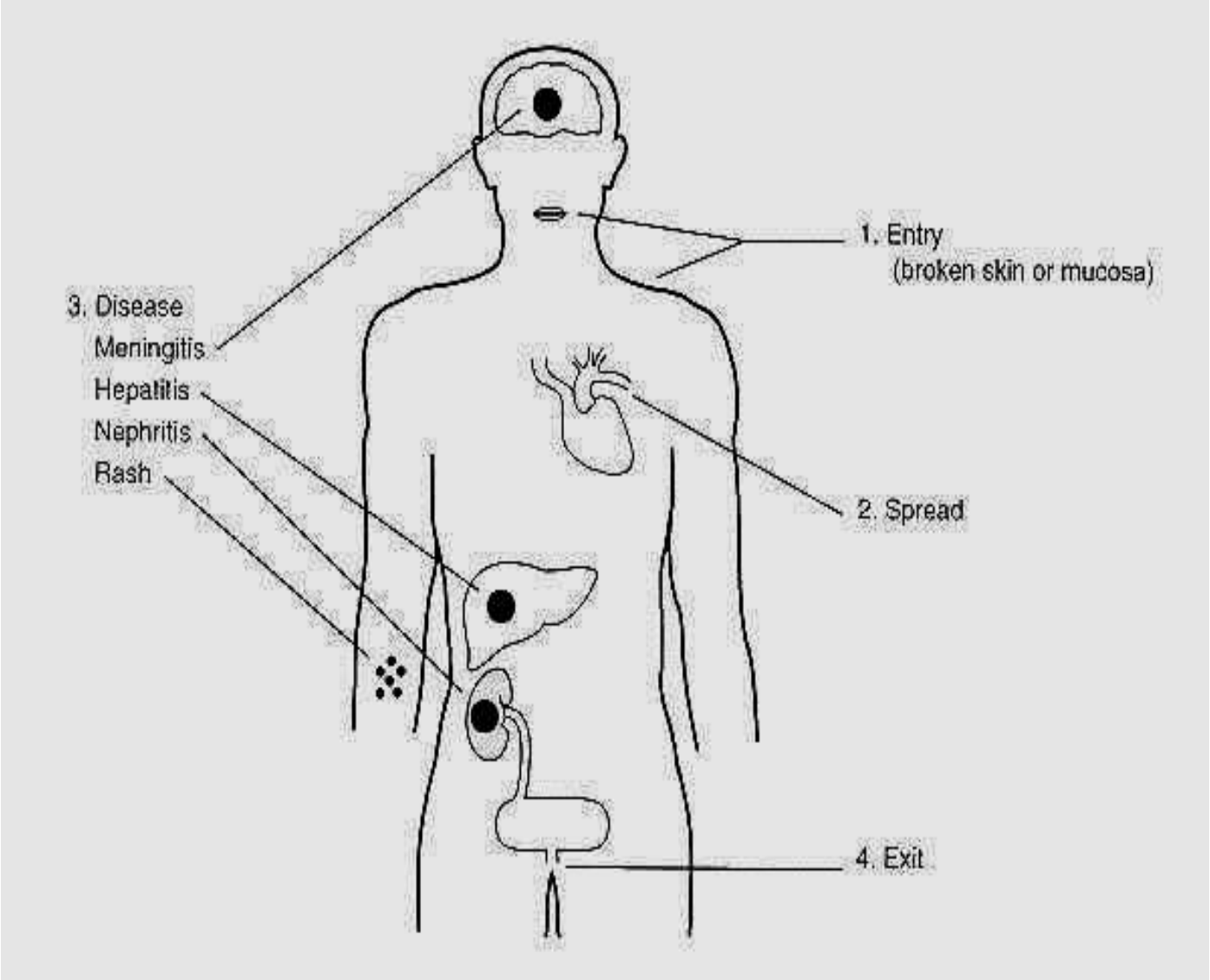
Leptospira – electron microscopy photo



Leptospirosis: characteristics of the disease

- Leptospira produces leptospirosis – zoonotic infection.
- Human infection is accidental following contacts with water or other materials contaminated with the excreta of animal hosts (cattle, swine, rodents could be the sources of the infection). The mechanism of the spreading of the infection is faecal-oral.

Clinical manifestations of leptospirosis



Leptospirosis: pathogenesis

- Leptospira are transmitted in water contaminated with infected urine from wild animals.
- Leptospira bacteria enter into the human organism with contaminated food or water (sometimes they enter through mucous membranes or breaks on the skin), invade the blood stream and then – parenchymatous organs (liver and kidney).
- The symptoms are: repeating fever and haemorrhage and necrosis resulted in dysfunction of:
- liver
- kidney

The CNS is frequently invaded and results in a clinical picture of "aseptic meningitis".

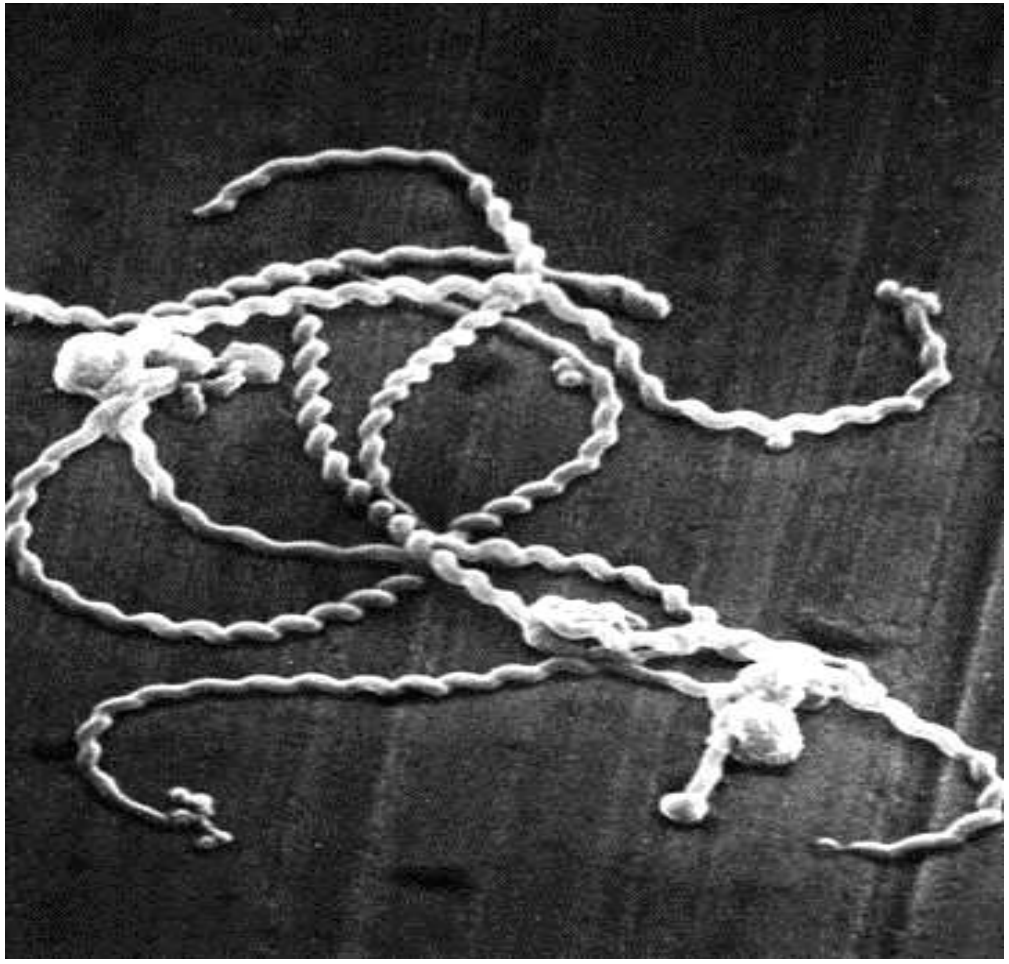
Kidney involvement results in the elimination of large numbers of bacteria in the urine – probably it is the main source of contamination and infection of humans.

Leptospirosis: laboratory diagnostics

- Dark-field examinations or thick smear stained by Romanovskyy-Giemsa technique show leptospira in fresh blood from early infections.
- Isolation of pure culture from the specimens get from
  - blood (early stage of disease)
  - urine (later stage)
  - liquor (in the case of CNS involvement
- Serological tests

  - leptospiral cultures clump in the presence of specific antibodies.

Leptospira - clump in the presence of specific antibodies



Leptospirosis: prophylaxis and treatment

Nonspecific prophylaxis:

- Control of the infection consists of preventive exposure to potentially contaminated water and reducing contamination by rodent control.

Specific prophylaxis:

- Killed polivalent vaccine
- Ig

Treatment:

- In very early infection antibiotics (penicillin, tetracycline) have some therapeutic effect but not eradicate the infection.

RICKETTSIA AND CHLAMYDIA. MYCOPLASMA.

Theme N26

Rickettsiae: classification

- Rickettsiales (order)
- Rickettsiaceae (family)

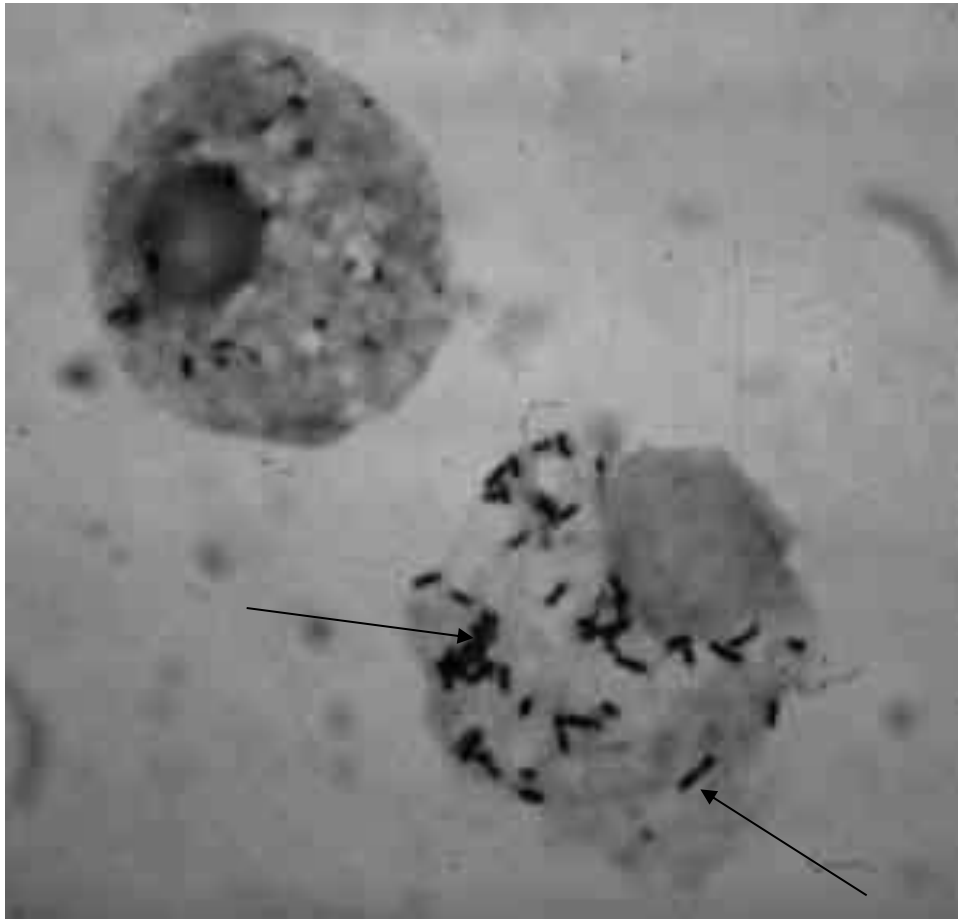
Genera:

  - Rickettsia
  - Coxiella
  - Rochalimaea

Rickettsiae: main characteristics

- Polymorphic appearing either as short and long rods, or as cocci and occur in short chains or in filaments
- With Romanovsky-Giemza stain technique they stain blue and with Zdradovsky stain they stain red
- They are obligate parasites but true bacteria showing all the structural features of bacteria
- Rickettsiae lost their ability to synthesize NAD
- Rickettsiae grow readily in the embryonated egg (yolk sac)

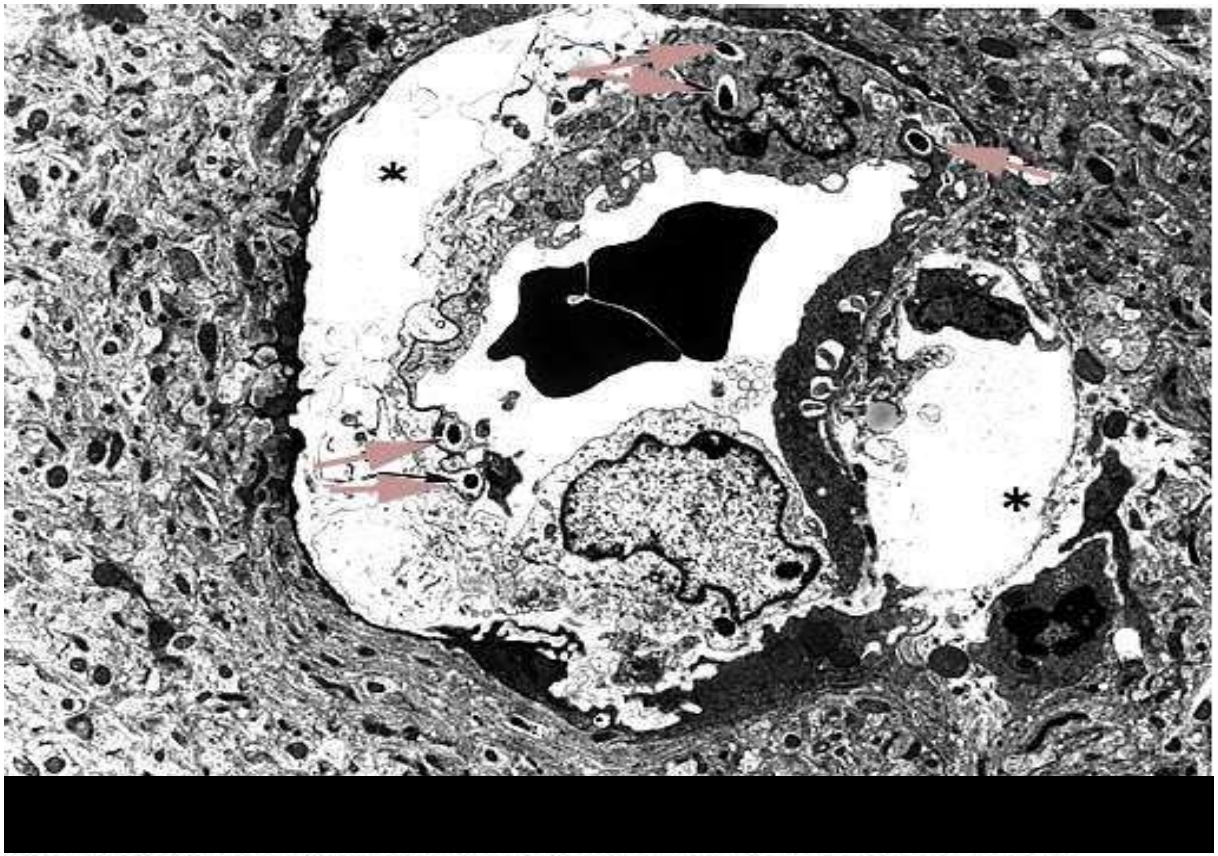
Rickettsia – intracellular parasites



Rickettsiae: characteristics of the disease

- All of these organisms are maintained in animal and arthropod reservoirs and, with the exception of *Coxiella*, are transmitted by arthropod vectors (*e.g.*, ticks, mites, lice or fleas).
- Humans are accidentally infected with these organisms.
- When bacteria transmitted to an unnatural host such as human , they cause disease.
- The bacteria multiply in endothelial cells of small blood vessels and cause rush appearance and fever development.
- The cells become swollen and necrotic , there is thrombosis of the vessels.
- *Coxiella burnetii infects a wide range of animals including goats sheep cattle and cats.* Infection of humans occurs by inhalation of airborne particles. The bacteria multiplies in the lungs and is disseminated to other organs. Pneumonia and granulomatous hepatitis are observed in patients with severe infections.

Rickettsia – growth in the endothelium



Growth of rickettsiae (arrows) in the endothelium results in damage to vascular integrity and thus the leakage of fluid into a vital organ such as the brain. The accumulation of fluid (edema) in the perivascular space (asterisks) may result in clinical encephalitis.

Rickettsiae: factors of pathogenicity

- haemolysins
- endotoxin
- factors of
  - adhesion
  - invasion

Rickettsiosises: typical groups and infectious agents

Typhus group

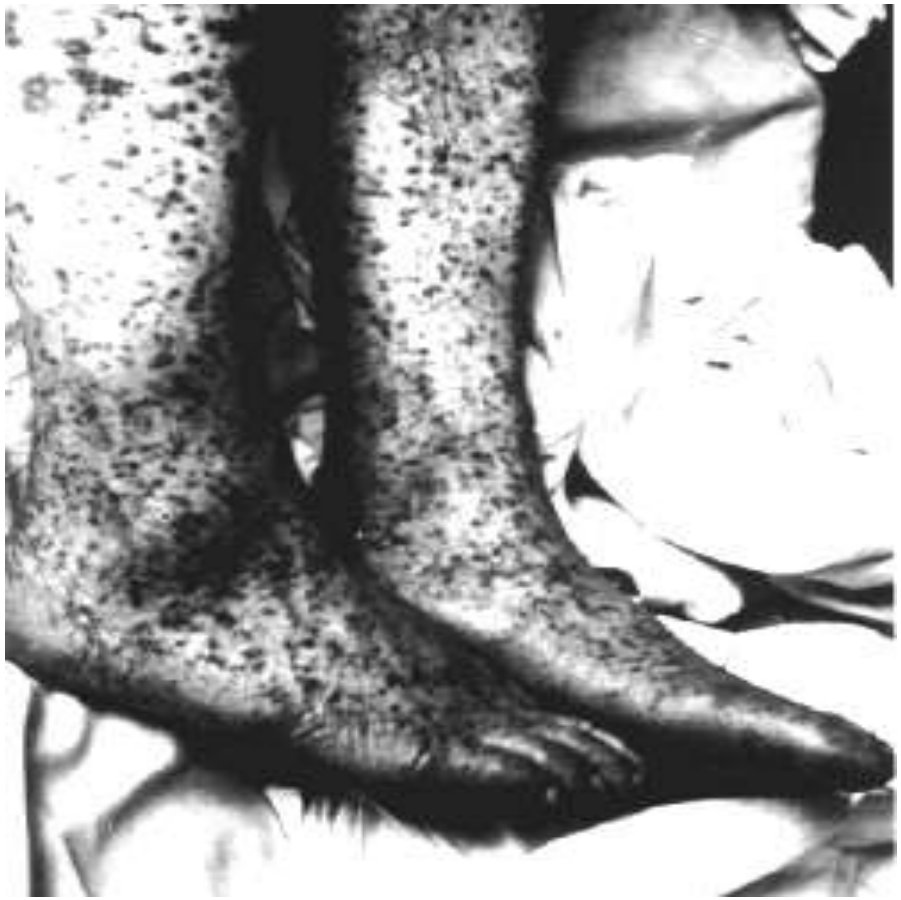
- Epidemic typhus has been known since the 16th century and it has long been associated with famine and war. It killed or caused great suffering in great number of people in the two World Wars (during the period from 1914-1922 three million died in Russia).
- Epidemic typhus, louse-borne - Rickettsia prowazekii (the causative agent was described by Prowazek in 1913).
- – severe systemic infection and prostration, mortality is about 6-30% during epidemics.
- Endemic typhus, flea-born - Rickettsia typhi – the disease is quite similar to epidemic but milder and rarely fatal.

Rickettsiosises: typical groups and infectious agents

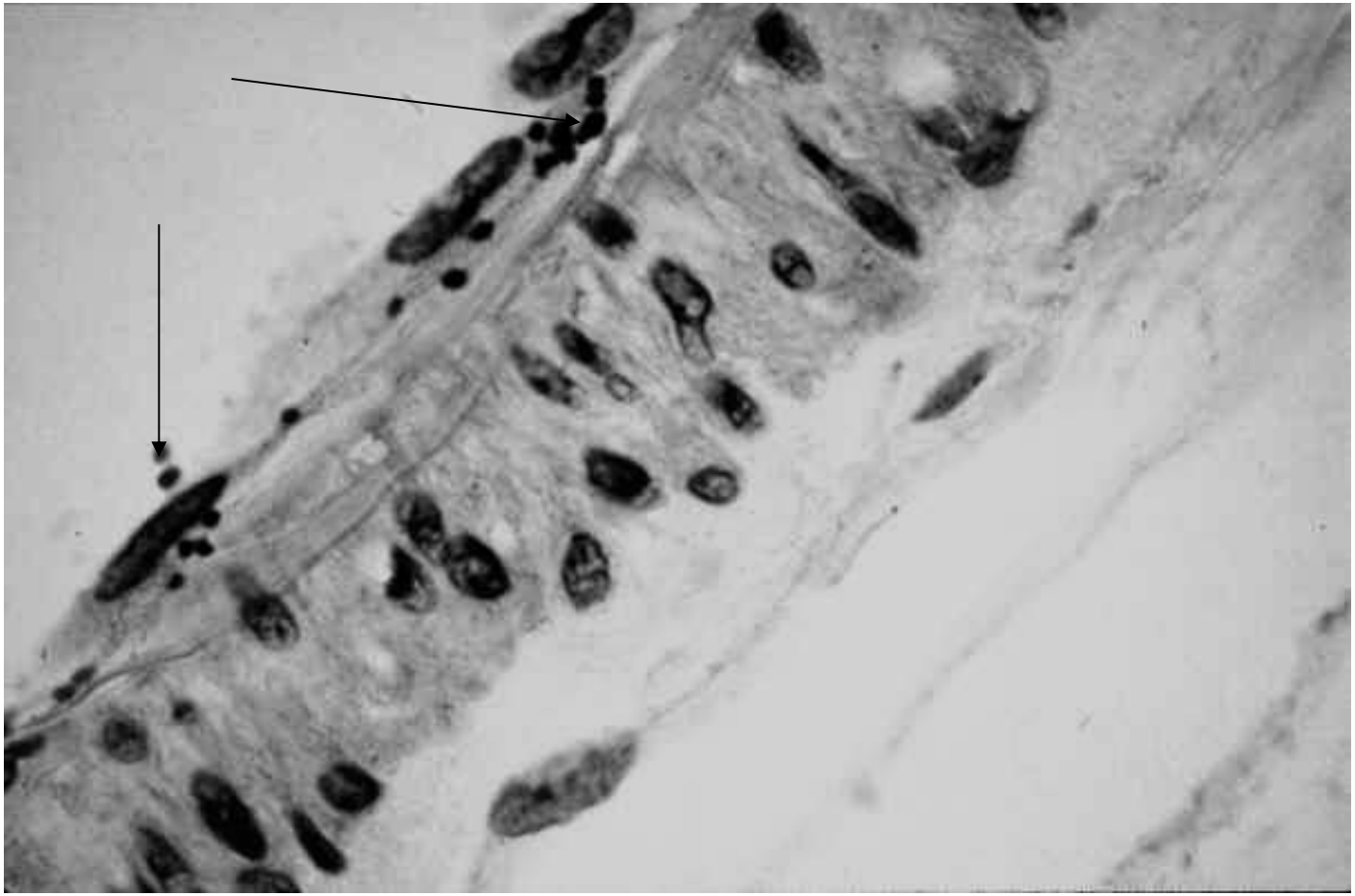
Spotted fever group

- Rocky Mountain spotted fever – **R. rickettsii** (the causative agent was described by Howard Ricketts at the beginning of 20th century).
- The disease resembles typhus clinically: the disease begins with the abrupt onset of fever, chills headache and myalgia usually 2-12 days after the tick bite. Rash usually appears (90% of cases). Complications: vasculitis can include respiratory failure, coma and acute renal failure. When untreated the mortality can achieve 60% especially in older age groups.
- Mediterranean fever – **R. conorii**
- Queensland tick typhus – **R. australis**
- North Asian tick-borne rickettsiosis – **R. sibirica**
- Rickettsial pox (Russian vesicular rickettsiosis) – **R. akari**
- Q fever (pneumorickettsiosis) - **Coxiella burnetii**
- Trench fever - **Rochalimaea quintana**
- Scrub typhus (Tsutsugamushi fever) – **R. tsutsugamushi**

Characteristic spotted rash of late-stage Rocky Mountain spotted fever



Immunohistological staining of Rickettsia rickettsii in endothelial cells of a blood vessel



Epidemic typhus: pathogenesis and pathology

Faeces of the human louse

⇓

penetration of rickettsiae into the blood vessels after the louse bites

⇓

adhesion on cholesterol containing receptors of

endothelial cells of the small vessels

toxinemia, thrombosis: rush, fever

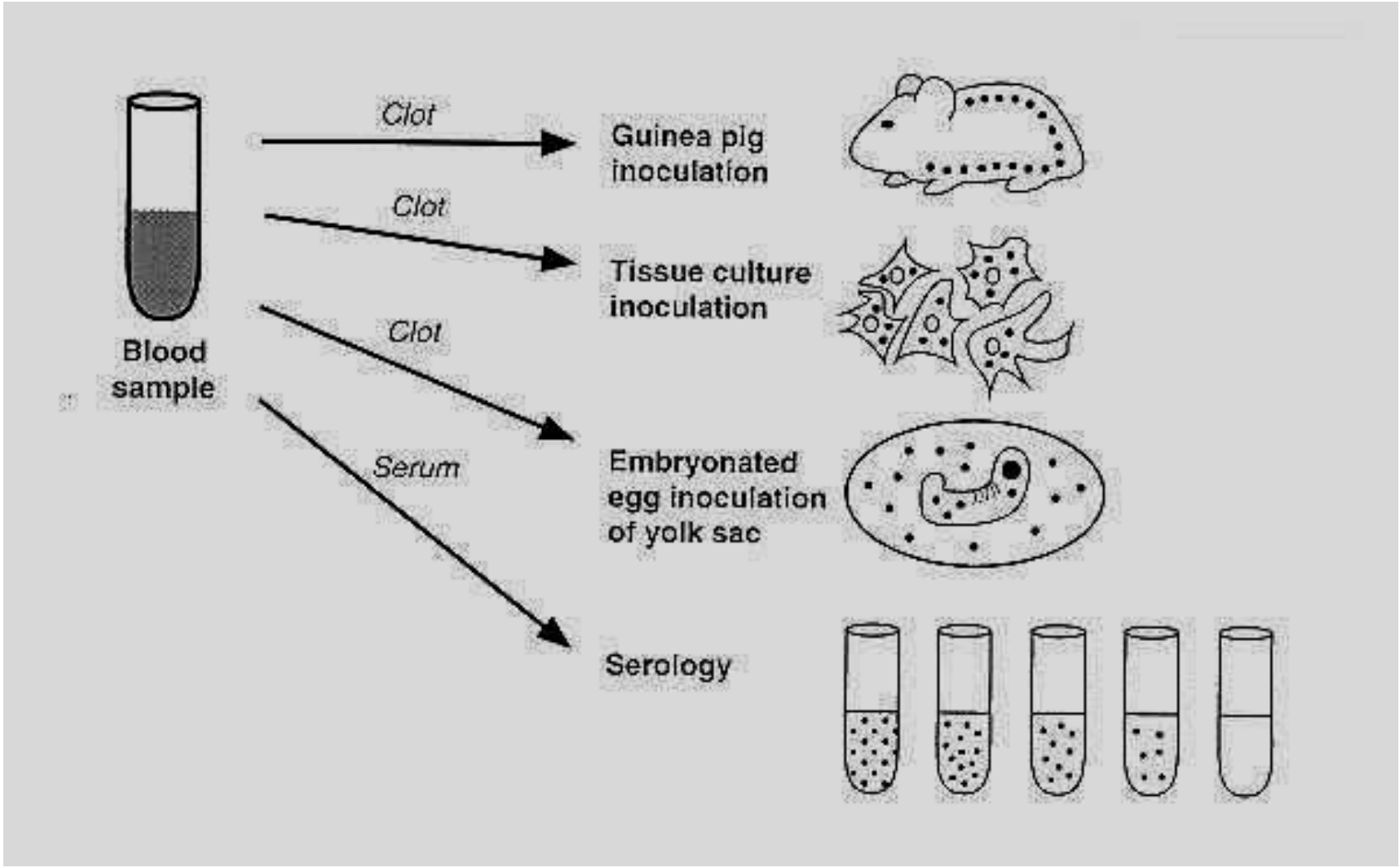
vasculitis in many organs: brain, myocardium, kidney

macrophages

Epidemic typhus: laboratory diagnostics

- Whole blood could be inoculated into guinea pig or egg to cultivate bacteria and use immunofluorescence to detect antigens.
- Serologic tests (RA, CF reaction) with use of the serum got from infected humans: help in diagnostics and demonstrate the course of the disease

  (an antibodies titre rise shows active stage of disease).


Blood sample
Clot
Guinea pig inoculation
Clot
Tissue culture inoculation
Clot
Embryonated egg inoculation of yolk sac
Serum
Serology

Epidemic typhus: prophylaxis and treatment

Specific prophylaxis:

active immunisation with use of **formalinised antigens** prepared from the yolk sacs of infected chick embryo,

a live vaccine

(strain E)

Chemoprophylaxis and treatment:

tetracycline and chloramphenicol suppress the growth of bacteria

Chlamydiae: classification

- Chlamydiales (order)
- Chlamydiaceae (family)
  - Chlamydia (genus)

  Species:

  C. trachomatis

  C. pneumoniae

  C. psittaci

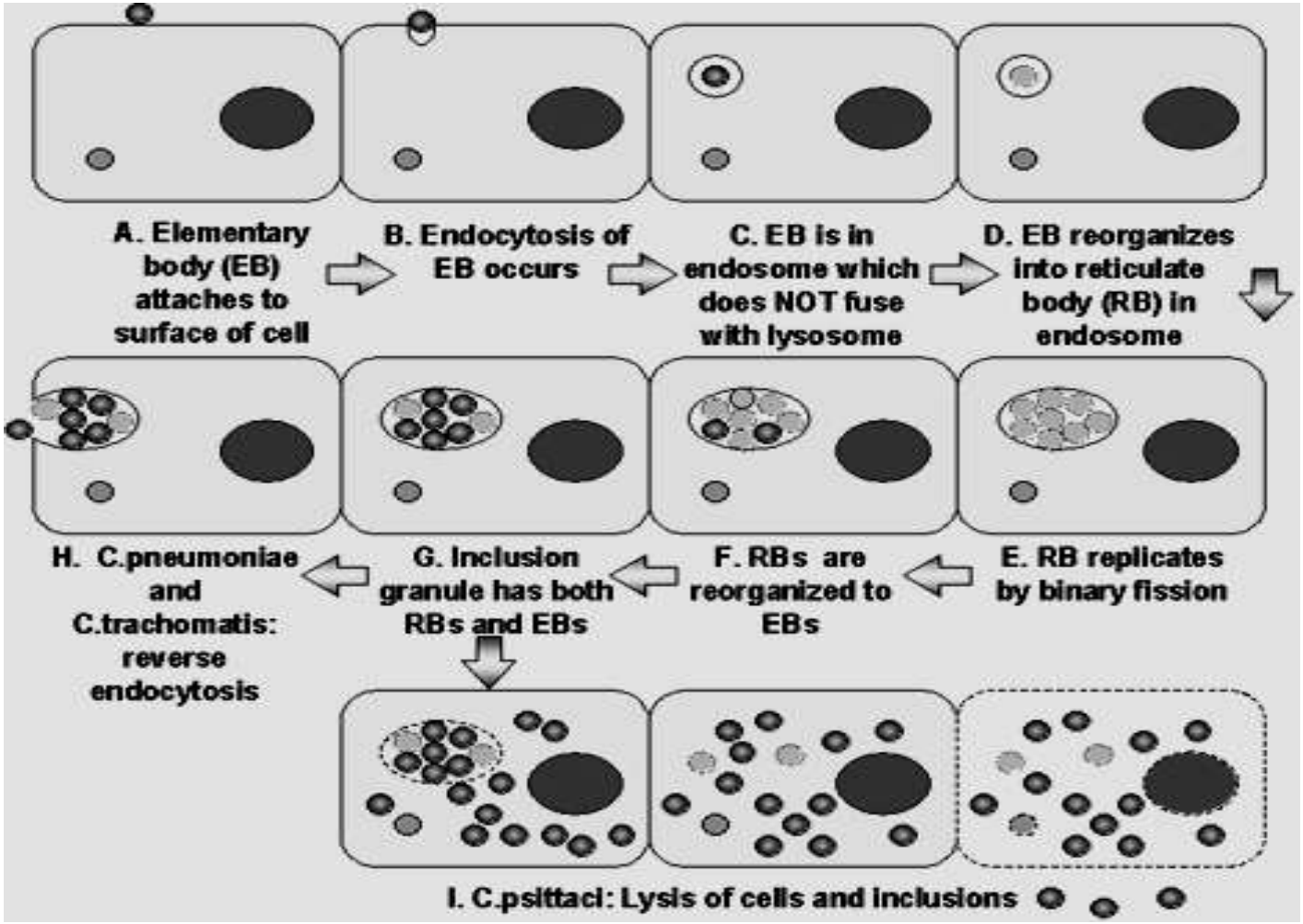
Chlamydiae: main characteristics

- Obligate intracellular parasites closely related to gram-negative bacteria.
- They lack important mechanisms for the production of metabolic energy (ATP synthesis).
- The defect restricts them to an intracellular existence where the host cell furnishes energy-rich intermediates.

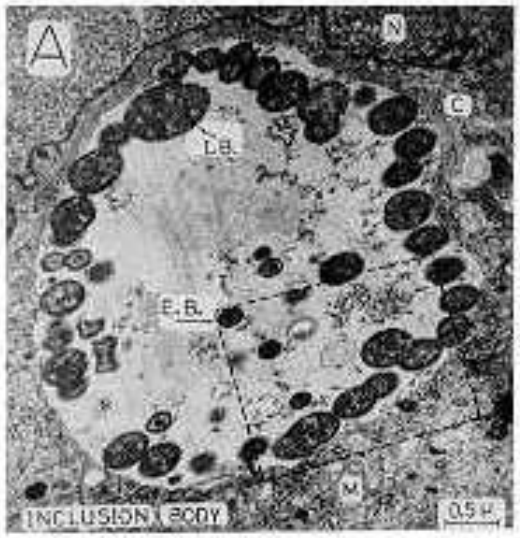
Chlamydiae: reproduction

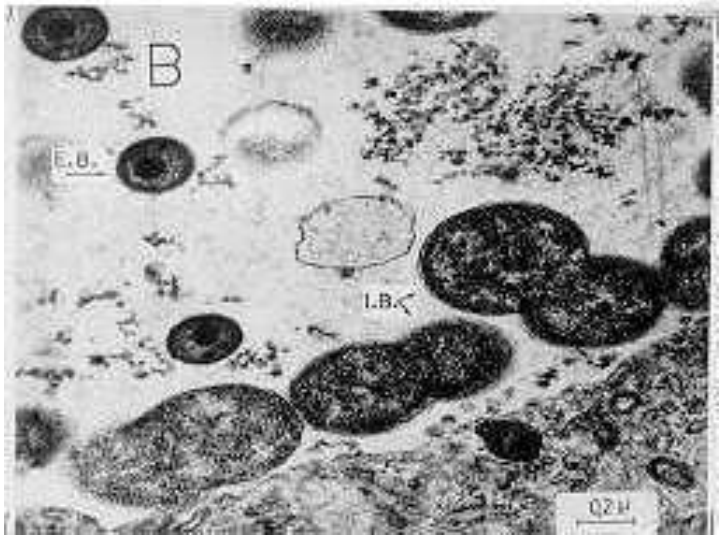
- The reproduction of chlamydiae presented by a the developmental cycle: the sequence of events starting from ”elementary body” following by “initial” and “reticular” bodies and finishing by the formation of a new generation of elementary bodies leaving the host cell.
- The result of the multiplication of chlamydiae are ‘inclusions’ in the host cell cytoplasm: the entire vacuoles filled with small particles of the bacteria.

Scheme of the developmental cycle of Chlamydia spp



Developmental cycle of Chlamydia spp (electron microscopy)

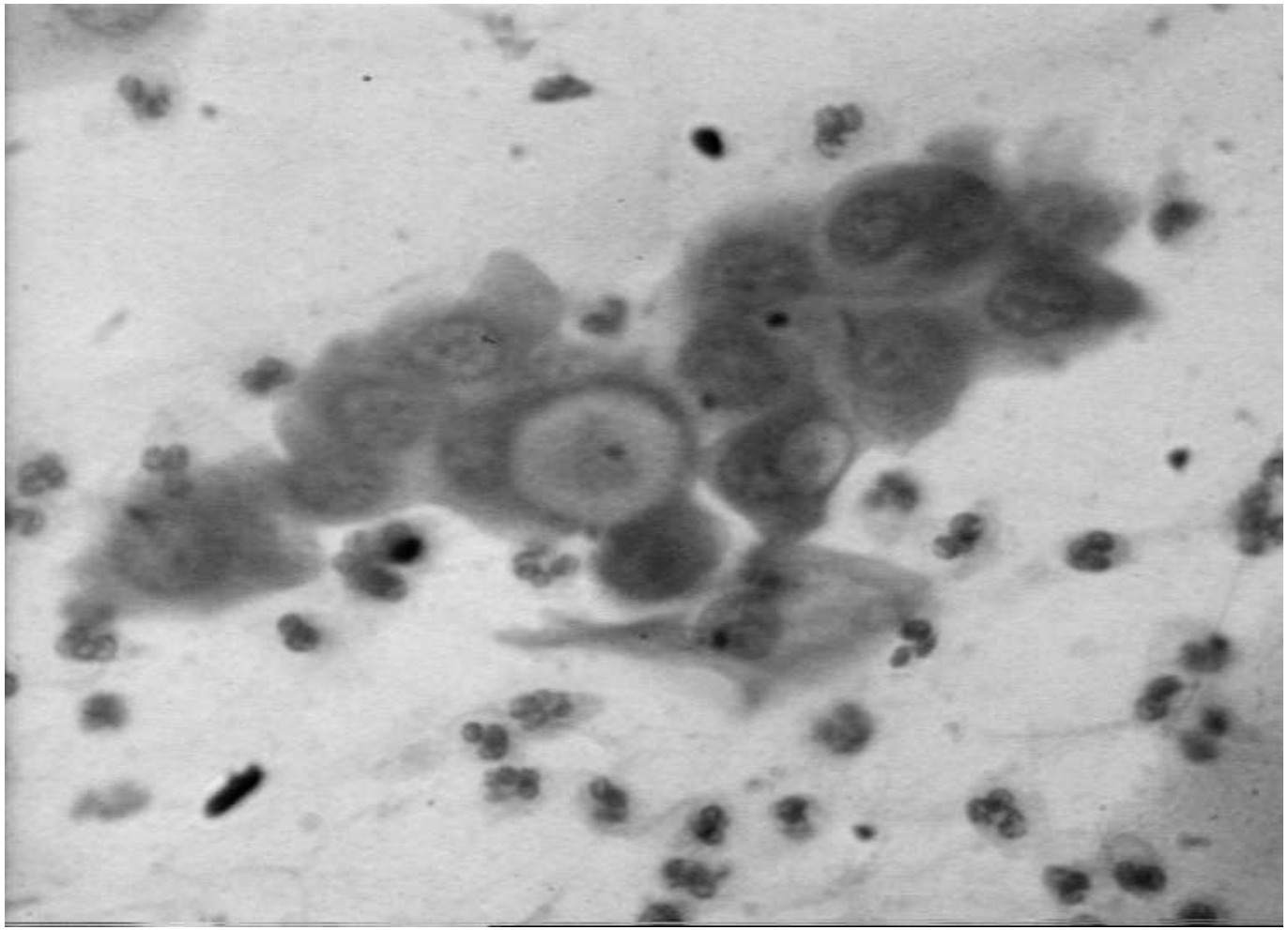


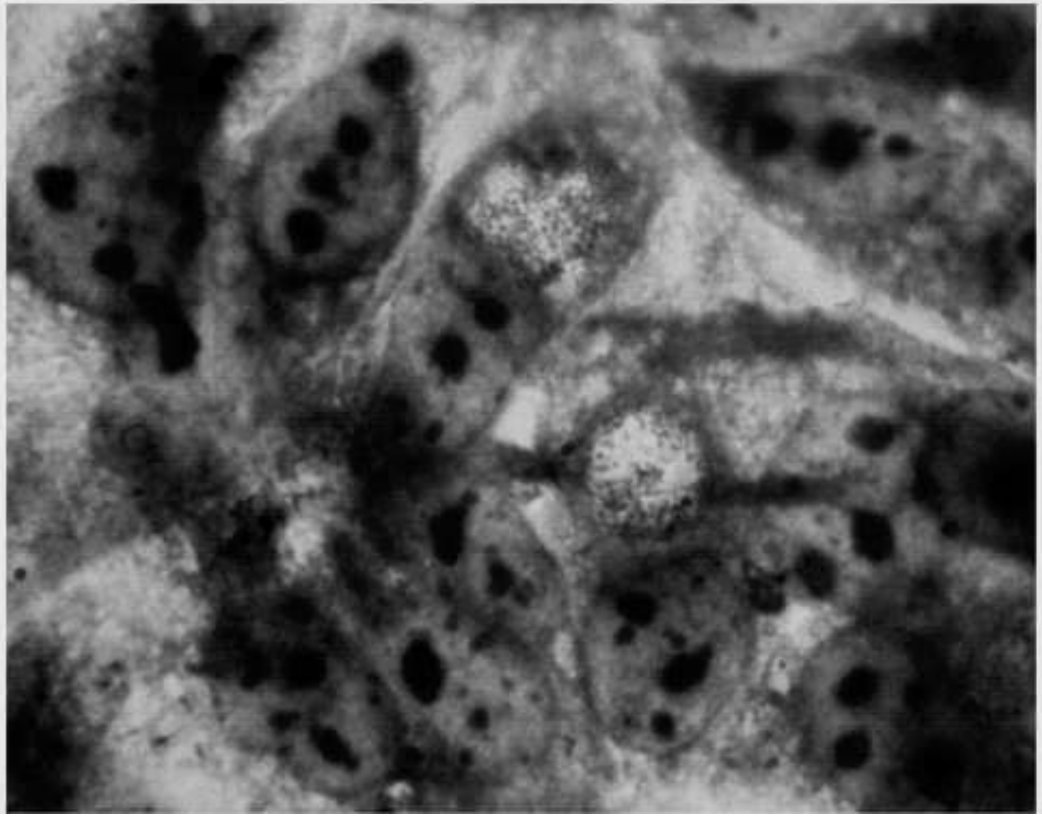


Chlamydiae: staining properties

- Single mature particles (elementary bodies) stain purple with Romanovsky-Giemsa’s stain.
- The gram reaction is negative.
- Fully formed intracellular inclusions are compact masses near the nucleus which are dark purple when stained with Romanovsky-Giemsa’s stain and if stained with dilute Lughole's iodine solution they appear brown because of the glycogen-like matrix that surrounds the particles (Chlamydia trachomatis).

Chlamydial inclusions



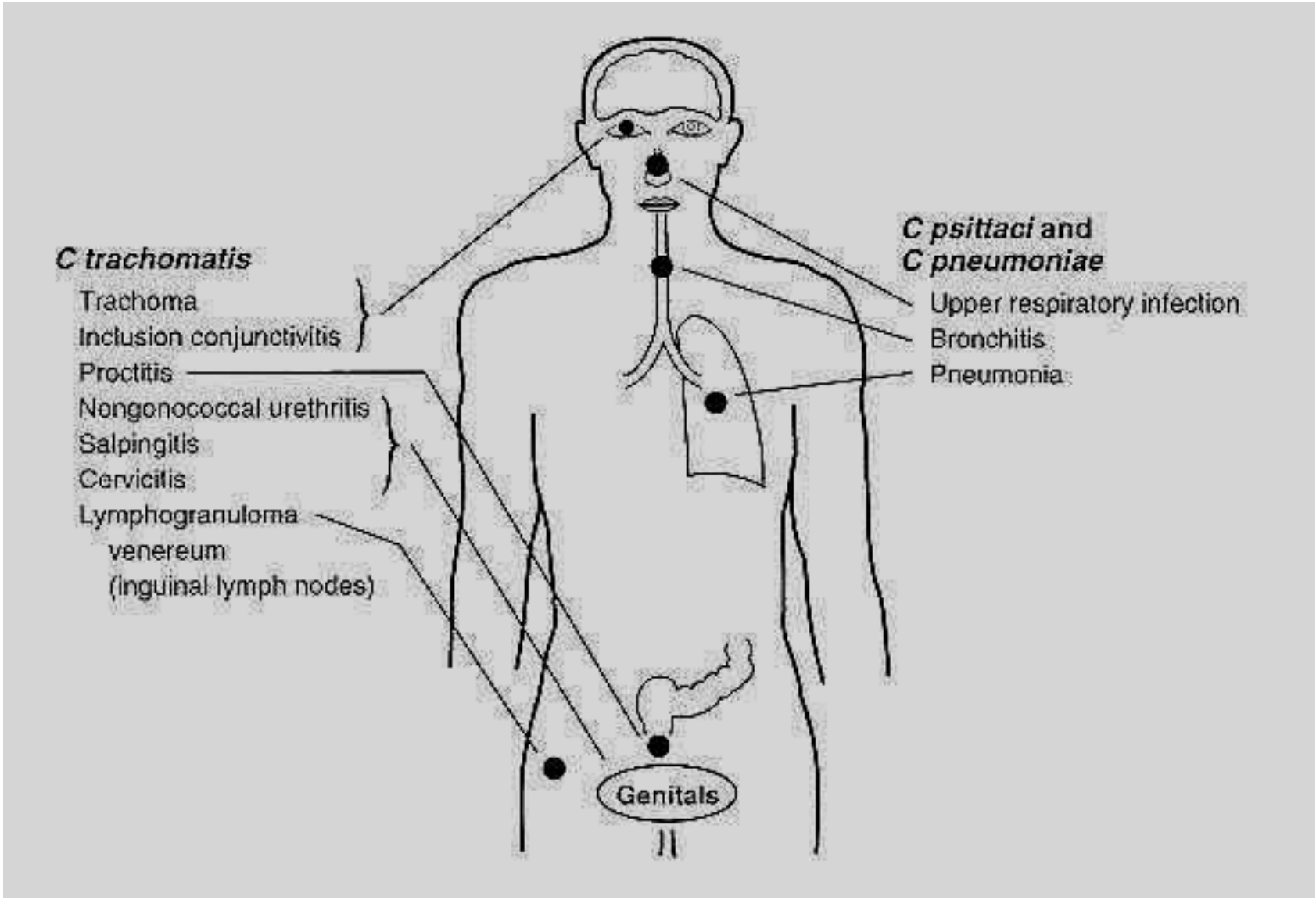


To be seen each cell are two inclusions with elementary bodies. (Giemsa stain)

Diseases due to chlamydiae: characteristics of the infectious agent and disease

On the basis of antigenic composition and intracellular inclusions Chlamydia are divided into three species:

- ***Chlamydia trachomatis*** (produces compact intracytoplasmic inclusions that contain glycogen)
  - immunotypes A – C – trachoma
  - immunotypes D – K – nongonococcal chlamydiosis
  - immunotypes L_1 – L_3 – lymphogranuloma venereum
- ***Chlamydia psittaci*** (produces intracytoplasmic inclusions that lack glycogen)
  - psittacosis in humans
- ***Chlamydia pneumoniae***
  - causes bronchitis, sinusitis, pneumonia and possibly atherosclerosis


C trachomatis
Trachoma
Inclusion conjunctivitis
Proctitis
Nongonococcal urethritis
Salpingitis
Cervicitis
Lymphogranuloma venereum (inguinal lymph nodes)
C psittaci and C pneumoniae
Upper respiratory infection
Bronchitis
Pneumonia
Genitals

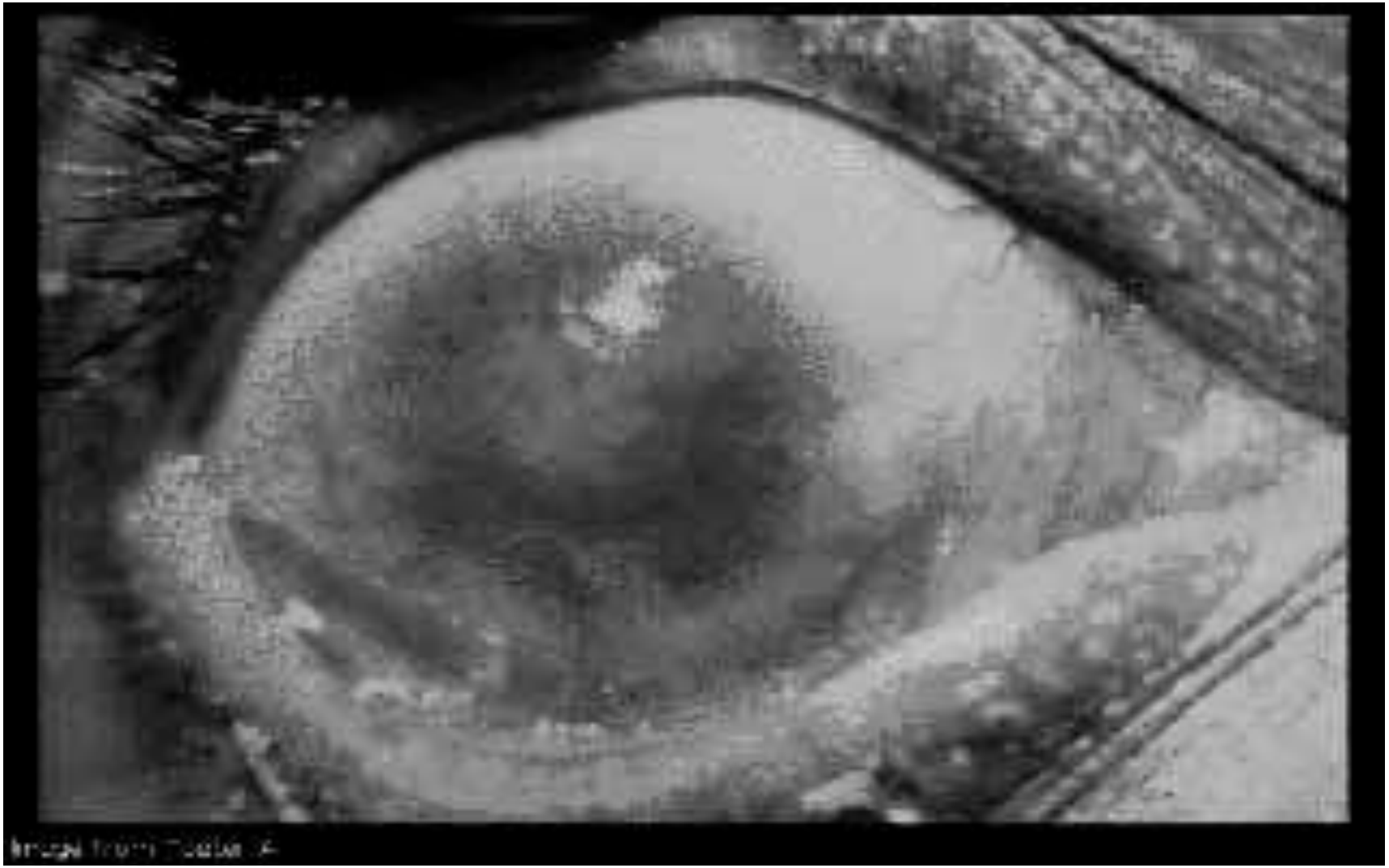
Trachoma: pathogenesis and pathology

- It is a chronic keratoconjunctivitis characterised by:
  - the development of follicles: conjunctival hyperaemia (lacrimation, mucopurulent discharge and irritation)
  - papillary hypertrophy
  - progression of pannus across the cornea leading to scar formation and sometimes to blindness

Trachoma is spread mechanically from eye to eye by contact way: by fingers and shared cosmetics, towels, etc.

The disease is most prevalent in Africa and Asia, particularly where hygienic conditions are poor.

Keratoconjunctivitis produced by Chlamydia trachomatis

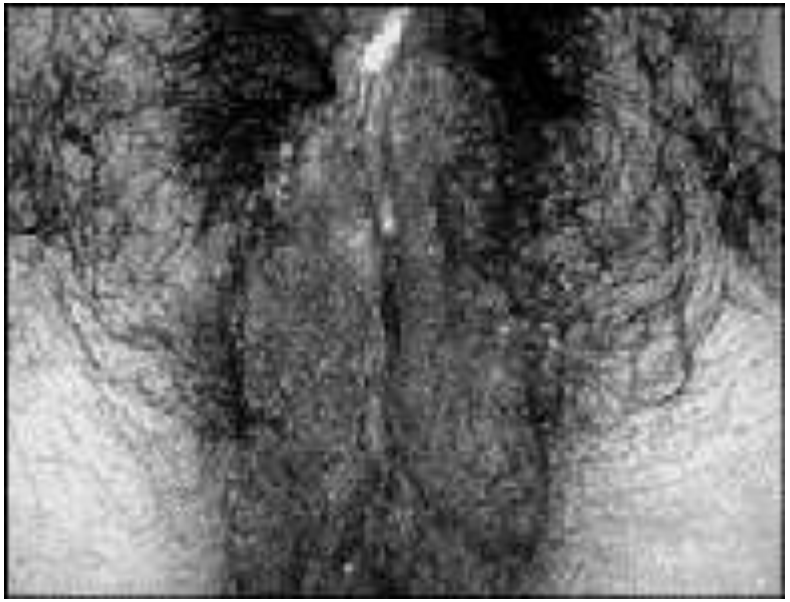


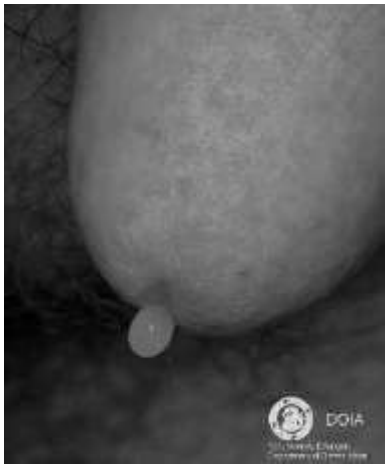
Urogenital chlamydiosis (nongonococcal urethritis): pathogenesis and pathology

- In females the infection is usually (80%) asymptomatic but symptoms can include cervicitis, urethritis, and salpingitis.
- In males, the infection is usually (75%) symptomatic. Clinical symptoms in males: dysuria, thin discharge and negative cultures for gonococci.
- Chlamydiae grow in the urethral epithelium of male and are the source of female cervical infection.
- Up to 40% of women with untreated (undiagnosed) chlamydiae will develop pelvic inflammatory diseases and about 20% of these women will become infertile.
- Women infected with chlamydia have a 3 - 5 fold increased risk of acquiring HIV.

That is sexually transmitted disease

Nongonococcal chlamydiosis





Lymphogranuloma venereum (LGV): pathogenesis and pathology

- The disease is spread by contact way (most often by sexual contact).
- The genital tracts and rectums of chronically infected persons serve as reservoirs of infection.

Lymphogranuloma venereum: pathogenesis and pathology

papule or vesicle appear on any part of external genitalia, anus or rectum (it is painless and heals in a few days)

↓

regional lymph nodes enlargement (they become painful)

↓

the nodes suppurate and discharge pus through sinus tracts (in females the perirectal nodes are involved with proctitis and anal discharge)

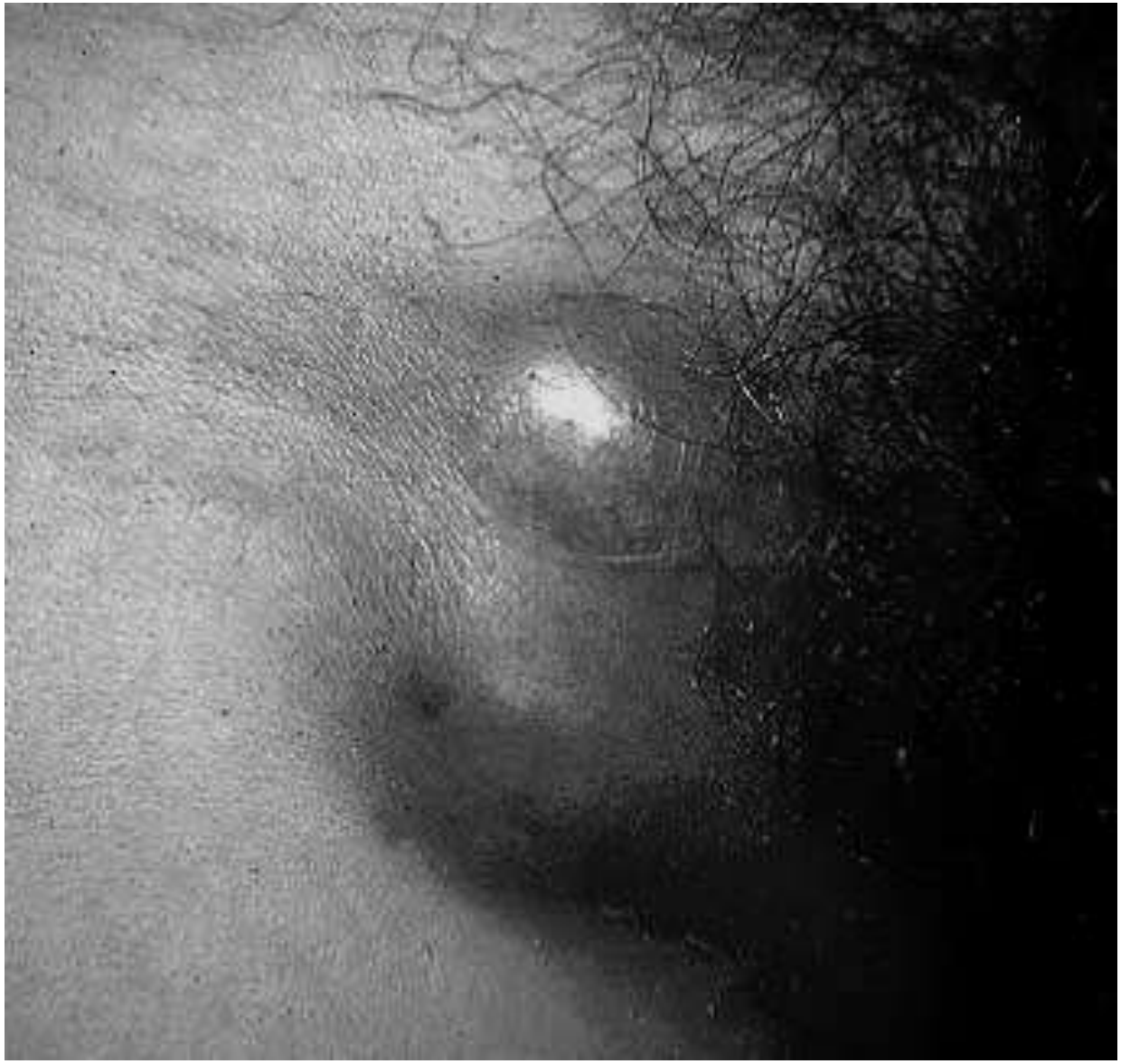
↓

chronic inflammatory process progresses to fibrosis, lymphatic obstruction (may lead to elephantiasis of penis or vulva)

↓

chronic proctitis of women may lead to fistula formation

Lymphogranuloma venereum – regional lymph nodes enlargement



Psittacosis: pathogenesis and pathology

- The disease of birds (parrot fever) that has also been called **ornithosis** from the Greek word for 'bird'.
- It can be transmitted to humans.
- The mechanism of the spreading is air-born: inhalation of infected dried bird faeces.
- The agent enters through the respiratory tract and causes patchy inflammation of the lungs.
- Liver, heart, spleen and kidney are often involved (as a result of bacteremia): they got enlarged and congested.

laboratory diagnostics of the diseases caused by chlamydiae

1. **Trachoma, urogenital chlamydiosis**
   - Romanovsky-Giemsa stain to find the intracellular inclusions in epithelial cells
   - Revealing of specific antigen in the infected epithelial cells with fluorescent antibodies
2. **Lymphogranuloma venereum**
   - Isolation of agent by inoculation of the specimens (pus, buboes or biopsy material) into the yolk sacs of embryonated eggs.
   - The CF reaction is the serologic test for the presence of antibodies in the serum of the patients (positive 2-4 weeks after onset of illness).
   - Skin hypersensitivity test.
3. **Psittacosis**
   - Complement fixation with group antigen (run in acute and later phases to establish an antibody rise).
   - Specimens (blood, sputum in fatal cases lung tissues) are inoculated into the yolk sacs of embryonated eggs and after recovering the pathogen the infection is confirmed by microscopic investigations and serologic identification.
   - Skin tests with group reactive antigen are used to confirm the diagnosis.

Chlamydial infections: immunity and treatment

The coexistence of latent infection, antibodies and cell-mediated reactions are typical for many chlamydial infections.

The tetracyclines and sulphonamides have been widely used for treatment. They could be used with good results, especially in the early stages of the infection.

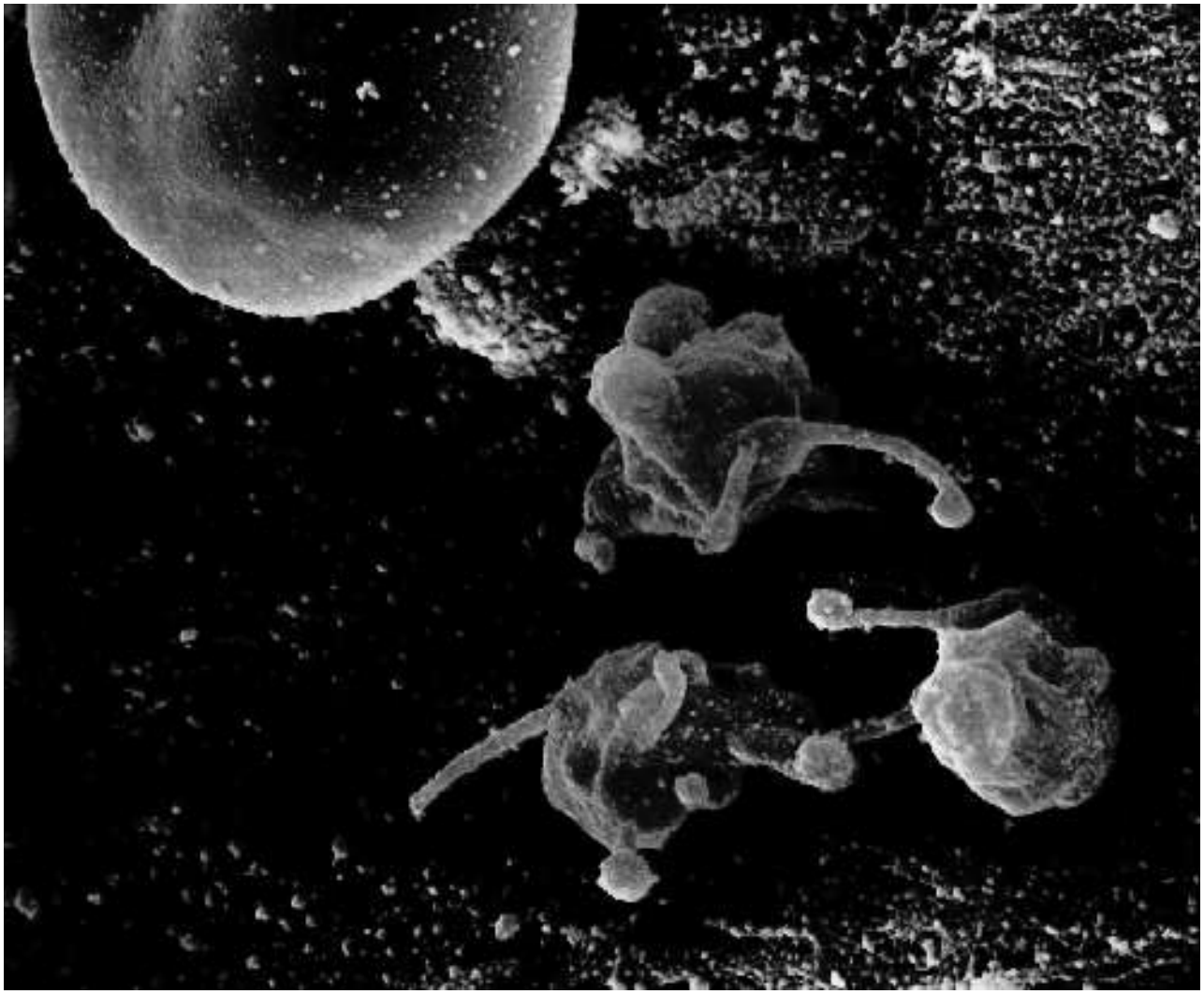
Mycopmasmas: classification

- **Tenericutes** (devision)
- **Mollicutes** (class)
- **Mycoplasmatales** (order)
- **Mycoplasmataceae** (family)
- **Mycoplasma** (genus)
- **Ureaplasma** (genus)

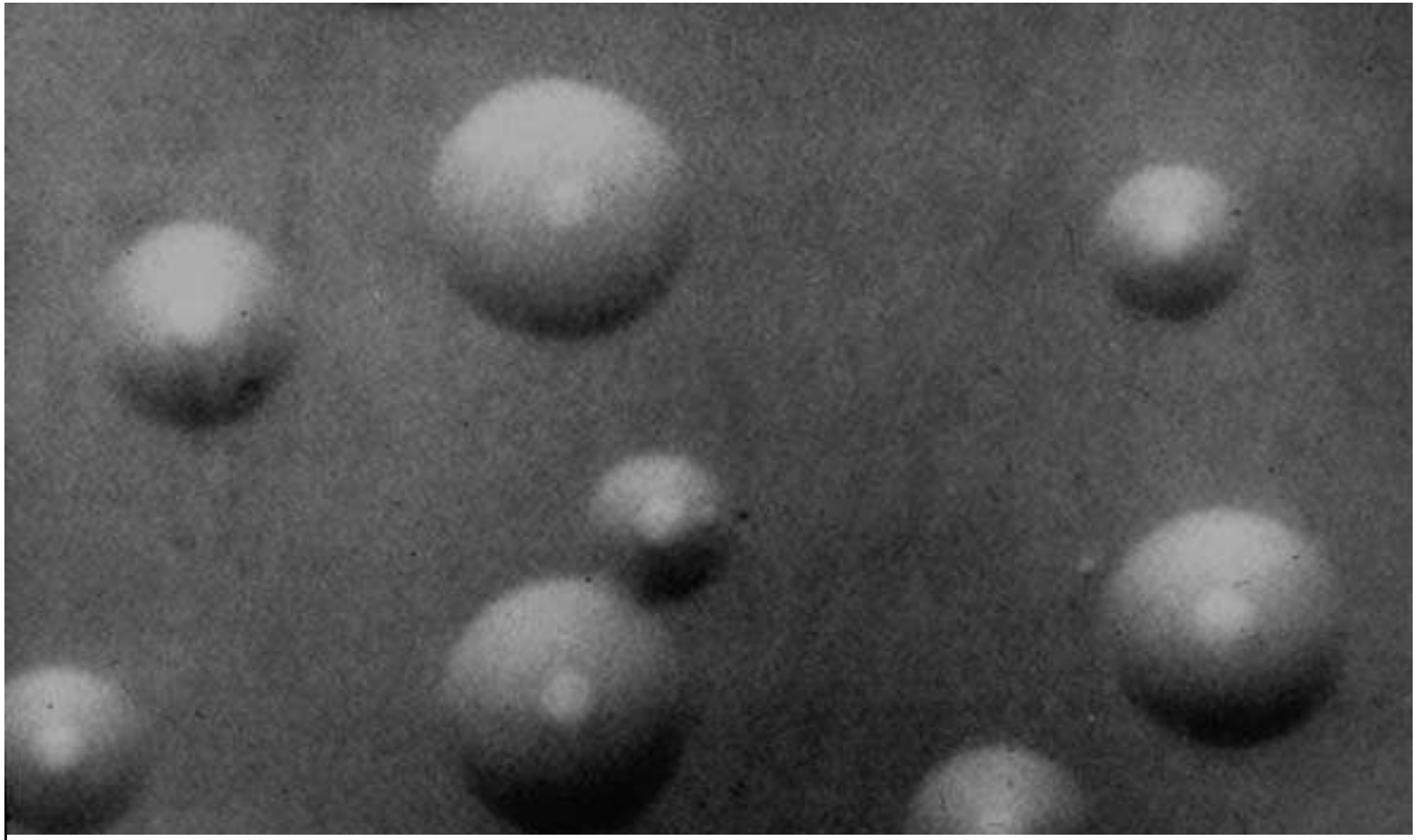
Mycoplasmas: main characteristics

- The smallest reproductive units: have a size of 125-250 nm
- Highly pleomorphic as they lack a rigid cell wall and are bounded only by triple-layered membrane
- Complete resistant to penicillin
- Can reproduce in cell-free media; on agar the centre of the whole colony is characteristically embedded beneath the surface (“fried egg” appearance)
- Growth is inhibited by specific antibodies
- All parasitic strains of mycoplasma require for growth cholesterol and in the cell culture they develop predominantly at the cell surface (are extracellular pathogens).
- Toxic metabolic products (hydrogen peroxide and superoxide) which are products of mycoplasma accumulate and damage host tissues (oxidized host lipids).

Mycoplasmas - highly polymorphic bacteria



"Fried egg" colonies of Mycoplasma bacteria on agar medium

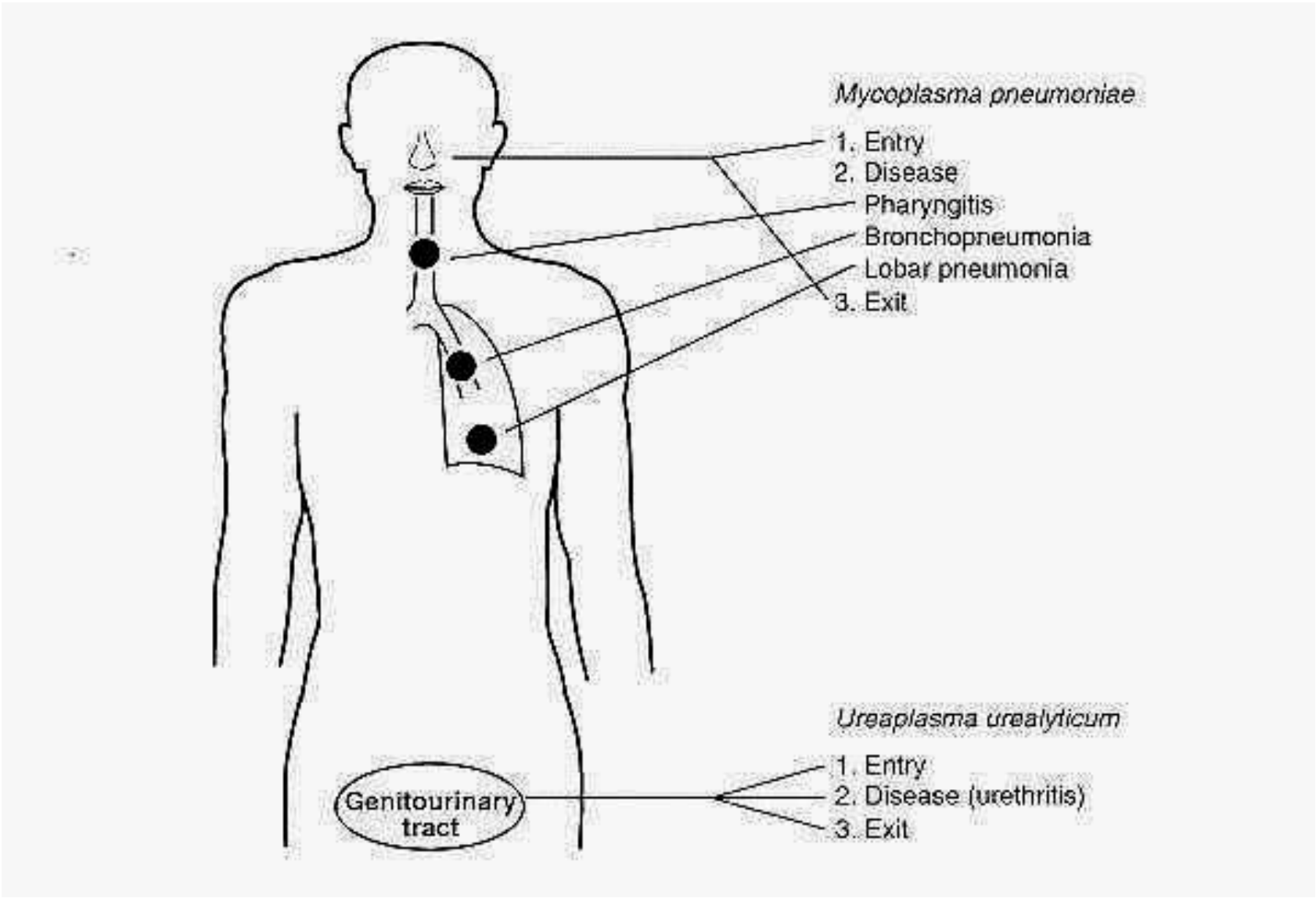


Diseases due to mycoplasmas: characteristics of the infectious agent and disease

In vivo mycoplasmas appear to be intracellular parasites with a predilection for mesothelial cells . In human respiratory tract infections mucous membranes are inflamed .

- ***Mycoplasma pneumoniae*** is the causative agent of nonbacterial pneumonia, the effects range from inapparent infection to mild or severe upper respiratory disease and bronchial pneumonia.
- ***Mycoplasma hominis*** can cause an acute, afebrile respiratory illness, pelvic inflammatory disease and pyelonephritis. Women can carry it in the genital tract.
- ***Ureaplasma uralyticum*** is found in some cases of urethritis and prostatitis in men who suffer from “nongonococcal urethritis” .

When individuals become sexually active, the rates of colonization by M.hominis and U.urealyticum increase. Approximately 15% are colonized with M. hominis and 45% - 75% with U. urealyticum. The carriers are asymptomatic.


Mycoplasma pneumoniae
1. Entry
2. Disease
Pharyngitis
Bronchopneumonia
Lobar pneumonia
3. Exit
Ureaplasma urealyticum
Genitourinary tract
1. Entry
2. Disease (urethritis)
3. Exit

Mycoplasmas: laboratory diagnostics

- Culture: the material (throat swabs, sputum, urethral or genital secretions) is inoculated onto special media following with microscopic investigations by immunofluorescence technique.
- Serology: CF tests can be performed with glycolipid antigens extracted from mycoplasmas, conterimmunoelectrophoresis in gel may be used.
- The tests that measure the inhibition of growth by antibodies are quite specific.

Mycoplasmas: immunity and treatment

- Antibodies develop in humans infected with mycoplasmas and rising antibody titre is required for diagnostic significance because of the high incidence of positive serologic tests in normal individuals.
- Many strains of mycoplasma are inhibited by a variety of antimicrobial drugs, but some strains are resistant to penicillin and cephalosporin. Tetracycline and erythromycin are the drugs of choice in mycoplasmal pneumonia.

PATHOGENIC FUNGI AND PROTOZOA.

Theme N27

Mycoses: laboratory diagnostics

1. Microscopic investigations
2. Growing of fungal culture
3. Immunological test
4. Molecular – genetic techniques in the case of systemic mycoses

Microscopic investigations in diagnosis of mycoses

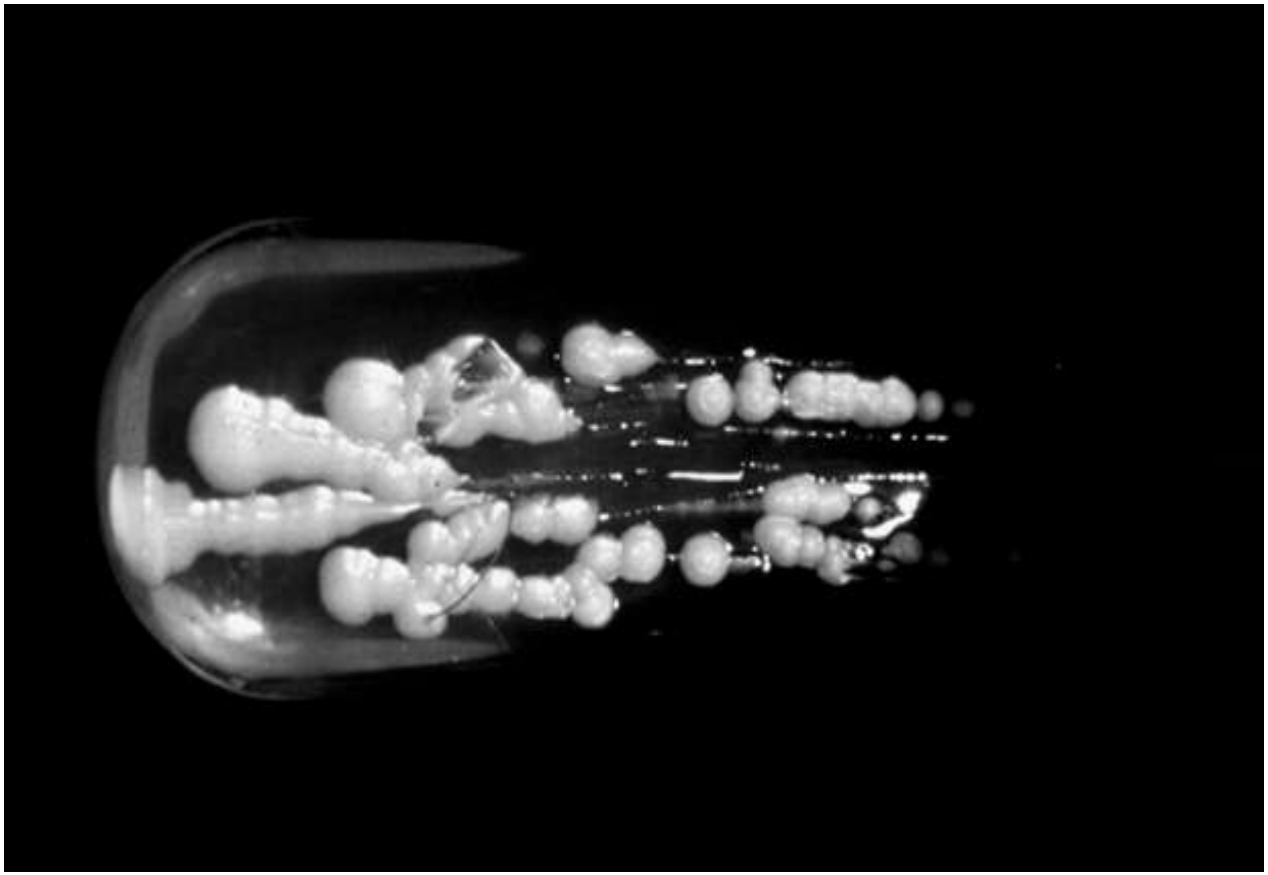
The basic method of diagnosis:

- microscopic investigations of wet mounts: hanging drop could be used to reveal the fungal structures in the semi-liquid specimens,
- use of 10% solution of KOH to visualise fungal structures in the specimens rich in keratin: pieces of skin, nails, hair, etc,
- microscopic investigation of stained smears:
  - Gram-stained (fungi are stained as gram-positive)
  - use of Schiff's stain (to reveal polysaccharides in the cell wall)
  - use of Romanovsky-Giemsa technique, etc
- fluorescent sera are usually used to reveal antigens of infectious agent – in direct immunoflurescence reaction.

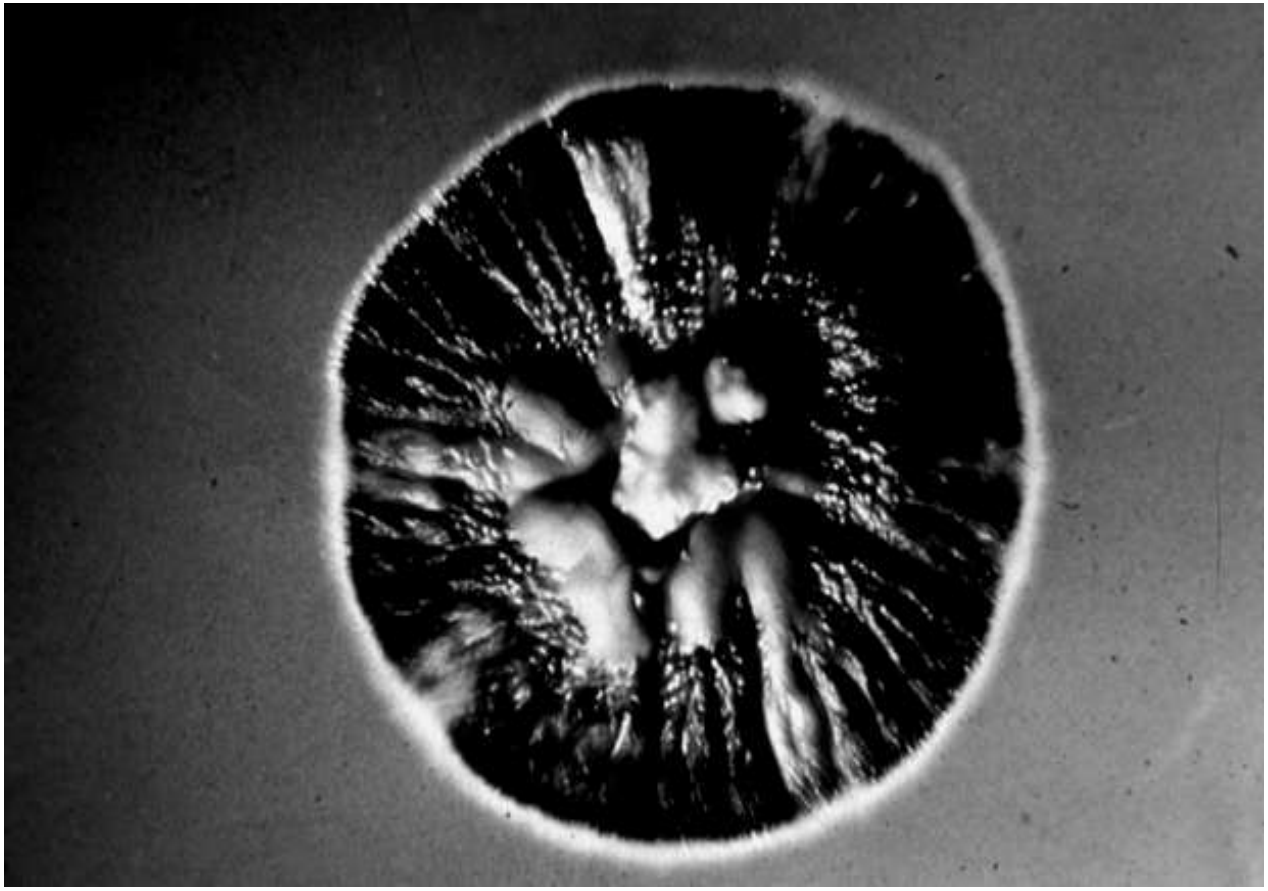
Cultivation of fungi

- The pH of mycological media usually ranges between 4.0 and 6.5 (bacterial pathogens usually are not capable of growing under these acid conditions).
- The standard laboratory culture medium is ***Sabouraud's agar*** (SAB) (peptone agar containing high concentrations of maltose and glucose).
- To make the media more selective for fungi different antibacterial agents are usually added (penicillin, streptomycin, etc)
- The fungal cultures are usually growing at different temperature regime to reveal possible dimorphism of the fungus(at 25^{o}C and 37^{o}C).
- Final identification involves characteristics of morphology and biochemical properties of the fungus.
- The method of cultivation takes about several weeks.

Character of growth of Candida (yeasts) on agar slant



Character of growth of mould on agar medium



Mycoses: classification

- Superficial mycoses (dermatomycoses, dermatophytoses)
- Subcutaneous mycoses
- Systemic mycoses
- Opportunistic mycoses

Superficial mycoses: aetiology

- Involve the keratin-containing structures of the body, and disease rarely spreads to other tissues.
- The major cases caused by Trychophyton, Microsporum and Epidermophyton species, which collectively referred to fungi dermatophytes.

Superficial mycoses: pathogenesis

- Infection occurs with Candida and hyphal fragments of fungi and restricted to keratinous structures (i.e., the stratum corneum, hair and nails).
- The virulence of these fungi is low.
- The ability to break down and metabolise keratin (connected with the ability of production of specific enzymes by fungi) is characteristic for all etiologic agents.

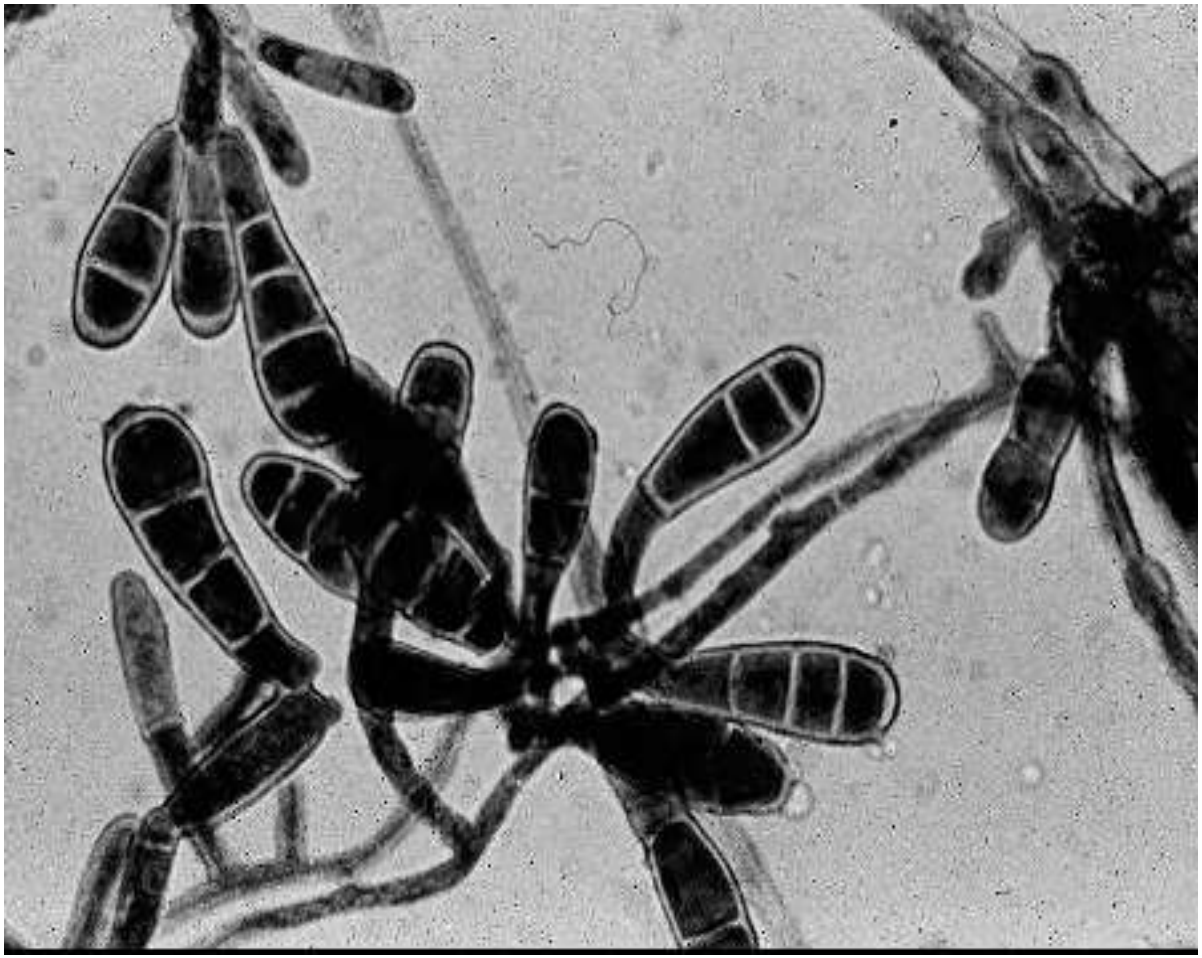
Dermatophytoses: characteristics of the diseases

- The disease is commonly known as ringworm and diagnostically is referred to as tineas.

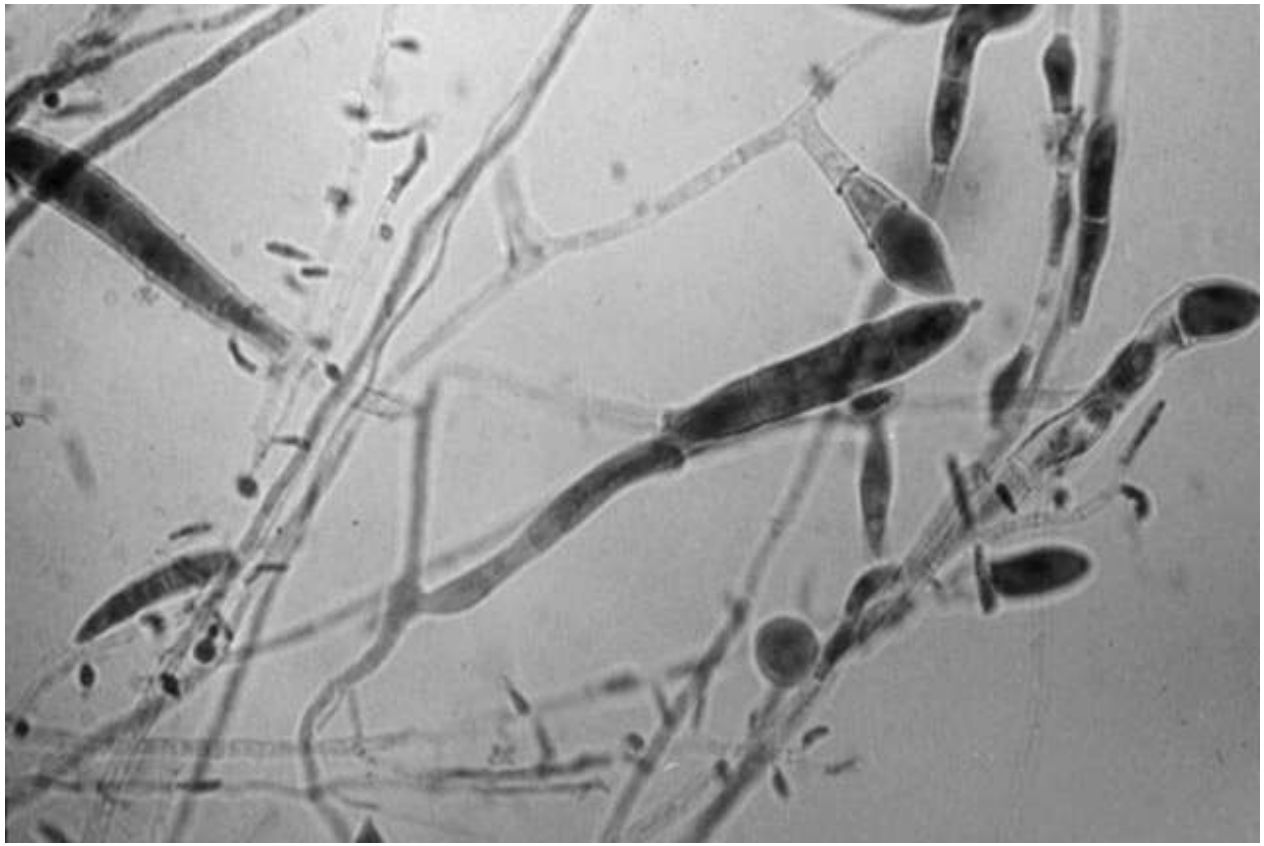
Some clinical features of dermatophyte infections

Skin disease	Location of lesions	Clinical appearance	Fungi most frequently responsible
Tinea corporis (ringworm)	Involves the trunk of the body	Circular patches with advancing red, vesiculated border and central scaling	Microsporum canis, Trichophyton mentagrophytes
Tinea capitis	Infection of hair and scalp	Circular bald patches with short hair stubs or broken hair within hair follicles	M. canis, T. tonsurans
Tinea cruris	Groin. Infection of the inguinal areas.	Erythematous scaling lesion in intertriginous areas.	T. rubrum, Epidermophyton floccosum
Tinea pedis (athlete's foot)	Interdigital spaces on feet	Acute: itching, red, vesicular. Chronic: itching, scaling, fissures.	T. mentagrophytes
Tinea unguium	Nail	Nails thickened or crumbling distally, discoloured, lustreless	T.rubrum, E. floccosum

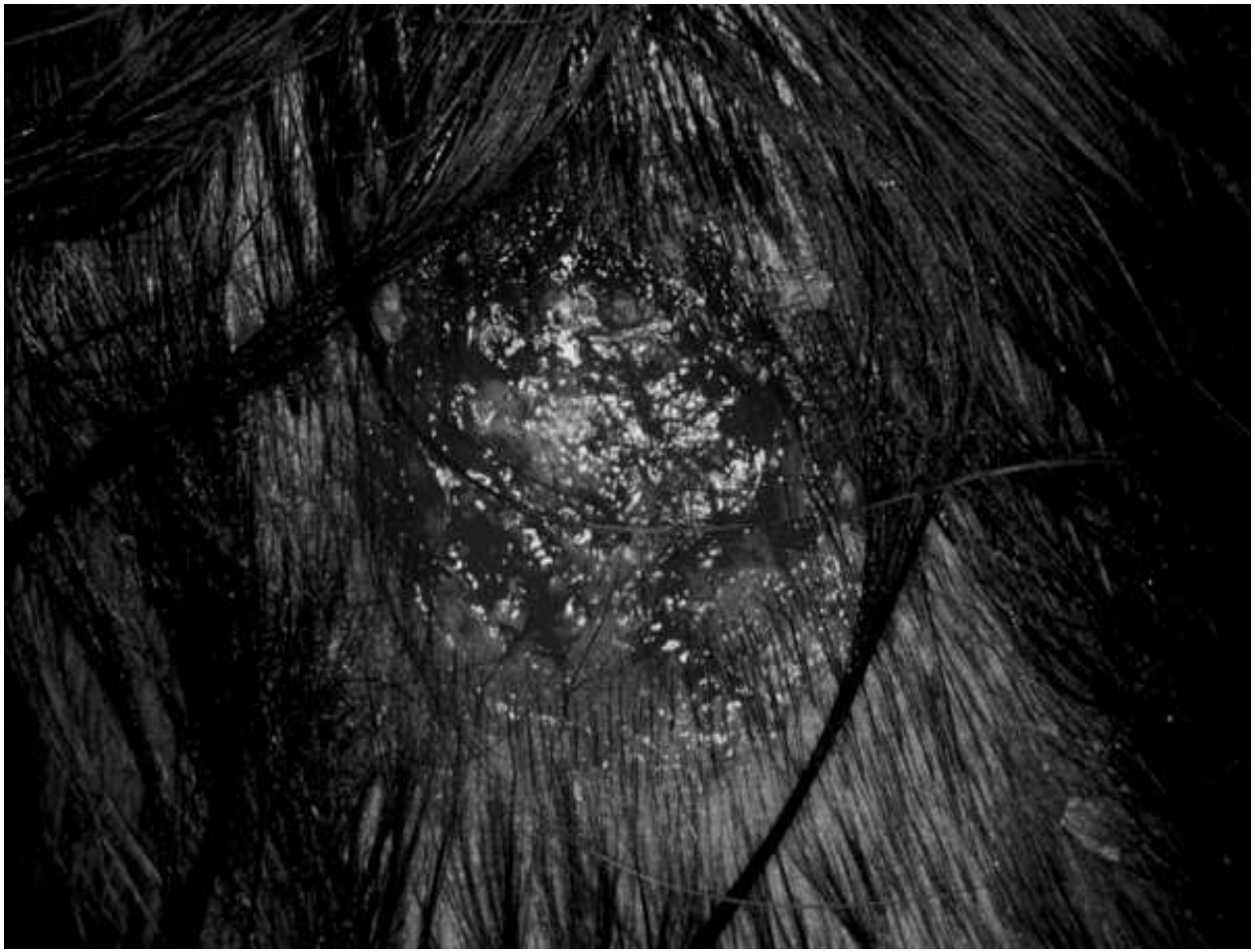
Epidermophyton floccosum



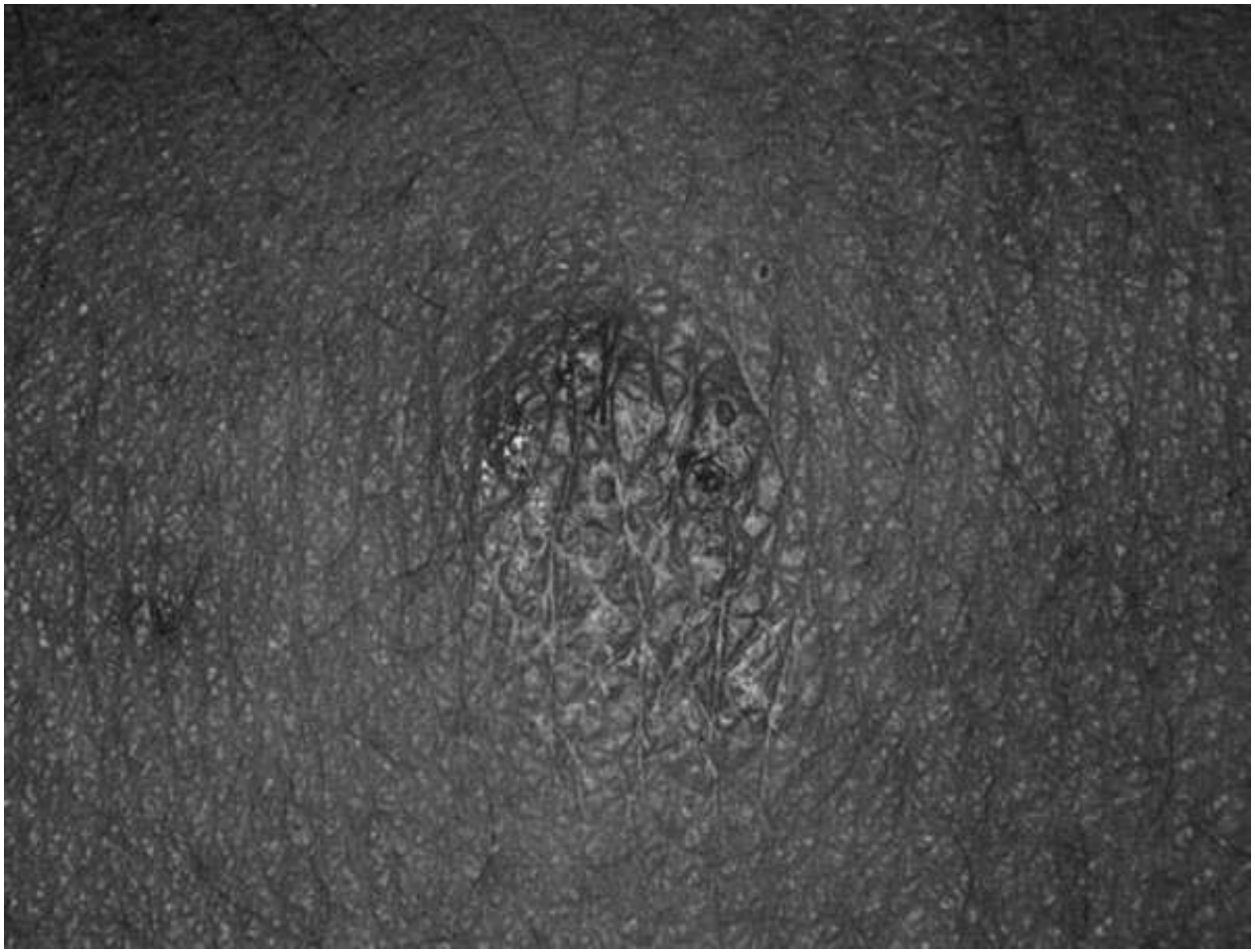
Trichophyton tonzurans



Tinea captis (ringworm of the scalp)

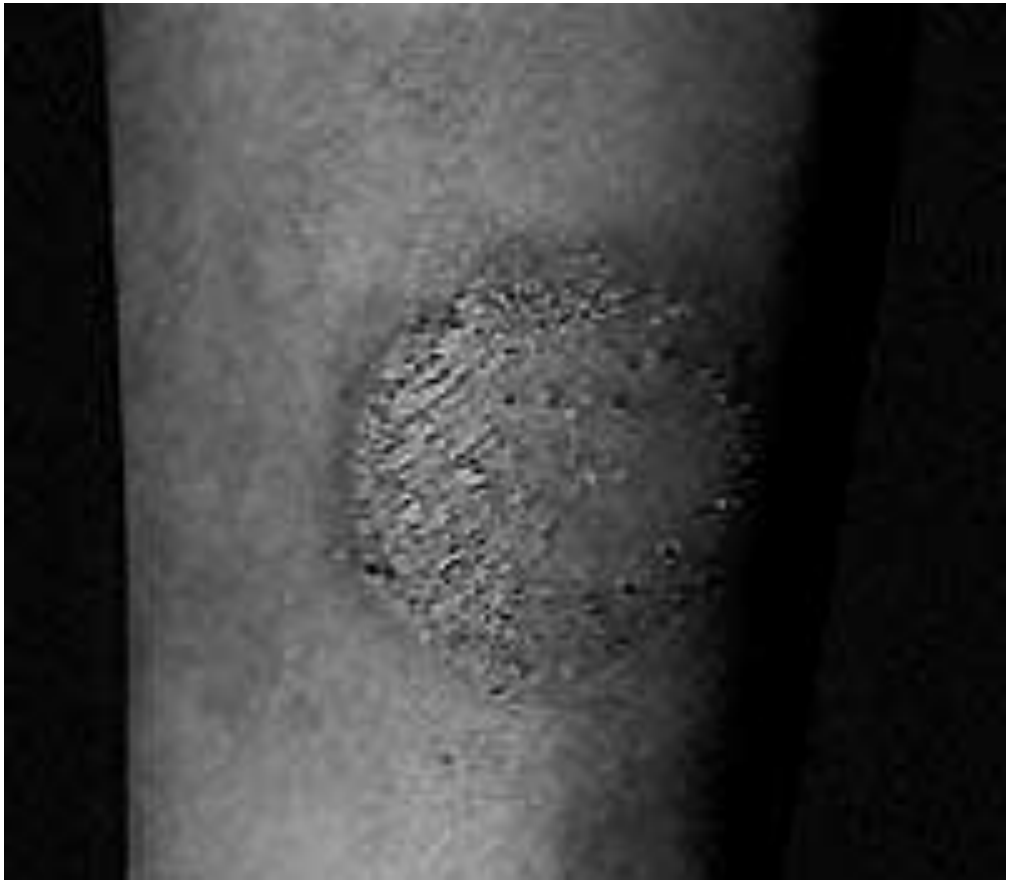


Tinea corporis: lesions on the skin

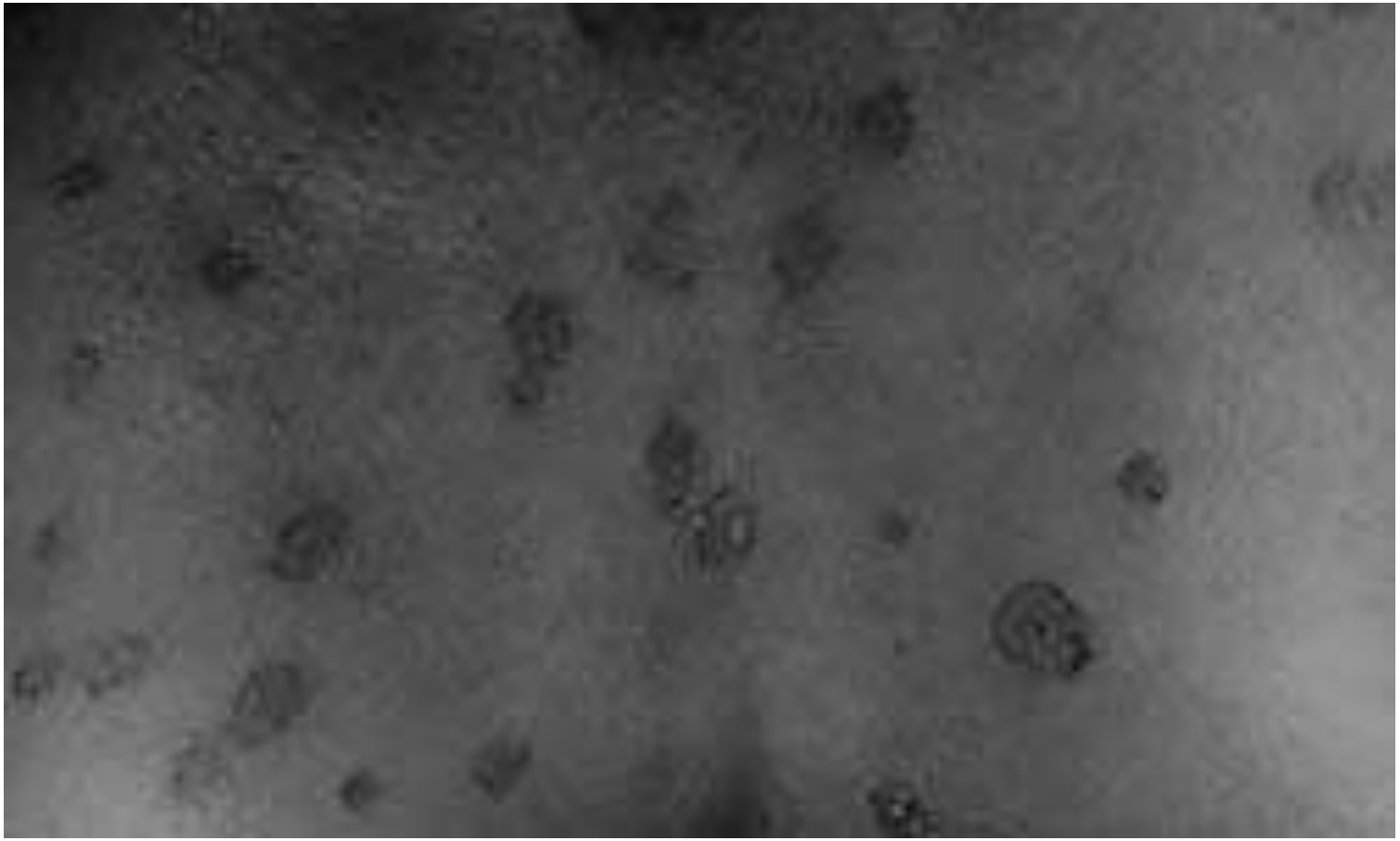


Microsporum canis

Microsporia: lesions on skin



Microsporia: disseminated centres



Superficial mycoses: laboratory diagnostics

Trichophyton

Microscopic examination:

Specimens are placed on a slide in a drop of 10-20% potassium hydroxide, covered with a coverslip and examined immediately. Results:

- Parallel rows of spores outside the hair shaft - ectothrix (usually zoophiles)
- Parallel rows of spores inside the hair shaft - endotrix (usually infect humans)

Culture:

specimens are inoculated onto Saburaud's agar slants, incubated for 1-3 weeks at room temperature.

Superficial mycoses: laboratory diagnostics

Microsporum

Microscopic examination:

- in hair - dense sheaths of spores in a mosaic pattern around the hair
- in skin or nails – branching hyphae or chains of spores are seen

Culture:

specimens are inoculated onto Saburaud's agar slants, incubated for 1-3 weeks at room temperature.

Infected hair under Wood's light produce

bright green fluorescence

Subcutaneous mycoses: main characteristics

- The fungi which cause subcutaneous mycosis grow in soil or on decaying vegetation.
- They have to be introduce into the subcutaneous tissue to produce disease.
- Lesions spread slowly from the area of implantation of fungi (usually by lymphatic draining).

Subcutaneous mycoses: sporotrichosis

- Sporotrichum schenckii:
- dimorphic fungus,
- lives on plants or wood and causes sporotrichosis, a chronic granulomatous infections when traumatically introduced into the skin.

Subcutaneous mycoses: chromomycosis

Several species of black moulds

- Phialophora verrucosa
- Fonsecaea pedrosoi
- Fonsecaea dermatitidis
- Cladosporium carrioni

- The fungi are dimorphic deuteromycetes.
- They are introduced by trauma into the skin, and over months or years, wartlike growths extend the lymphatics of the effected area.
- Histological preparations of the lesions show dark-brown granulomas within leucocytes or giant cells.

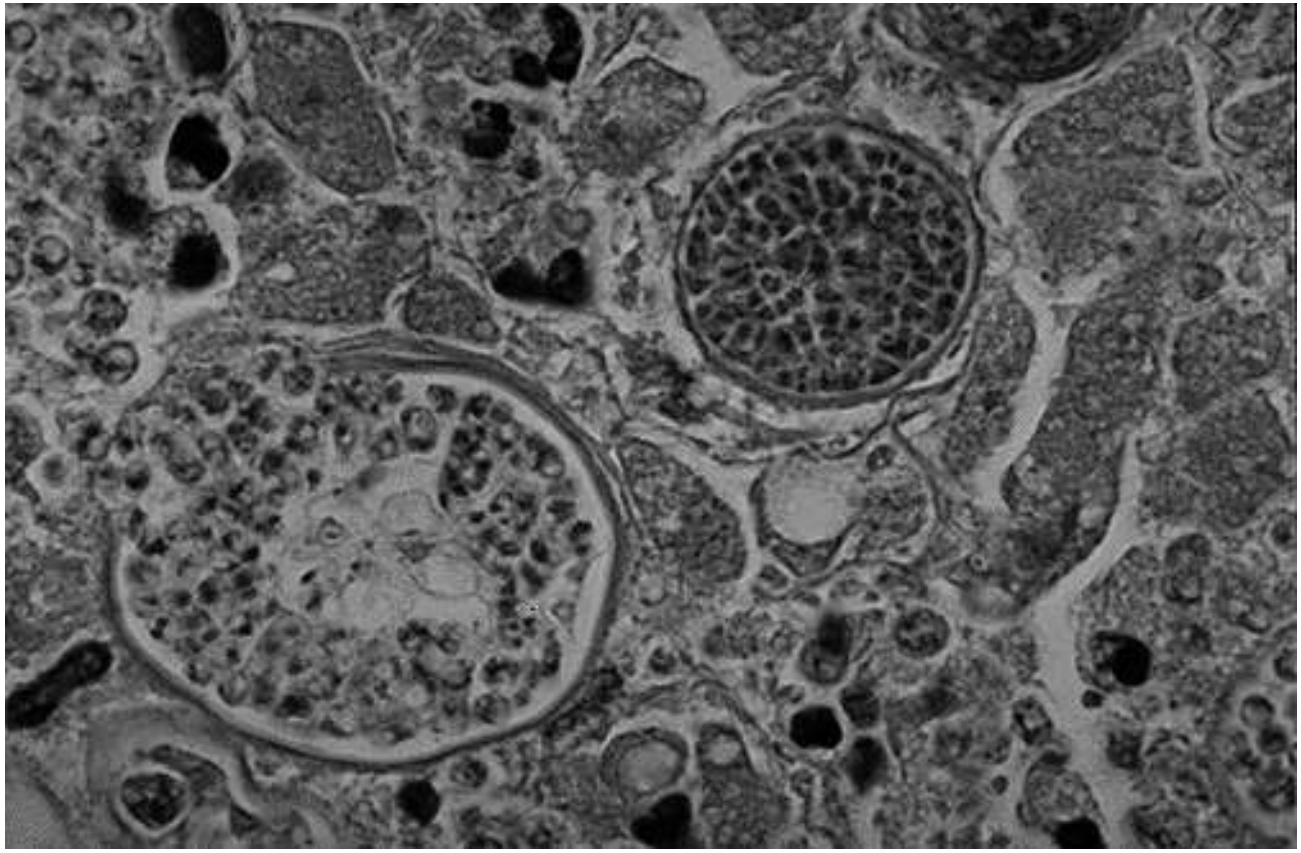
Systemic mycoses: main characteristics

- The mycoses caused by soil dimorphic fungi.
- Infection is acquired by inhalation and most infections are asymptomatic.
- Dissemination of infection may occur to any organ.
- The fungi cause disease in specific predisposed persons, and in these patients usually fatal infection takes place.

Systemic mycoses: coccidioidomycosis

- Coccidioides immitis: dimorphic fungus.
- The infection is endemic in some regions of the southern USA and Latin America and it is usually self-limited.
- Infection is acquired through the inhalation of air-borne spores. Dissemination is rare but may be fatal. Disseminated disease is comparable to tuberculosis, with lesions in many organs, bones and the CNS.
- In histological sections of tissue, in pus, or sputum it appears as a spherule with a thick wall and filled with endospores which are released into infected tissue upon rupture of the wall.

Coccidioides immitis in liver tissue (the wall of one of the spherule is ruptured)



Systemic mycoses: blastomycosis and paracoccidioidomycosis

- Dimorphic fungi.
- Blastomyces dermatitidis causes chronic granulomatous disease.
- Paracoccidioides sp. infects lungs and could be disseminated to the spleen, liver, mucous membranes and skin.

Opportunistic mycosis: main characteristics

- Fungi which cause opportunistic mycoses usually do not induce disease but may do so in persons who have altered host defence mechanisms.
- They may infect any or all organs of the body.

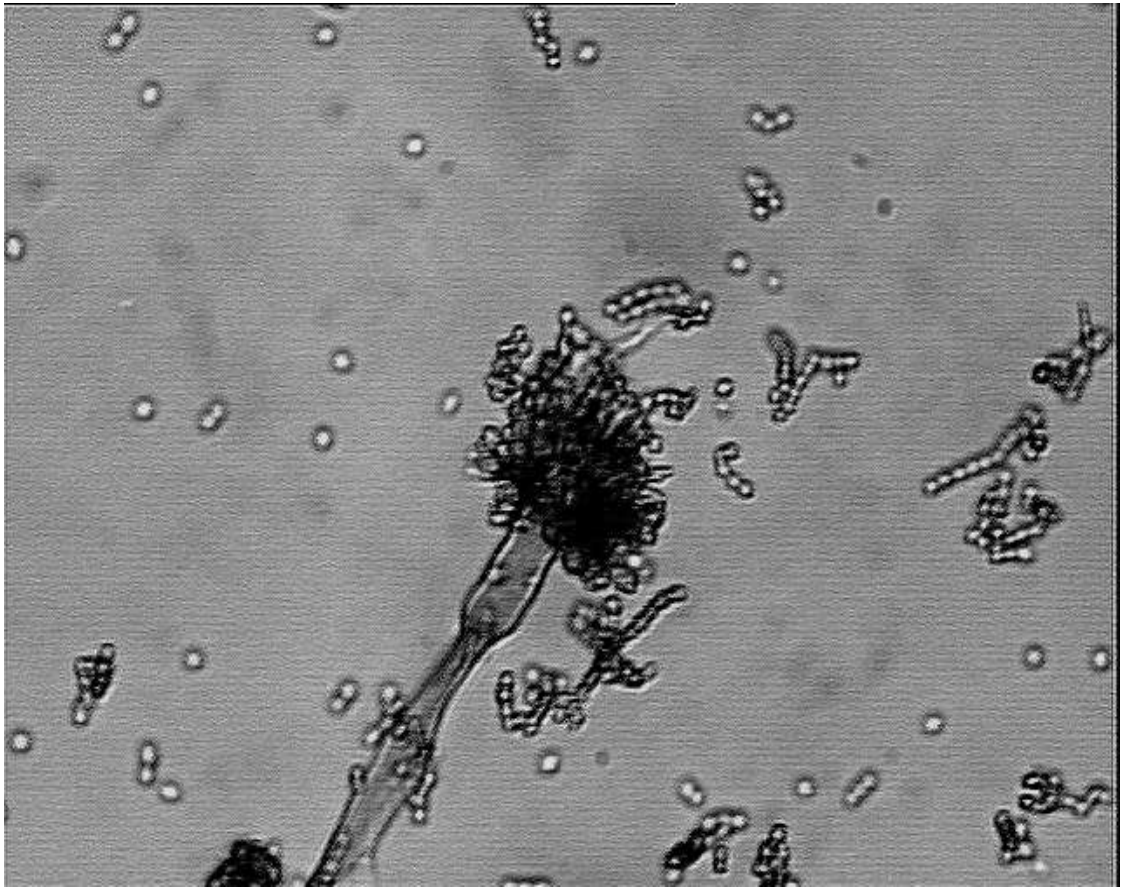
Asperigillosis: classification of pathogens and main characteristics of disease

Various species of genus Aspergillus cause the disease:

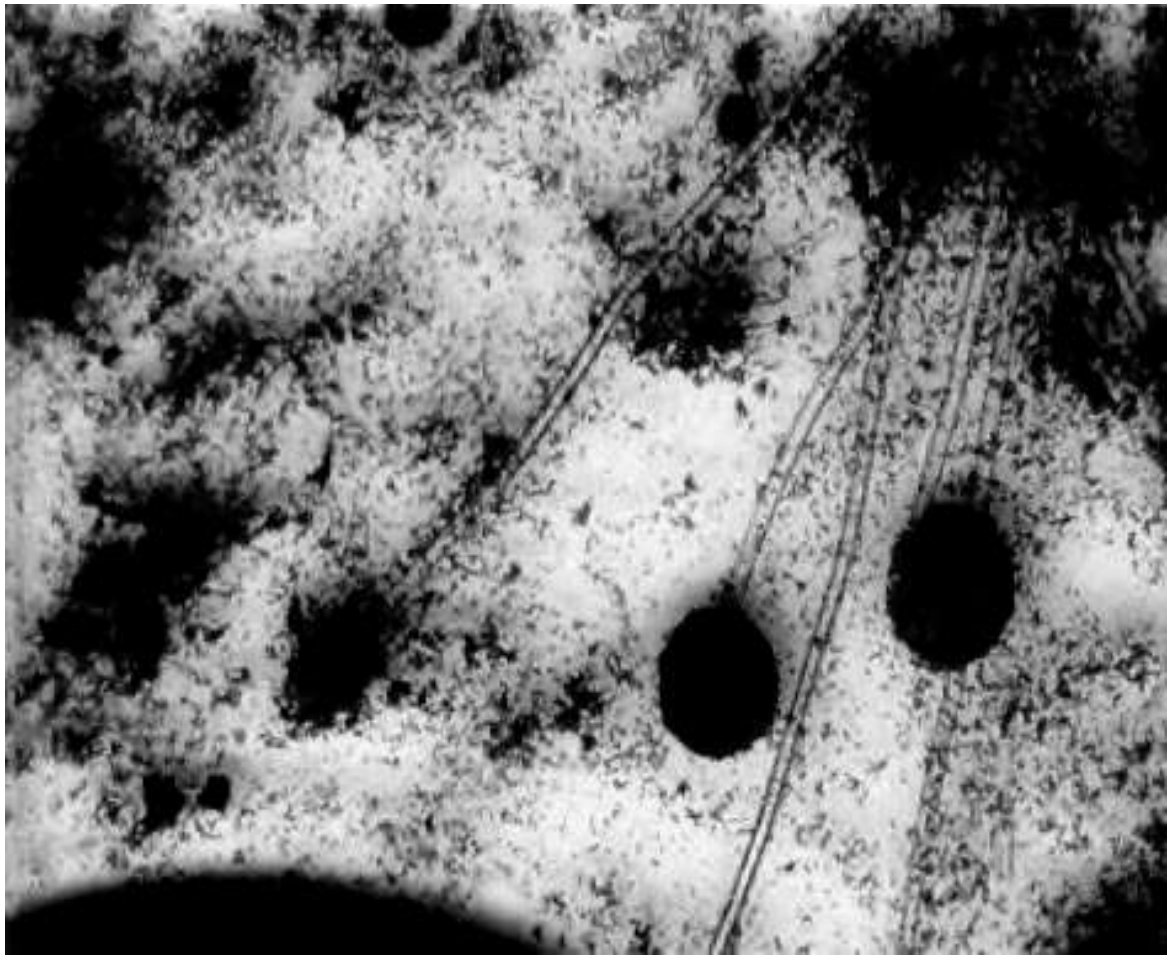
- A. fumigatus
- A. flavus
- A. niger
- A. nidulans

The fungi are opportunistic invaders in immunodefficient persons and cause pulmonary aspergillosis – necrotising pneumonia.

Aspergillus fumigatus



Aspergillus niger



Candida infections (candidiasises): classification of pathogens and main characteristics of disease

Among yeast cells Candida pathogens are:

– more frequently – C. albicans

– rare – C. tropicalis

– very rare – other species of the genus (>100)

The fungi cause the following infections localised in:

– female genitalia,

– skin,

– lungs,

– chronic mucocutaneous candidiasis.

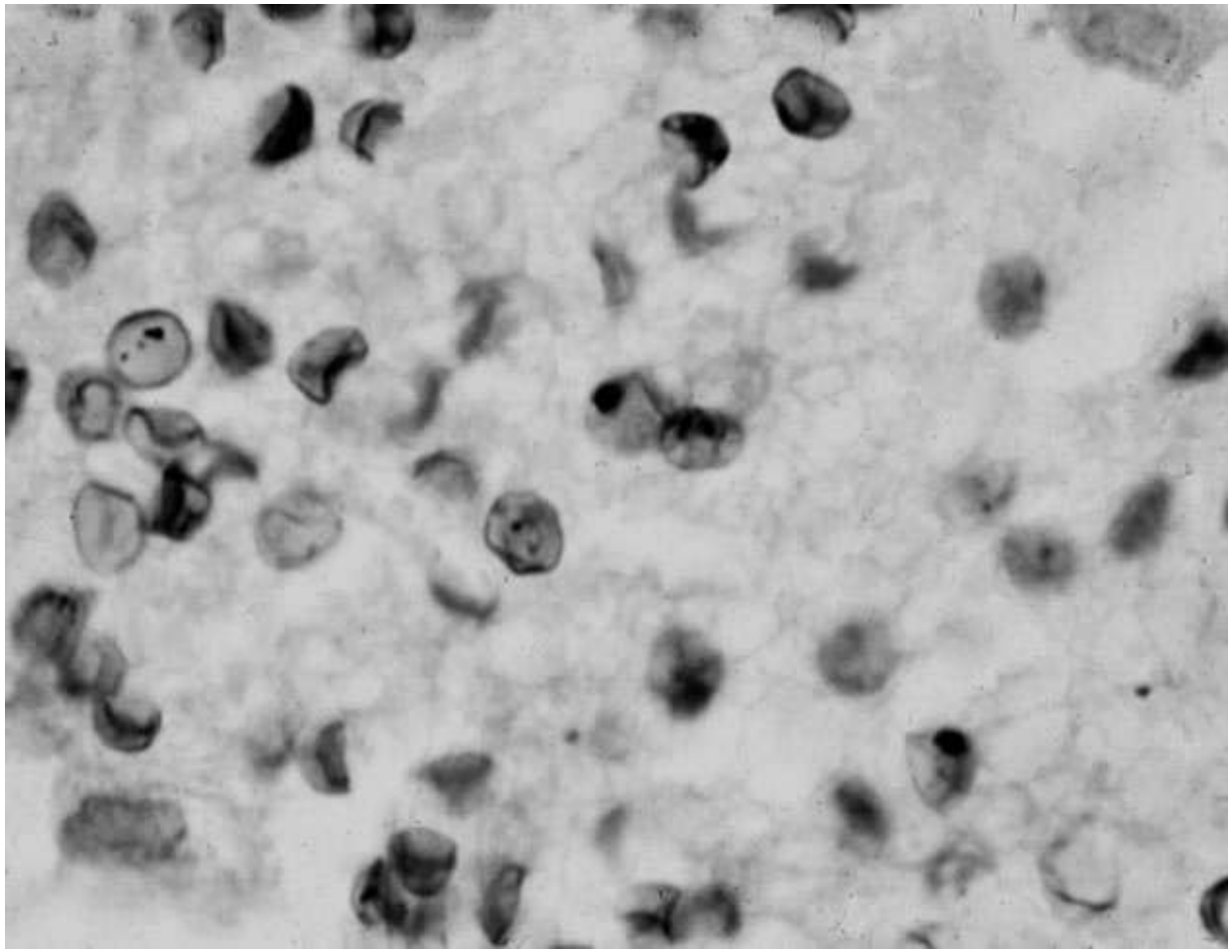
Candidiasises: laboratory diagnostics

1. Microscopic examination:
   - diagnostics of skin or nails specimens
   - revealing of pseudohyphae and budding cells in the spesimens.
2. Culture:
   - the specimens are cultured on Sabouraud's agar
   - to confirm diagnosis and to identify the species of the fungus
3. Serology (revealing of antibodies):
   - it is applied in visceral form of the disease and includes:
     - agglutination reaction
     - CF reaction
4. Skin test:
   - used in the case of visceral form.

Infections caused by Pneumocystis spp: main characteristics.

- Pneumocystis carinii the fungus related to the yeasts.
- Widely distributed among animals in nature: rats, dogs, mice without causing disease in them.
- May cause interstitial plasma cell pneumonia in immuncompromised people.

Pneumocystis carinii



Infections caused by Pneumocystis spp.: laboratory diagnostics

- The fungus is usually discovered in tissue after autopsy or lung puncture biopsy by use of combination of Romanovsky-Giemsa staining with hematoxylin and Schiff stain.

Medical Parasitology

Medical Parasitology: characteristics of the discipline

- Medical Parasitology has included the study of three major groups of animals: parasitic protozoa, parasitic helminths (worms), and those arthropods that directly cause disease or act as vectors of various pathogens.
- Infections of humans caused by parasites number in the billions and range from relatively innocuous to fatal. The diseases constitute major human health problems throughout the world.
- Our main interest of the subject are protozoan parasites and helminths of medical importance whose identification depends upon microscopic study.

Parasites: classification and morphology

- The parasites of human combined in the phylum Protozoa are classified in 4 groups:
- Mastigophora, or flagellates
- Sarcodina, typically amoeboid
- Sporozoa, parasites with complex life cycle
- Ciliata, ciliated protozoa.

Laboratory diagnostics of infections caused by parasites: classification of the methods

1. Microscopic examination.
2. Cultivation of parasites.
3. Immunological tests
   - Serological tests
   - Skin tests

Laboratory diagnostics of infections caused by parasites: microscopic investigations

Stool (faeces)

A. Specimens:

- At least 3 portions of liquid stool collected over 10 days (in the case of infections caused by amoebas – 6 portions got during 14 days)
- Necessary to avoid contamination of the specimens by water or urine (causes death of parasites)
- The specimens have to be collected at least 8 days after the treatment was terminated
- Immediate examination of fresh specimens have been done to detect trophozoites – motile form of parasites

Laboratory diagnostics of infections caused by parasites: microscopic examination

Stool (faeces)

B. Examination of the specimens

- Examination by unaided eye
  - to reveal blood and mucous in stool (in diagnostics of infections caused by amoebas)
- Microscopic investigation
  - wet mounts (placed under the cover slide)
    - revealing of trophozoites
    - staining by Lugohl to detect cysts
  - stained smears
    - more frequently by hematoxylin-eosin staining
      - **to detect parasites**
      - **to differentiate parasites**

Laboratory diagnostics of infections caused by parasites: microscopic examination

Blood specimens

A. Collecting of the specimens:

Use of capillary or of venous blood mixed with anticoagulating preparation.

Examination of the specimens should be done as quickly as possible.

Laboratory diagnostics of infections caused by parasites: microscopic examination

Blood specimens staining

B. Examination of the specimens usually Romanovsky-Giemsa staining could be applied

Laboratory diagnostics of infections caused by parasites: microscopic examination

Tissue specimens: depend on the characteristic features of parasites, they could be:

- skin specimens stained with histological stains,
- sputum:
  - wet mounts
  - smears stained by Romanovsky-Giemsa stain.

Laboratory diagnostics of infections caused by parasites: cultivation techniques

- Could be done only in special laboratories.
- Practically all parasites could be cultivated:
  - with use of special media,
  - in cell cultures.

Laboratory diagnostics of infections caused by parasites: serological tests

- The most available diagnostic tests.
- Used more frequently.

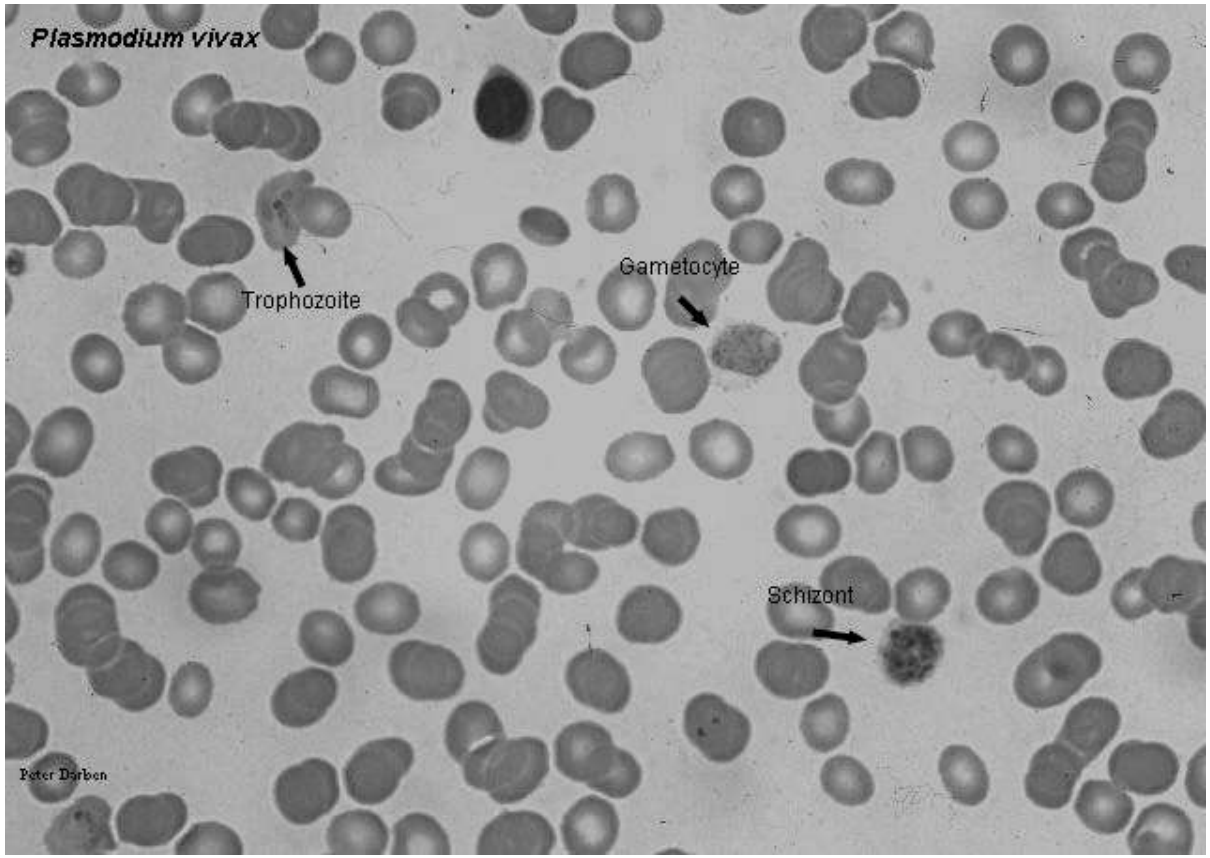
Malaria: classification of parasites

- Type – Apicomplexa
- Genus – Plasmodium

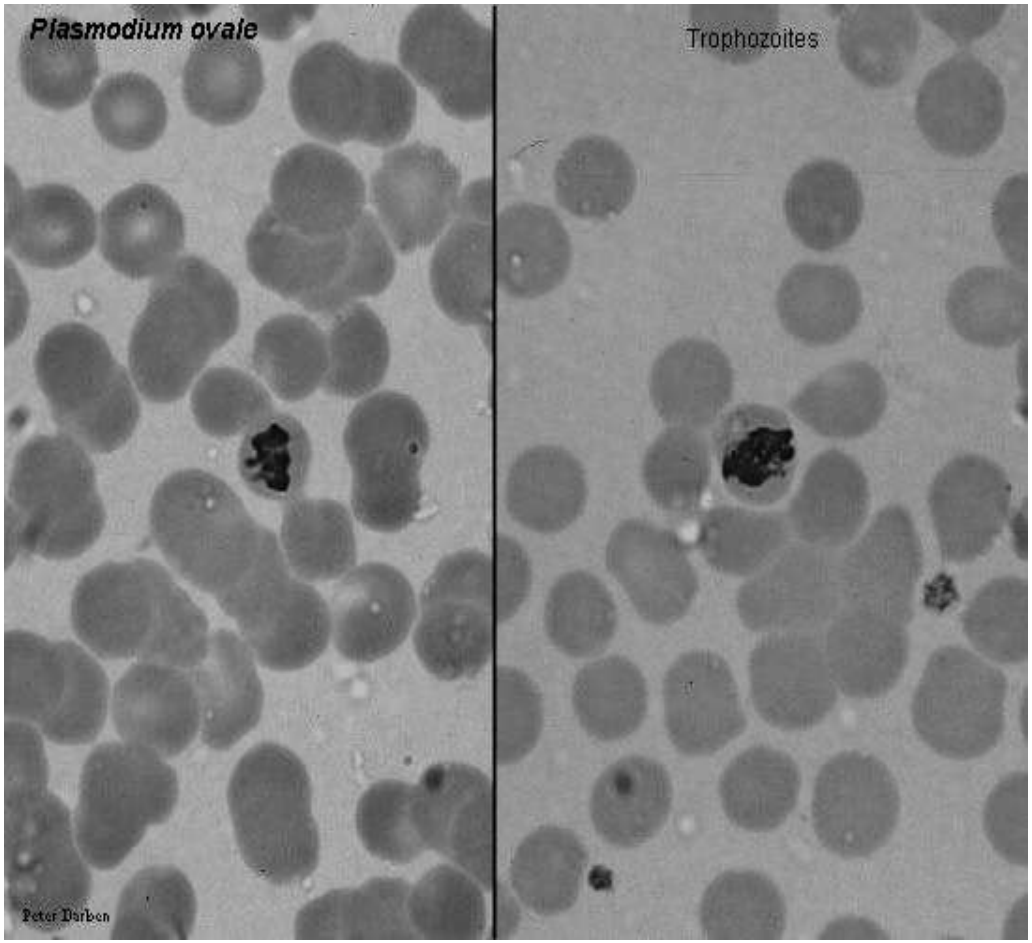
Species – human parasites:

1. P. vivax – three-days malaria
2. P. malariae – four-days malaria
3. P. falciparum – tropical malaria
4. P. ovale – malaria-ovale

Plasmodium vivax



Plasmodium ovale



Malaria: main characteristics of the disease

- ***Epidemiology:*** malaria is distributed worldwide throughout the tropics and subtropics. Parasites contained in the salivary glands of infected mosquitoes are injected into a human host when the mosquito feeds. The parasites rapidly invade liver parenchymatous cells, where they mature and multiply.
- ***Pathogenesis:*** the symptoms of malaria are associated with the rupture of erythrocytes by parasites. In severe falciparum malaria red cells containing parasites may obstruct capillaries and postcapillary venules, leading to local hypoxia and the release of toxic cellular products. Obstruction of the microcirculation in the brain (cerebral malaria) and in other vital organs is thought to be responsible for severe complications.
- ***Disease:*** symptoms are fever, chills, sweating, headache, weakness, etc. Later, severe disease may develop, with severe anaemia, renal failure, and multi-system failure. As the disease progresses, some patients may develop the classic malaria paroxysm with bouts of illness alternating with symptom-free periods.

Malaria: laboratory diagnostics

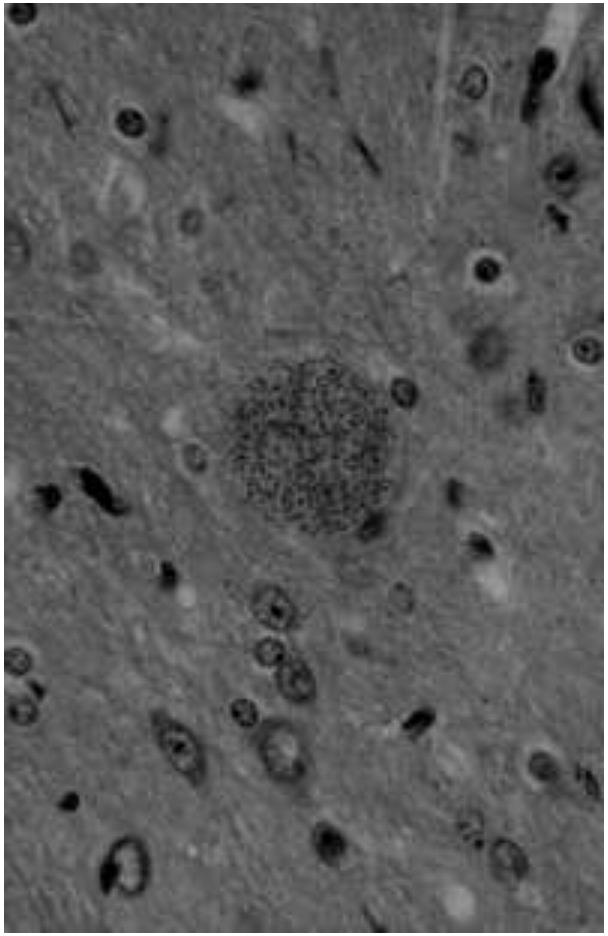
1. Microscopic examination of smears stained by Romanovsky-Giemsa technique to determine the species by:
   - morphological peculiarities of parasites
   - morphology of damages found in erythrocytes
2. Serological tests (in some cases) – immunofluorescence, agglutination reactions, ELISA.

Toxoplasmosis: aetiology

Type – Apicomplexa

Toxoplasma gondii

Toxoplasma gondii



Toxoplasmosis: main characteristics of the disease

- ***Epidemiology:*** members of the cat family (Felidae) are the definitive hosts; many mammals and birds serve as intermediate hosts. Infection is contracted by ingesting either oocysts or meat containing live organisms. Organisms enter the intestinal epithelium and can spread to many host tissues.
- ***Pathogenesis:*** host cells are destroyed by active multiplication of *T. gondii*. Necrotic foci may result. Congenital infection often involves the retina and brain; focal chorioretinitis may result in impaired vision.
- ***Disease:*** infection is often asymptomatic. Immunocompetent individuals may present with fever, lymphadenopathy, muscle aches, and headache. Congenitally infected children may suffer impaired vision and mental retardation. Immunosuppressed patients may have central nervous system disease (encephalitis). Brain involvement may lead to large necrotic abscesses.

Toxoplasmosis: laboratory diagnostics

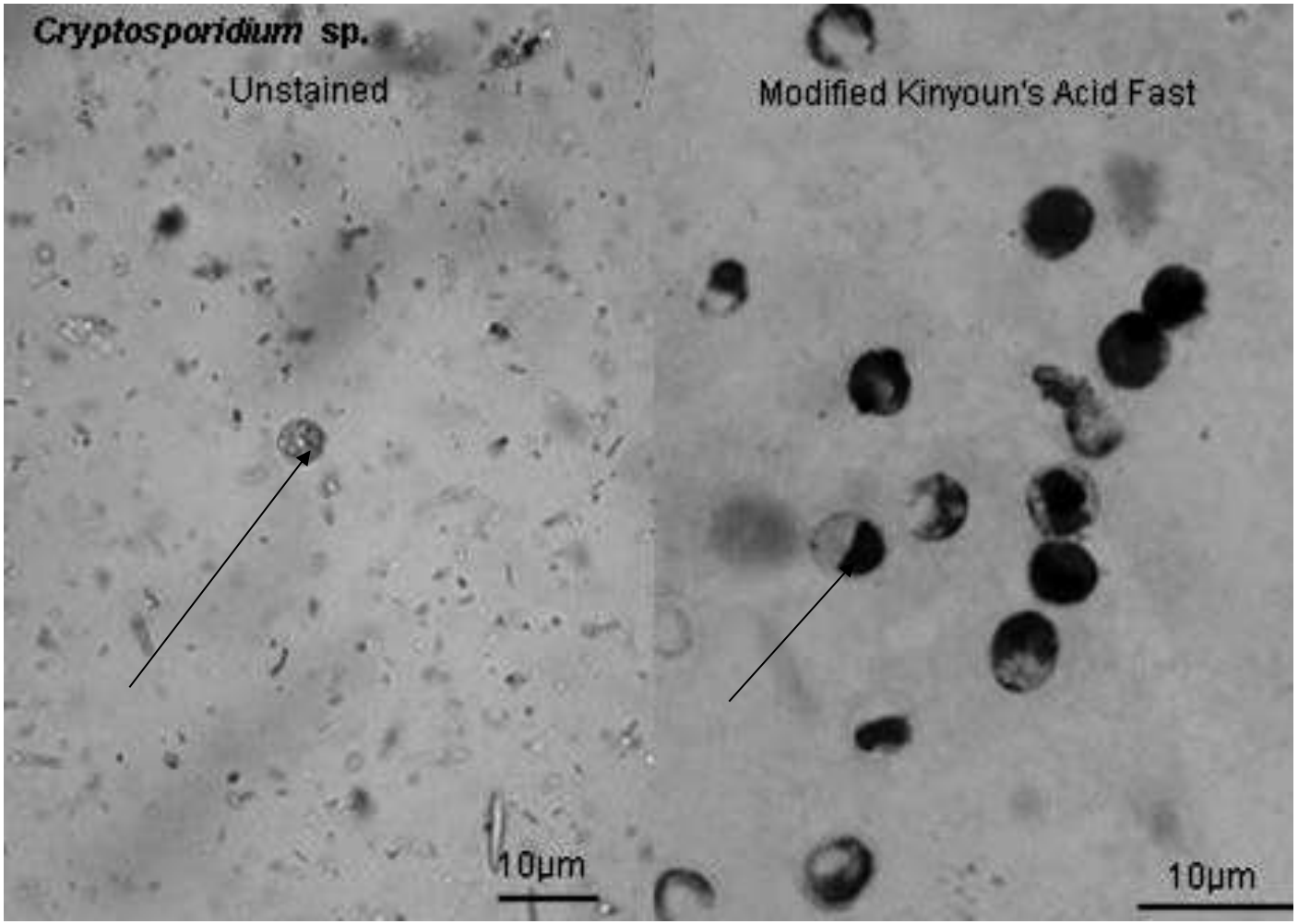
1. Serologic tests (revealing of antibodies in CF test, immunofluorescence and latex-agglutination tests).
2. Skin test with toxoplasmin:
   - It is positive starting from the 4^{th} week of the disease.
   - It could be a result of infection which occurred in the past.
3. Isolation of toxoplasmas could be carried out by inoculation of laboratory animals.

Infections caused by Cryptosporidia: aetiology

Type – Apicomplexa

Criptosporidium parvum

Criptosporidium parvum



Infections caused by cryptosporidia: laboratory diagnostics

Revealing of cysts:

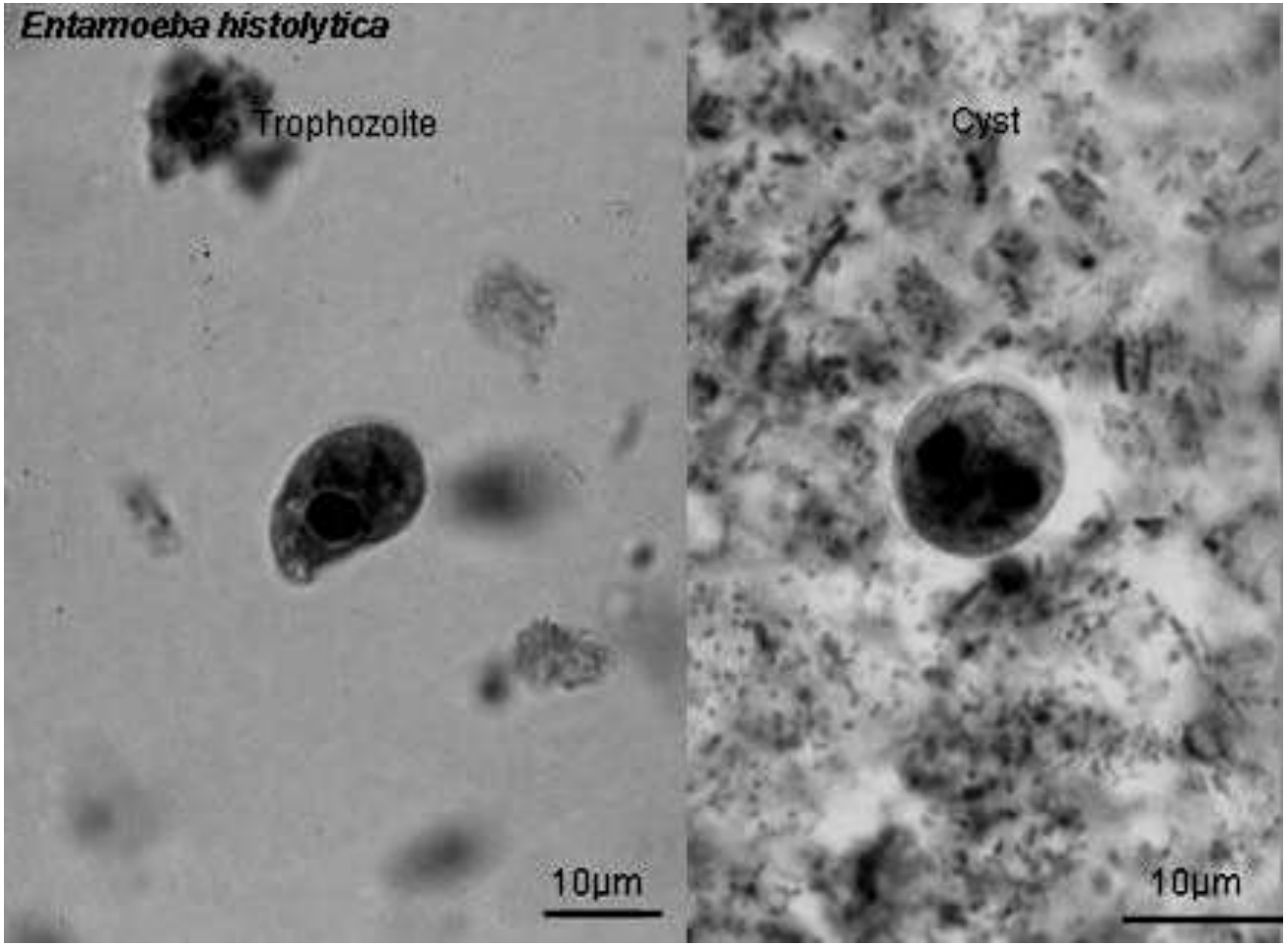
- in stool – by preparing of dried smears stain by Ziehl-Neelsen technique
- in biopsy material (stomach, bile ducts and gallbladder) – stain by Romanovsky-Giemsa technique.

Amoabiasis: aetiology

Type – Sarcomastigophora

Entamoeba histolytica

Entamoeba histolytica



Amoebiasis: main characteristics of the disease

- ***Epidemiology:*** faecal-oral transmission of cysts involves contaminated food or water. Amoebas can be transmitted directly by sexual contact involving the anus. 0.5 to 50% of the population world wide harbors *E. histolytica* parasites with the higher rates of infection being in underdeveloped countries.
- ***Pathogenesis:*** the amoeba possess the ability to adhere to colonic mucosal lining cells of colon and to colonize them without invasion of mucosa. In the case of invasion of the mucosa they produce ulcers that sometimes progress by direct extension or by metastasis. Metastatic infection first involves the liver. Extension or metastasis from the liver may involve the lung, brain, or other viscera.
- ***Disease:*** acute or chronic diarrhoea, which may progress to dysentery:

✓ **Acute**: frequent dysentery with necrotic mucosa and abdominal pain.

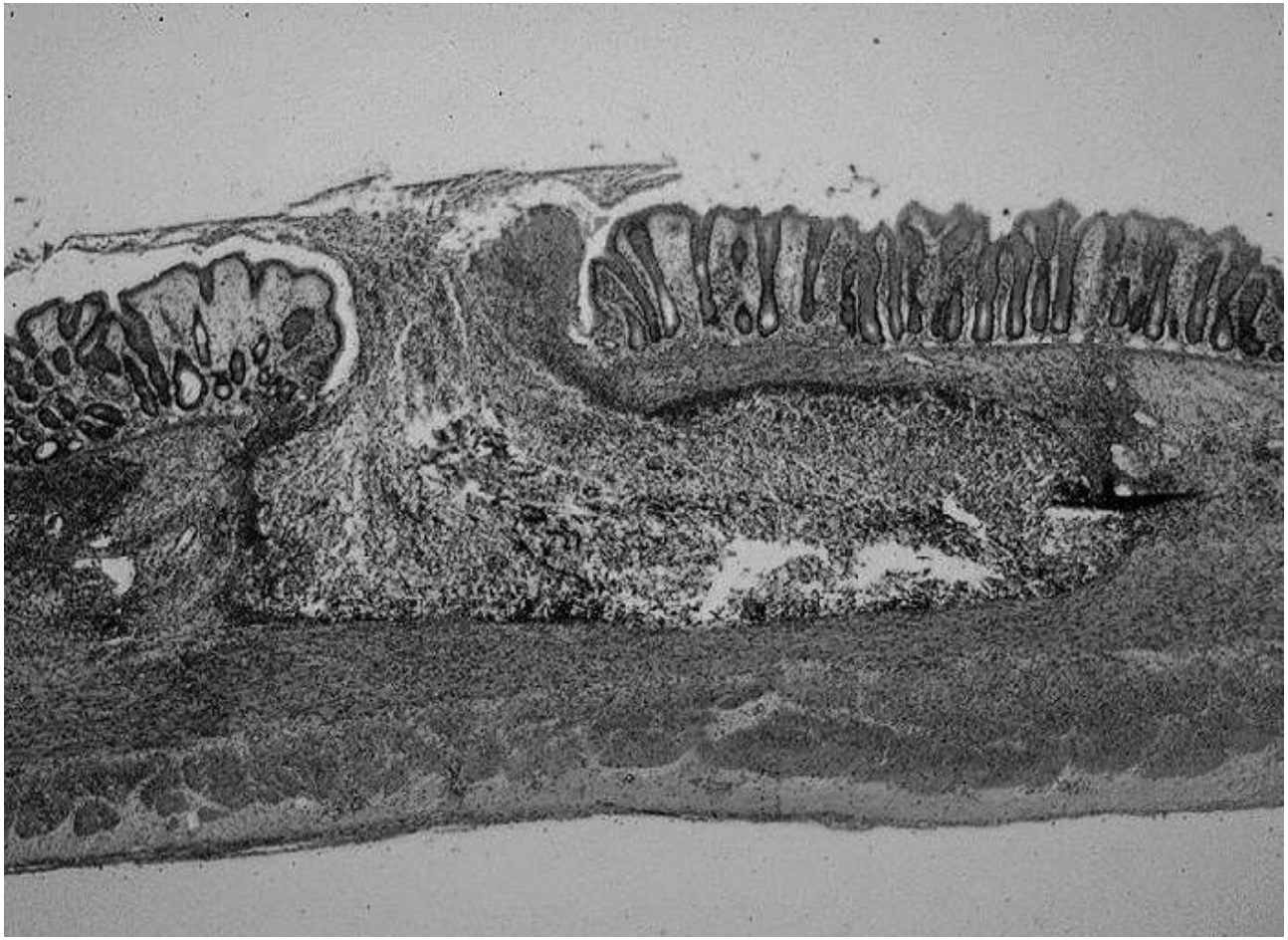
✓ **Chronic**: recurrent episodes of dysentery with blood and mucus in the feaces. Extraintestinal disease may be present as a complication problem (e.g., liver, lung or brain abscess, or skin or perianal infection).

Amoebiasis: laboratory diagnostics

Microscopic investigations of:

- the wet mounts prepared with use of effected tissues,
- the stool specimens (in the case of severe amoebiasis): finding cysts in the stool.

Histopathology of a typical flask-shaped ulcer of intestinal amebiasis



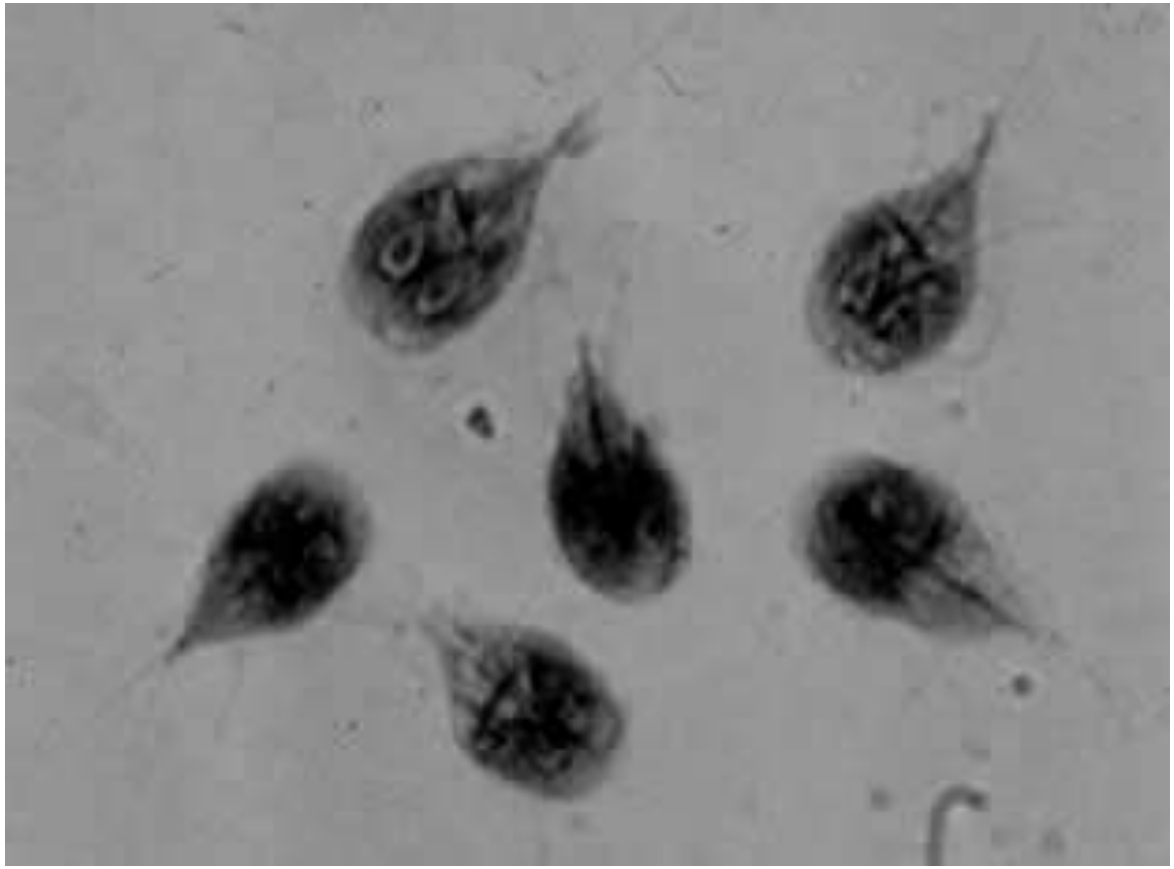
Giardiasis: aetiology

Type – Sarcomastigophora

Lamblia intestinalis (Giardia lamblia)

(giardiasis)

Lamblia intestinalis (Giardia lamblia)



Giardiasis: main characteristics of the disease

- ***Epidemiology:*** is cosmopolitan and common, especially in children. Humans are infected by ingestion of faecally contaminated water or food containing Giardia cysts. It is the most frequent protozoan intestinal disease in the US.
- ***Pathogenesis:*** large number of parasites attached to the bowel wall cause irritation and inflammation of the duodenal or jejunal mucosa with consequent acute or chronic diarrhoea. Flattening of the mucosal surface results in malabsorption of nutrients.
- ***Disease:*** malaise, weakness, weight loss, abdominal cramps. The bile ducts and gallbladder may be also invaded causing cholecystitis.

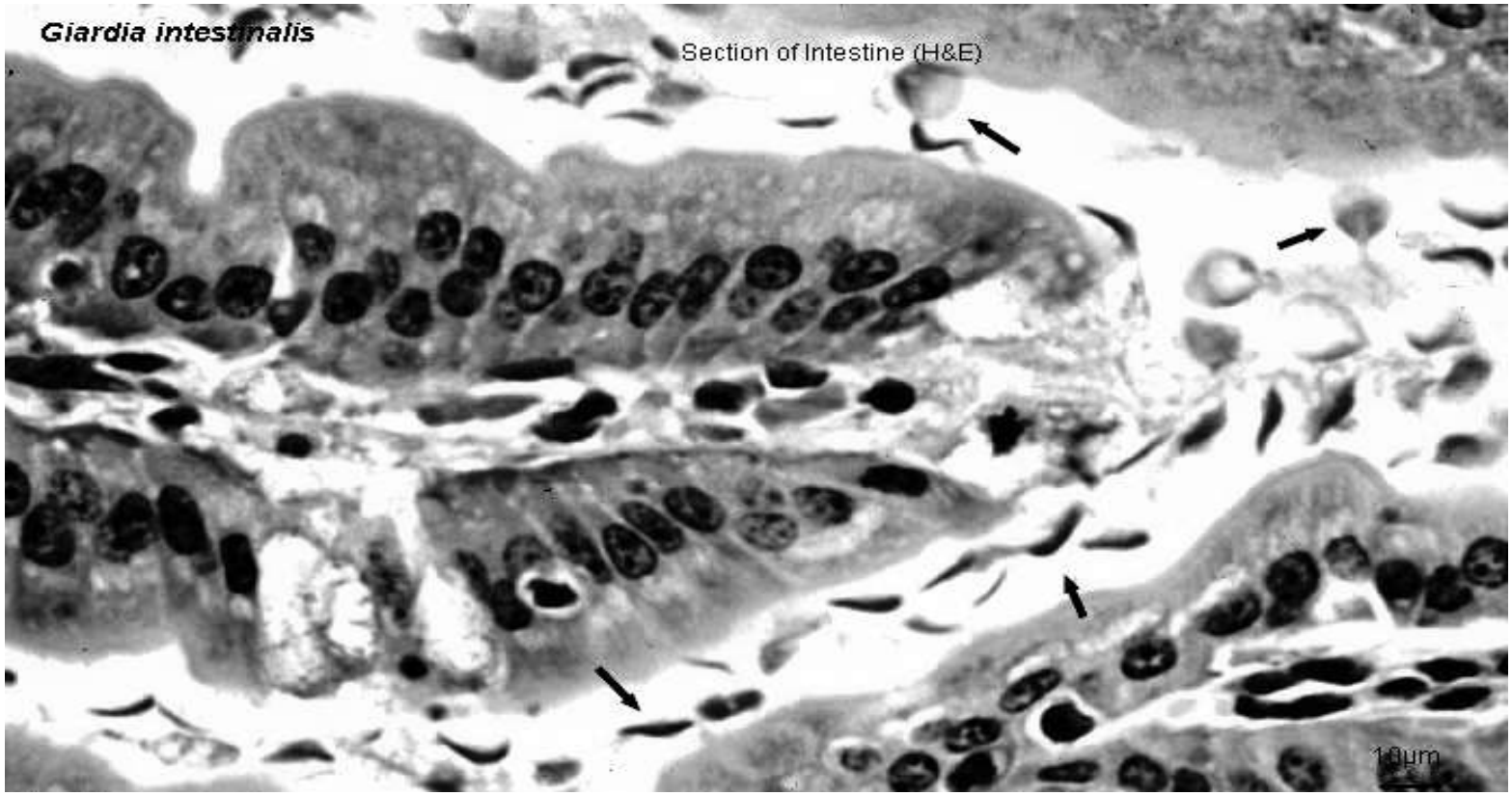
Giardiasis: laboratory diagnostics

Microscopic investigation of the specimens:

- stool
- duodenal contents

- wet mounts,
- wet mounts after adding the lughole solution.

Giardia trophozoites in section of intestine



Trichomoniasis: aetiology

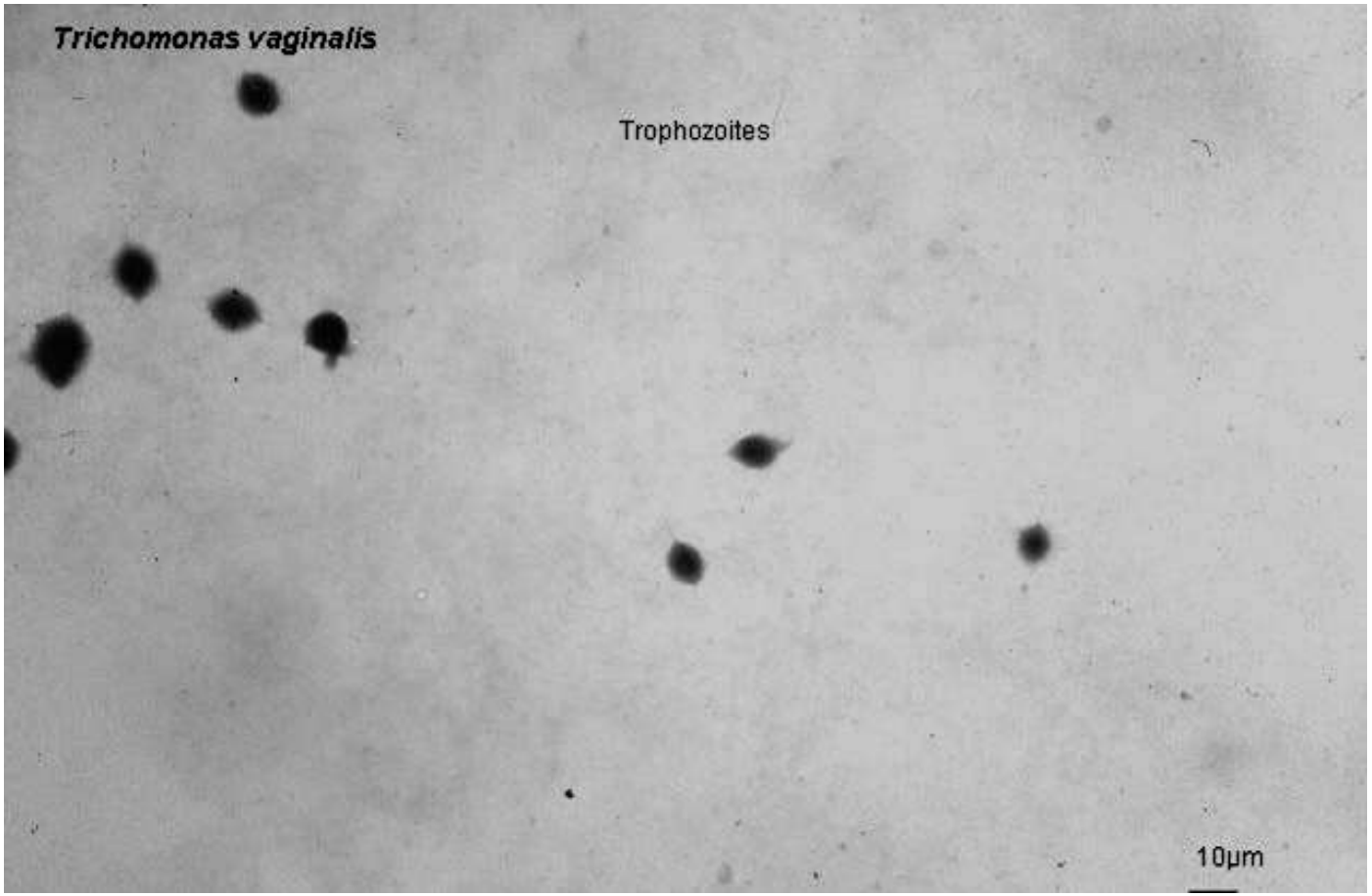
Type – Sarcomastigophora

Trichomonas vaginalis – only this parasite is pathogenic for humans.

Trichomoniasis: main characteristics of the disease

- ***Epidemiology:*** a flagellated protozoan parasite *Trichomonas vaginalis* is responsible for one of the most widespread sexually transmitted urogenital disease in women trichomoniasis or "trich" .
- ***Pathogenesis:*** the microorganism causes low-grade inflammation by mechanisms that are not clear but may involve mechanical irritation.
- ***Disease:*** vaginitis, with foul-smelling discharge and small hemorrhagic lesions, may be present; frequency of urination and painful urination are common. This infection is usually asymptomatic in men.

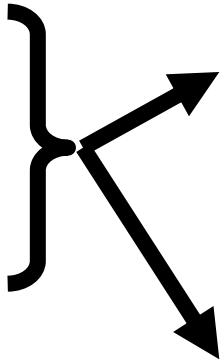
Trichomonas vaginalis



Trichomoniasis: laboratory diagnostics

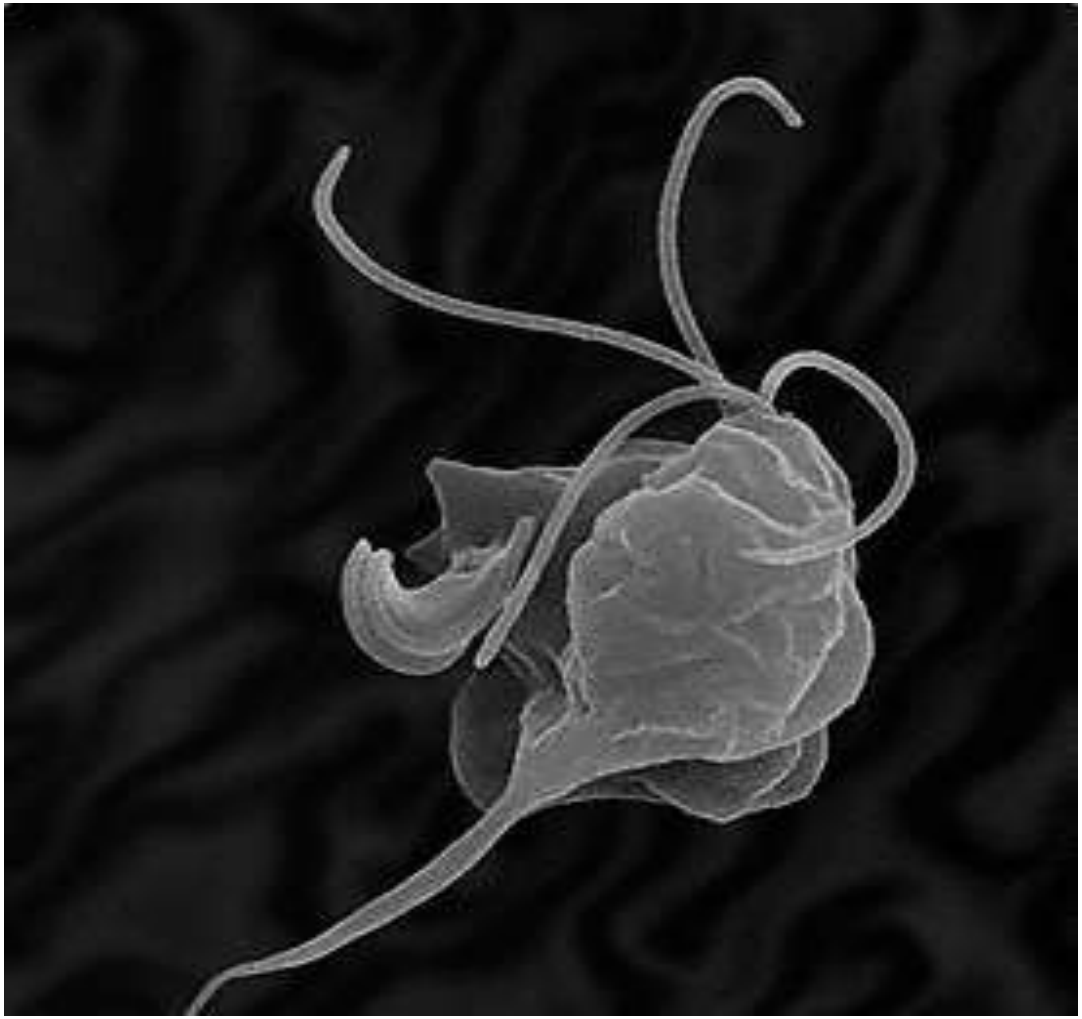
Microscopic examination of specimens:

- vaginal secretion
- urethral secretion

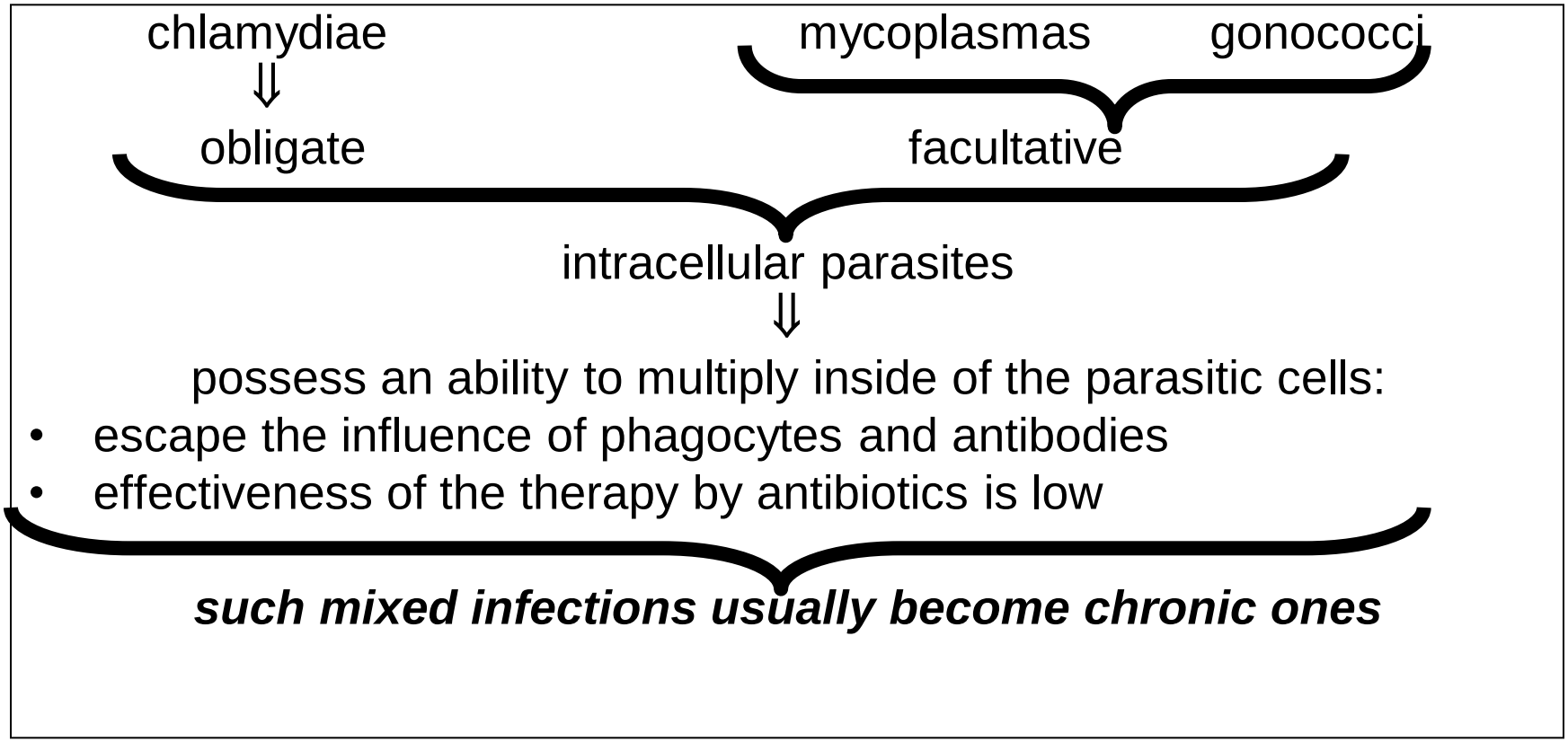


- wet mounts (to reveal motile trichomonads),
- dried smears stained with hematoxylin or Romanovsky-Giemsa stains.

Trichomonas vaginalis - parasitic protozoan that causes trichomoniasis (vegetative phase called trophozoite)



Infections caused by associations of parasites with chlamydiae, mycoplasmas and gonococci



Clinical microbiology

Clinical microbiology: definition of the term

It is a division of medical microbiology

which includes the study of:

- infectious diseases which occur in hospitals where the patients are getting treatment in the case of non-infectious diseases,
- these infections called hospital-acquired (iatrogenic or nosocomial) ones.

Clinical microbiology: main aspects of the study

The main aspects are:

- study of hospital-acquired infections:
  - their aetiology
  - pathogenesis
  - specificity of the immune response
- development and applying of the methods which include:
  - laboratory diagnostics
  - specific treatment
  - prophylaxis

Clinical microbiology: methods

- The methods which are used by clinical microbiology include usual methods characteristic for medical microbiology but the main method is:
  - ***the method of growing of bacterial culture and the quantitative aspect of the method (the number of the bacterial cells in the specimens is especially important)***

Clinical microbiology: subjects of the study

- common opportunistic pathogens (microorganisms)
- hospital environment

Opportunistic microorganisms: the main groups

1. Gram-positive cocci (staphylococci and pneumococci).
2. Gram-negative enteric rods.
3. Pseudomonades.
4. Fungi Candida.
5. Fungi of the genus Pneumocystis.

Opportunistic microorganisms: main characteristics

1. Ecological grouping
   - free-living microorganisms
   - the patient's indigenous micro-flora
2. Conditions for the realisation of the pathogenicity by opportunistic microorganisms:
   - high adaptive capacities in microbial population
   - production of endotoxin
   - production of toxic enzymes
3. Characteristics of the opportunistic population:
   - heterogeneity
   - changeability
4. Specificity of the methods applied for the diagnosis of nosocomial infections:
   - it is necessary to analyse many different strains even if they are belonging to the same species
   - it is necessary to pay special attention to high resistant strains (bacteria resistant to drugs, antiseptics and disinfectants).

Opportunistic infections: definition of the term

- The infections caused by opportunistic pathogens (more frequently not by one representative of opportunistic micro-flora but by the associations of the several ones).

Opportunistic infections: their appearance

- These infections can occur everywhere.

Opportunistic infections: conditions for their development

1. High inocula of pathogens.
2. Debilitation of hospitalised patients.
3. Infection by more virulent pathogens: unusually high virulent opportunistic microorganisms.

Opportunistic infections: specific features

- Tropism to many organs.
- Clinical symptoms usually dependent on the site of localisation of the infectious process: symptomatology may be vague (unclear) or atypical.
- Opportunistic diseases often are getting:
  - chronic
  - generalised
  - could result in septicopyemia
- Problems for the therapy:
  - drug resistance of wide spectrum
  - low resistance of human organism to the infection (immune compromised patients)
- The infections may be endogenous (caused by own indigenous microorganisms).
- The hospital – acquired infections are dominant among the opportunistic ones.

Opportunistic infections: laboratory diagnostics

Rapid diagnosis of opportunistic infections is important to a favourable prognosis for the patient (early start of the treatment).

laboratory diagnostics includes:

- Aetiology - isolation and identification of the opportunistic pathogen, that is especially important for the diagnostics in the cases when the pathogen has been isolated:
  - in high titre,
  - it demonstrates unusually high virulence,
  - it has been isolated from unusual site in human organism.
- The status of the immune response of the patient should be taken into consideration.
- Epidemiological aspects should be considered such as:
  - the source of the infection
  - factors of the transmission of the infection.

Hospital-acquired infections: definition of the term

- Infections which develop in patients due to medical manipulations for example, invasive procedures (intravenous cannulation, urinary cauterisation, surgery, etc) which are carried out:
  - in hospital
  - in ambulatory
  - at home.

Hospital-acquired infections: the reasons for their spreading

- The widespread and frequent use of therapeutic and prophylactic antimicrobial agents (drugs) provide selective pressure for the proliferation of drug-resistant microorganisms.
- Increase of the number of invasive methods of therapy and medical tests:
  - diagnostics that is accompanied by breakdown of physical barriers such as skin and mucous membranes,
  - surgery,
  - use of the immune suppressive drugs,
  - aging of the human population, survival of immune compromised persons,
  - high frequency of non-infectious underlying chronic diseases.
- Changes which occur in the hospital environment:
  - larger inocula of opportunistic pathogens which are circulating in hospital surroundings,
  - increase in the number of visits the hospitals by patients.

Hospital-acquired infections: aetiology

- Opportunistic microorganisms.
- Highly pathogenic microorganisms:
  - Hepatitis B virus
  - AIDS
  - Influenza virus
  - Viruses which cause acute respiratory and enteric infections
  - Salmonellae and Escherichiae in children
  - Adenoviruses (especially conjunctivitis)
  - Herpes and Cytomegalovirus infections
  - Chlamydia and Mycoplasma (urethritis)
  - Fungi producing dermatomycosises

Hospital-acquired infections: clinical ecological variants of pathogens

The pathogens found in the hospital surroundings are characterised by the next properties:

- highly resistant to numerous antimicrobial agents,
- highly resistant to antiseptics and disinfectants,
- highly resistant to the factors of innate immunity of the human organism.

Hospital-acquired infections: condition for their development

- Infection which is developing due to the medical manipulations

+

- Debilitation of hospitalised patients: their susceptibility to the infection

Hospital-acquired infections: specificity of pathogenesis

- Clinical manifestation and the composition of pathogens are due to the site where the invasive diagnostics or corrective, and maintenance procedures where applied.

Hospital-acquired infections: specificity of the immune response

- Usually patients are debilitated or immune compromised.
- Even in normal the immune response developed against opportunistic microorganisms is lower then one formed against high pathogenic microbes as the majority of opportunistic pathogens is belonging to human normal micro-flora.
- In the course of the disease the immune deficiency manifestations are increased in debilitated patients (the results are generalisation of the infectious process or development of chronic disease).

Hospital-acquired infections: diagnostics

The diagnostics includes:

investigation of the possible sources of the infectious agents:

- patient
- medical personnel and other patients
- hospital surroundings as they are possible factors of transmission of the pathogens

The diagnosis ***"hospital-acquired infection"*** could be stated when:

- hospital variant of pathogen has been isolated from the patient (even if the source of the infections and the factors of transmission where not found)
- when infectious process appeared as a result of the contacts with hospital personnel after passing the period of time equal to the incubation period (it takes 2 to 3 days in the case of opportunistic infections).

Hospital-acquired infections: prophylaxis

- Examination of patients and hospital personnel in connection with possible carriage of the agents of hospital infections.
- Examination of hospital living areas and therapeutic preparations to reveal their possible contamination with hospital opportunistic and iatrogenic pathogens.
- Maintaining a clean and disinfected environment.
- Treatment of patients, physicians and other hospital personnel who are shown to be carriers of potential pathogens by applying specific chemotherapy to eliminate the carrier state (to cure the patients and personnel completely).