BACTERIA – THE CAUSATIVE AGENTS OF RESPIRATORY TRACT DISEASES

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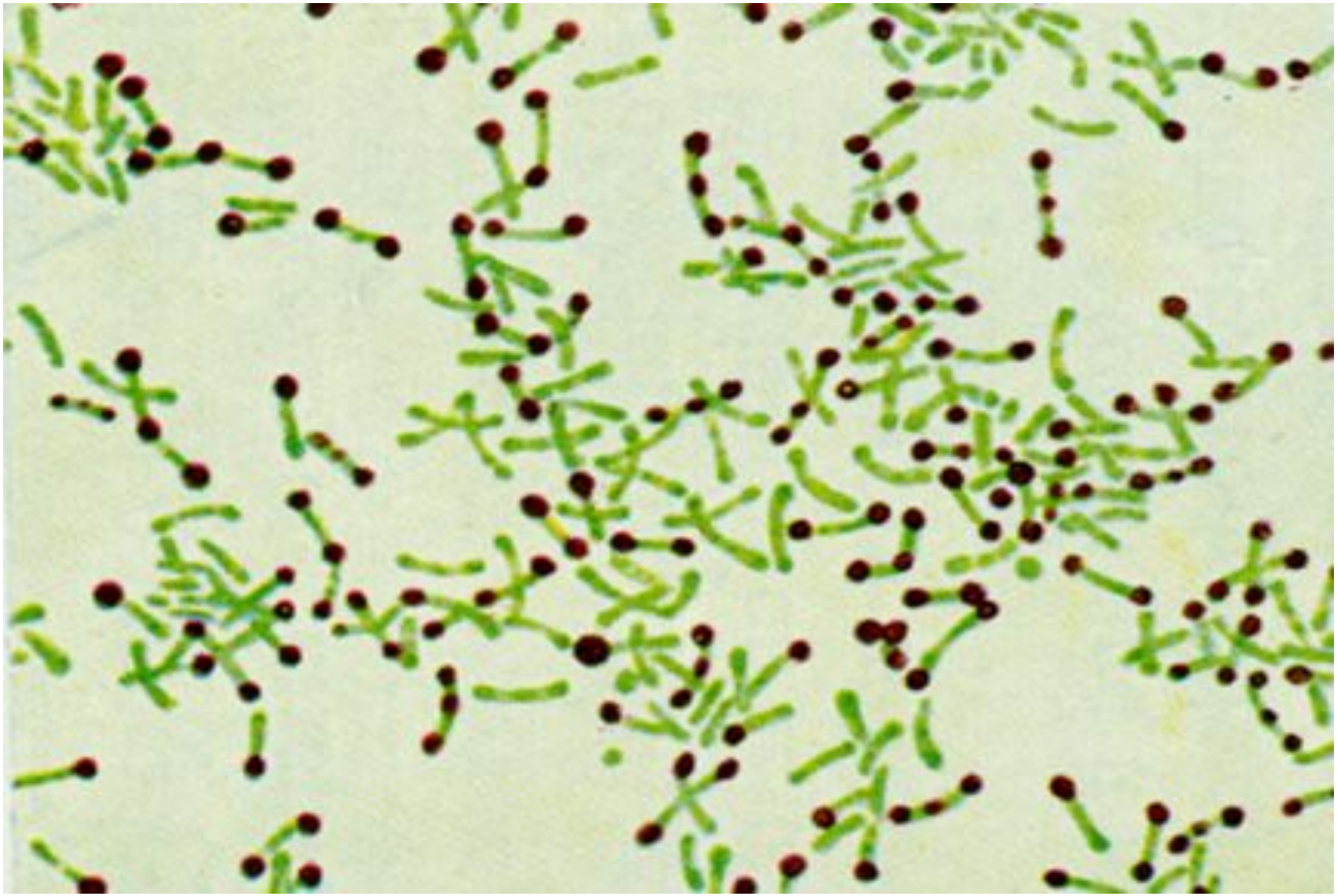
DIPHTHERIA

- Is **acute infectious disease caused by <u>Corynebacterium diphtheriae</u> and characterized by a primary lesion, usually in the upper respiratory tract, and more generalized symptoms resulting from the spread of bacterial toxin throughout the body.**

DIPHTHERIA

- The disease was first described in the 5th century BC by **Hippocrates.**
- The bacteria was discovered in **1882 by Edwin Klebs.**
- **F.Loeffler- 1884** –isolate in pure culture
- **E.Roux -1888-** separate the toxin
- **G.Ramon – 1923-** diphtheria toxoid

Cor. diphtheriae



Cor. diphtheriae

- **are Gram-positive, rod-shaped bacteria, stains more intensely at its ends (volutin).**

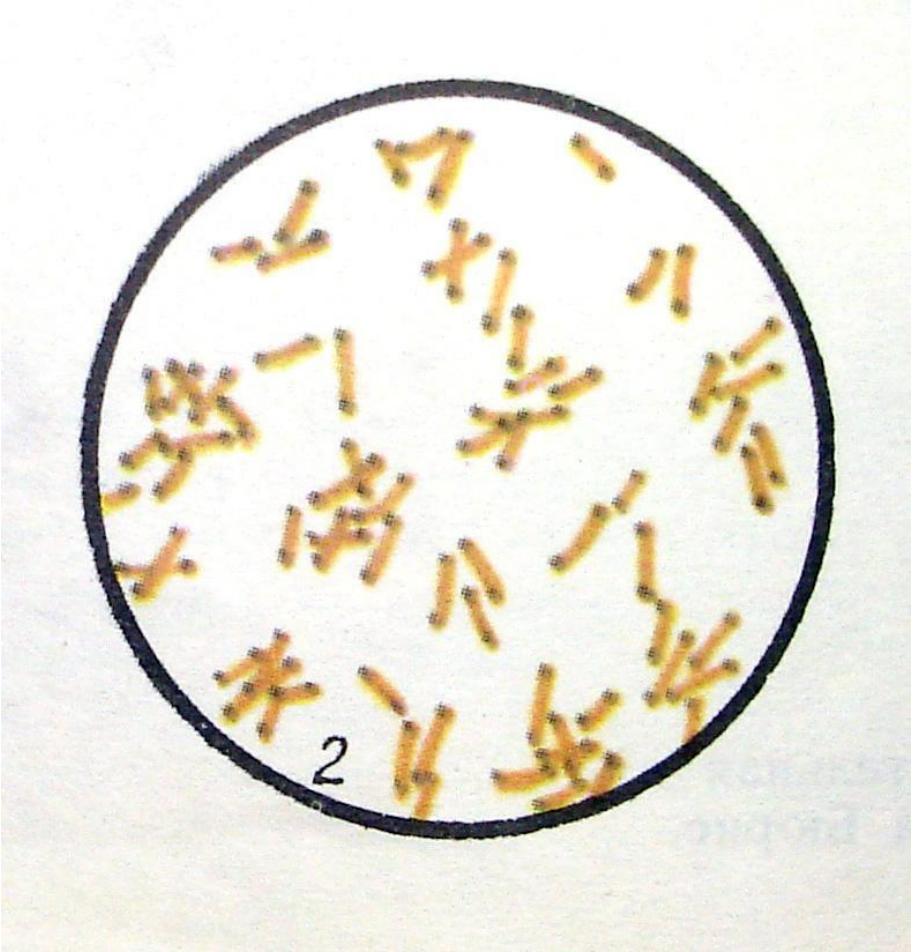
They have the characteristic of forming irregular, club-shaped or V-shaped arrangements in normal growth. The cytoplasm is granular.

They do not form spores.

Outer layer of the cell wall forming a microcapsula.

Nonmotile.

Neisser s stain



C. diphtheriae is a gram-positive, non-motile rods or somewhat pleomorphic organism. The club-shaped forms are long and slender with swollen ends, especially when stained with methylene blue or Neisser's stain (this reveals intensely stained volutin granules). On a slide, these microbes often arrange in pairs at acute angles to each other (V, L, X-arrangements). The non-pathogenic and opportunistic corynebacteria (diphtheroids) are rods arranged in parallel (“fence-like”) clusters. These bacteria possess irregular swellings only at one end of the cell. NOTE

Physiology of Cor. diphtheriae

- **Aerobes or facultative anaerobes**
- **T_{opt} = 37º C**
- **Grows readily on media with protein, sugar.**
- **Serum agar**
- **Blood agar**
- **Roux's media (coagulated horse serum)**
- **Loeffler's media (serum, sugar broth)**

C. diphtheriae grows much more readily on coagulated serum agar, on whose slope there is a creamy growth within 12 h. On blood tellurite agar (Klauberg agar) the three biotypes of C. diphtheriae - gravis, mitis, and intermedius - form different colonies. The gravis type forms relatively large, grayish flat, rough colonies with radial lines and wavy edges. Colonies of the mitis type are small, lustrous, black, with a smooth surface. The three biotypes also differ biochemically. If typical colonies are obtained, a pure culture is then identified by fermentative and toxigenic properties.

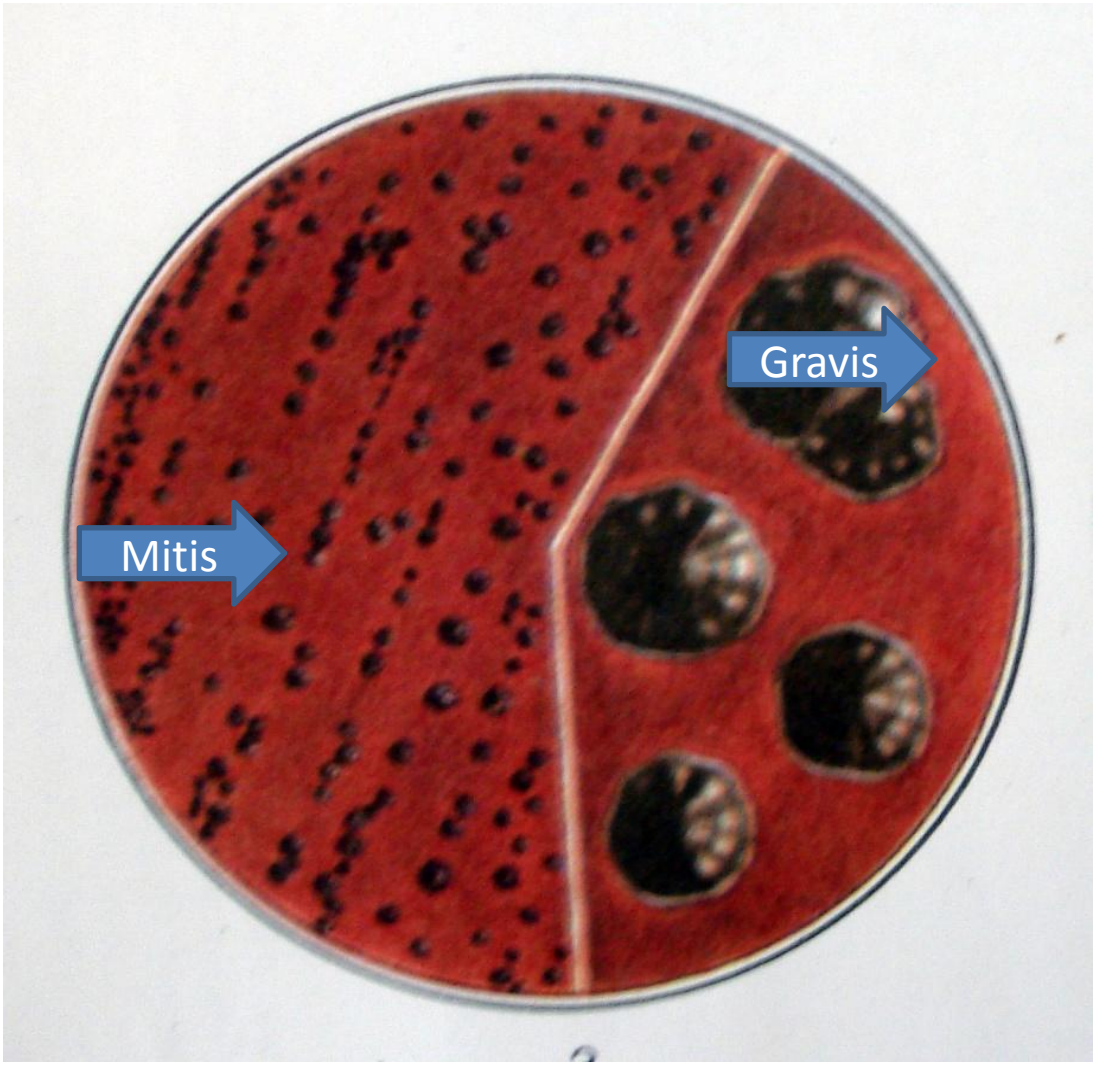
Physiology of Cor. diphtheriae

- **Glucose «+»**
- **Do not coagulate milk**
- **Do not break down urea**
- **Indol «-» H_2S «+»or «-»**
- **Reduce nitrates to nitrites**
- **Potassium tellurite is also reduced**

Biovars of Cor. diphtheriae

Biovars	GRAVIS	MITIS
Morphology	Short rods with granules	Long, curved rods with granules
Colony on tellurite blood agar	Large, rough, greyish with crenated edges. Rosette-like.	Small, smooth, shiny, black
Growth in broth	Surface pellicle	Diffuse turbidity
Glycogen and starch fermentation	Positive	Negative
Toxicity	Highly toxic	Less toxic

Tellurite agar



The gravis type form relatively large, grayish flat, rough colonies with radial lines and a wavy edges.

Colonies of the mitis type are small, black, with a smooth surface.

Antigens

- **K – antigen , type specific, thermolabile, surface protein.**
- **O – antigen , group specific, thermostabile, somatic polysaccharide.**
- **The diphtheria exotoxin is a complex of more than 20 antigens.**

Resistance

- **Cor. diphtheriae are relatively resistant to harmful environmental factors.**
- **They survive for 1 year on coagulated serum, for 2 months at room t, for several days on children's toys.**
- **Cor. diphtheriae are killed by t=60-100ºC and by 1% phenol in 10 min.**

Diphtheria toxin

- **Is a protein with 2 subunits:**
- **A – have enzymatic activity**
- **B- is responsible for binding the toxin to the cells**

- **Is complex of more than 20 antigens**

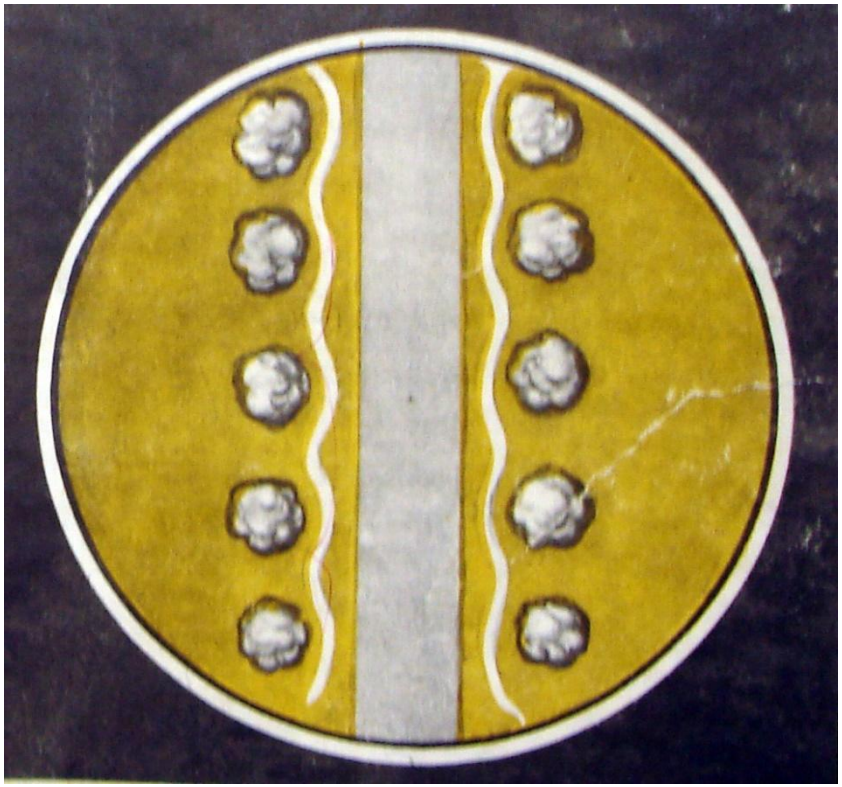
- **Is lable, destroyed easily by exposure to heat, light, O_2.**

Diphtheria toxin can cause myocarditis, polyneuritis, and other systemic toxic effects.

Diphtheria toxin

- **The toxigenicity of the Cor. diphtheriae depends on the presence in it of corynephages (tox+), which act as the genetic determinant controlling toxin production.**
- **The diphtheria toxin acts by inhibiting protein synthesis.**
- **The toxin causes local necrotic changes and the resulting fibrinous exudate, together with the disintegrating epithelial cells, leucocytes, erythrocytes and bacteria.**

Diphtheria toxin



Virulence Factors and Pathogenesis of Diphtheria The serious effects of diphtheria in man are caused by the diphtheria exotoxin, and only testing the organism for toxigenicity can definitively determine whether it is pathogenic or not. The diphtheria exotoxin is a polypeptide, which attaches to the cell membrane, allowing to enter the cells, where it catalyses a reaction that stops the synthesis of the polypeptide chains and the most profound effects are on the myocardium, peripheral nerves, and kidneys. C diphtheriae may have different portals of entry: they can colonize the pharynx (espessially the tonsillar regions), the larynx, the nose, the conjunctiva and occasionally the genital tract and the skin (wounds). After adhesion the microbes multiply locally without invading deeper tissues or spreading through the body. The microbe destroys epithelial cells with ulcer formation. This is covered with a necrotic exudate forming a “false membrane.”

DIPHTHERIA

- **Sources of infection are patients and carriers.**
- **The disease is transmitted by an air-droplet route.**
- **Transmission by various objects (toys) and foodstuffs (milk) contaminated with Cor. diphtheriae is also possible**.

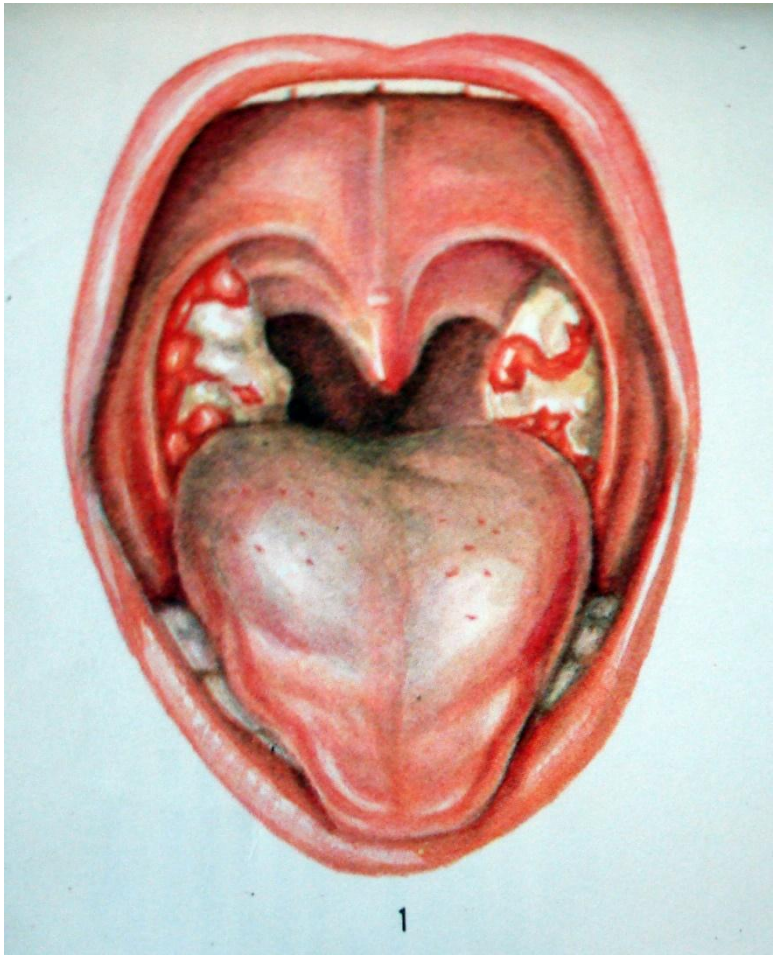
Diphtheria. Clinical Manifestations

The typical membrane on the throat or on other parts of the body, e.g., the skin, is the result of an inflammatory reaction to the presence of multiplying C.diphtheriae. This film soon becomes dark and malodorous, and bleeding occurs on attempting to remove it from tonsils surface. When the larynx is involved, it can result in life-threatening respiratory obstruction. The microbe produces exotoxin that passes into the bloodstream and the lymphatics. This toxemia causes severe generalized effects: myocarditis, polyneuritis, nephritis and suprarenal failure.

Clinical forms

- 1. **Anterior nasal diphtheria**, in which the membrane appears inside the nostrils. Almost no toxin is absorbed from this site, so there is no danger to life and complications are rare.
- 2. **Tonsillar diphtheria -** the most common type, in which the infection is limited mostly to the tonsillar region. Most patients recover if properly treated with diphtheria antitoxin.
- 3. **Nasopharyngeal diphtheria** - the most often fatal form, in which the tonsillar infection spreads to the nose and throat structures, sometimes completely covering them with the membrane and causing toxemia.

Tonsillar diphtheria



Clinical forms

- 4. **Laryngeal diphtheria** - usually resulting from the spread of the infection downward from the nasopharynx to the larynx.
- 5. **Extra-respiratory diphtheria**, consisting of those forms of the infection that affect parts of the body other than the respiratory tract – ex. the **skin and wound**.

Diphtheria. Microbiological Diagnosis

SPECIMENS. Swabs from the nose, throat, or other suspected lesions must be obtained.

Diphtheria. Microbiological Diagnosis

Bacterioscopical examination

Preparation of smears (Gram and Neisser staining) -

- Gram-positive rods.
- The club-shaped long and slender cells with swollen ends, cells arranged in pairs at acute angles to each other (V, L, X-arrangements).

Diphtheria. Microbiological Diagnosis

Bacteriological examination

1. Inoculation of blood- tellurite agar (Klauberg medium)
2. Subculture on coagulated serum medium
3. Identification of pure culture and differentiation from the diphtheroids
4. Determination of toxigenicity (IHA, ELISA, precipitation test)

Diphtheria. Treatment and Prevention

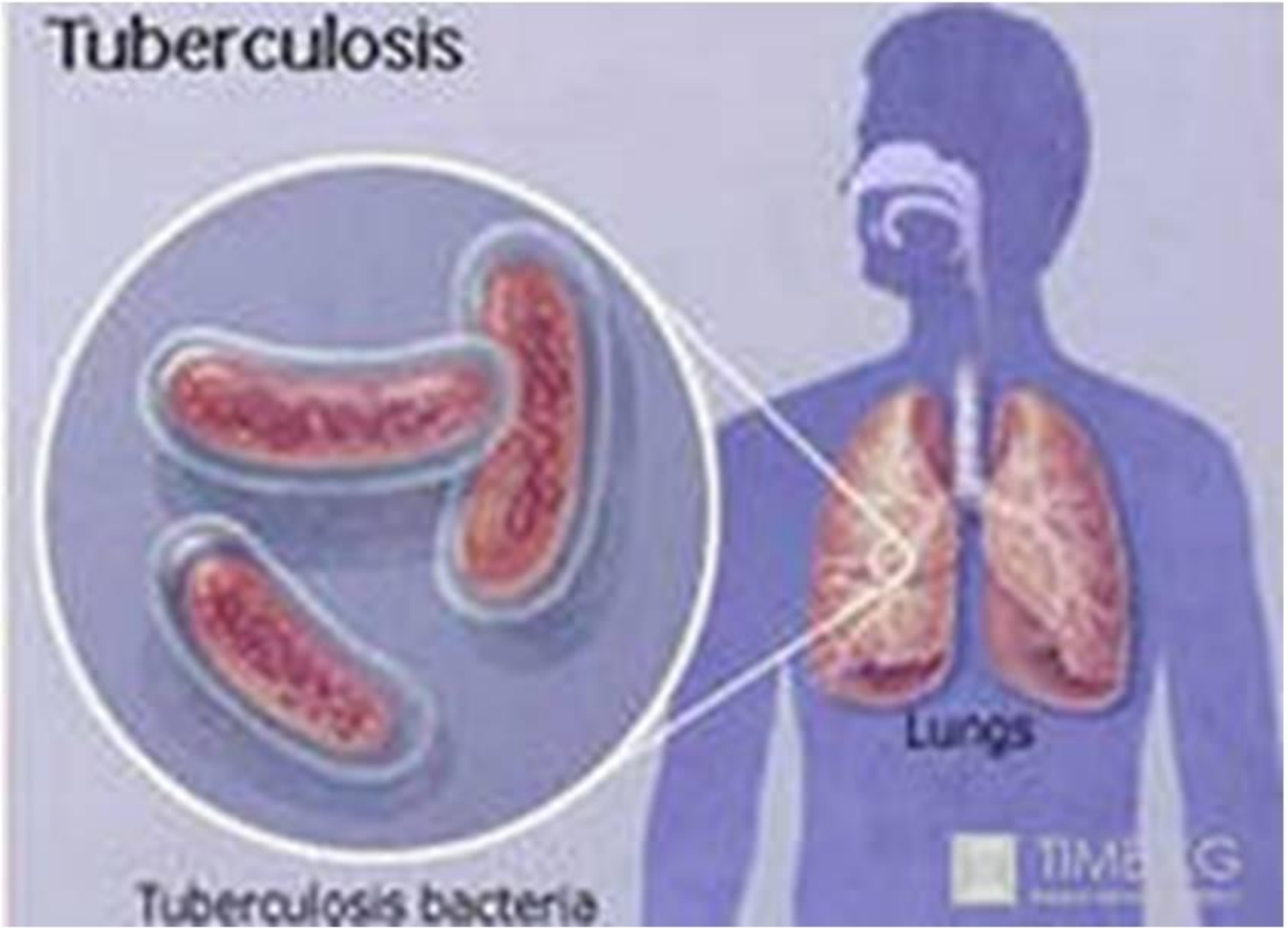
Only diphtheria antitoxin can neutralize diphtheria exotoxin, and it can do so only before the toxin reaches and damages tissue cells. Therefore, it must be given as soon as possible after C. diphtheriae begins to multiply in a patient's throat, on clinical suspicion, and before bacteriological confirmation. C. diphtheriae is nearly always sensitive to penicillin or to erythromycin. These will rid the patients of the organisms. Almost carriers can be cleared with antibiotics.

Active immunity.

The diphtheria toxoid is usually given, along with the tetanus and pertussis vaccine, as DPT (diphtheria -pertussis-tetanus vaccine) to infants between 3 and 6 months old. The usual course is three doses. A booster dose of the diphtheria tetanus (DT) vaccine is given at school entry. Passive immunity. Contacts of a patient may be protected by antitoxin. This may be useful when there is a danger of cross-infection in a ward from a missed case, or in home contacts of a patient.

- **Diphtheria toxoid (for stimulating antitoxin production)**
- **AB – tetracycline, erythromycin**
- **antitoxin**

- **Pertussis-diphtheria vaccine**
- **Diphtheria toxoid**
- **Pertussis-diphtheria-tetanus vaccine**

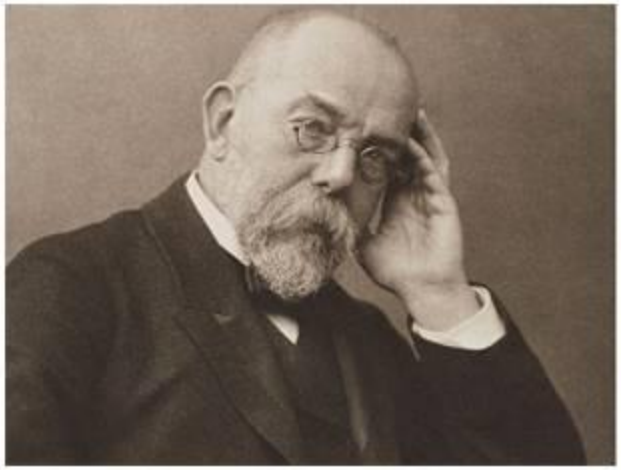

Tuberculosis
Lungs
Tuberculosis bacteria

The genus Mycobacterium belongs to the family Mycobacteriaceae. Mycobacterium contains about 50 species that are normally environmental saprophytes, although some species cause opportunistic disease of animals and man. The group of pathogenic mycobacteria includes M.leprae that causes leprosy, and the tubercle bacilli. There are three species of tubercle bacilli: M.tuberculosis, the human tubercle bacillus; M. bovis, the bovine tubercle bacillus; M. africanum, a rather heterogeneous type found in Equatorial Africa.

Mycobacterium tuberculosis

- Family: Micobacteriaceae
- Genus: Mycobacterium
- Species: M. tuberculosis, M. bovis.

M. Tuberculosis, R. Koch 1882



Morphology

Morphology

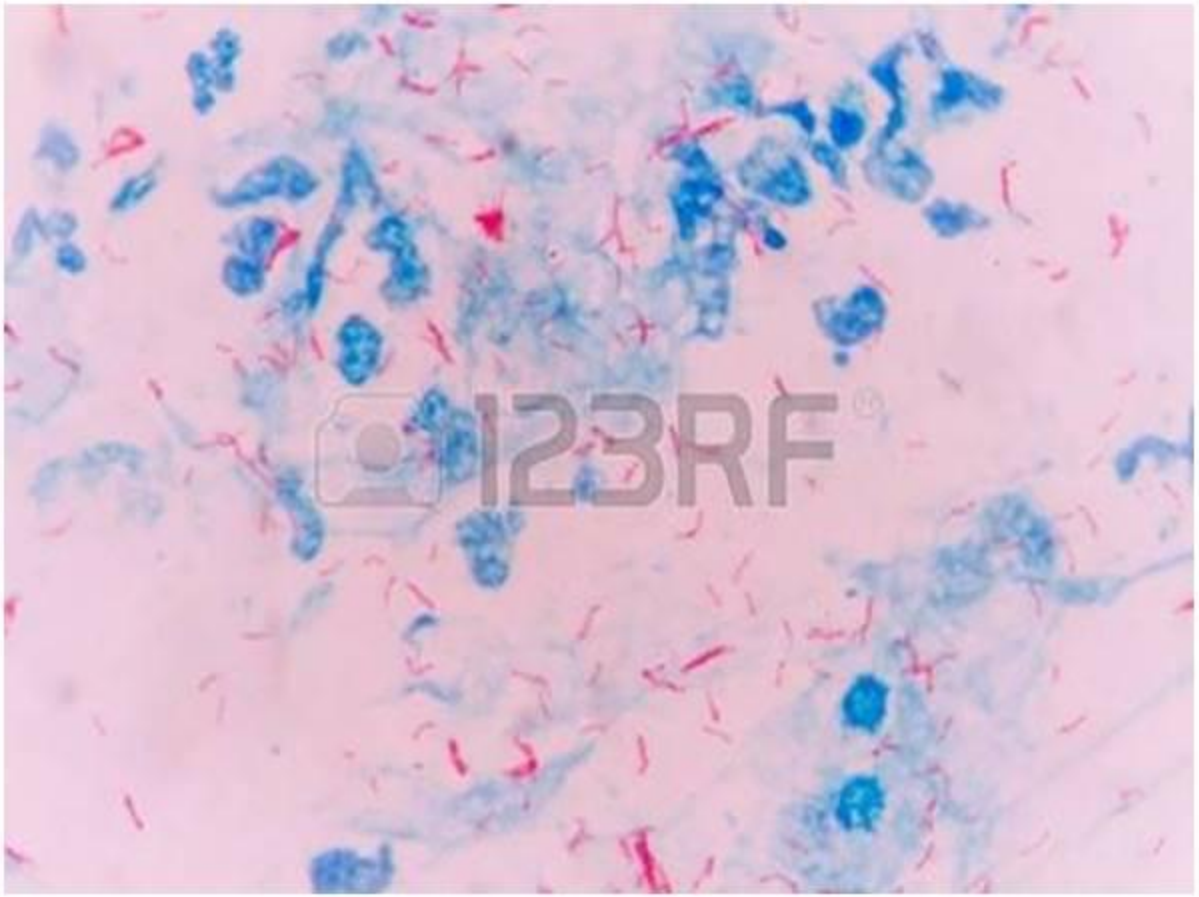
Gram positive slender, straight rods

- High lipid content in cell wall

Nonsporing, nonmotile, non capsulated.

- Stain: Ziehl-Neelsen – red rods, acid fast.
- Cell wall are complex: peptidoglycan layer is thick, arabinolactan-mycolic acid, lipids, polypeptides. The surface glycolipids and phenolic glycolipids are highly antigenic.

M. Tuberculosis, Ziehl-Neelsen stain

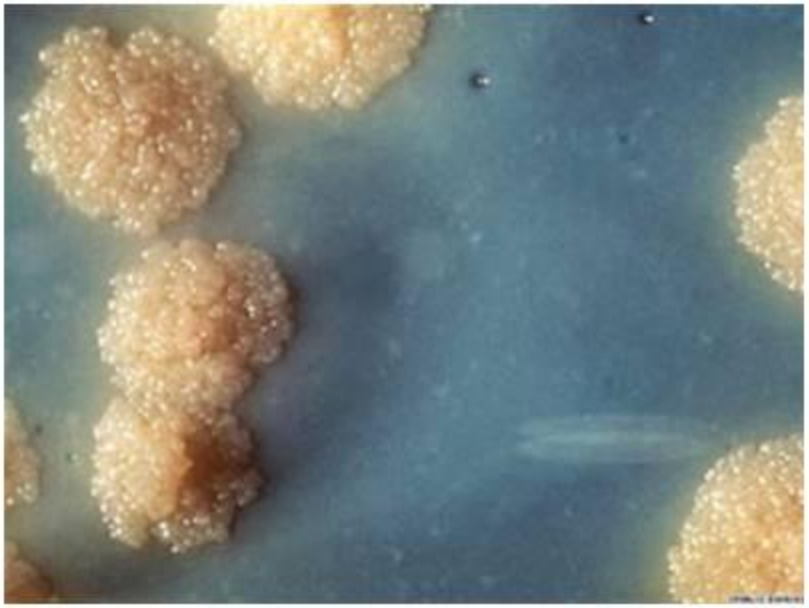


Cultural properties

- Obligate aerobe or microaerophilic.
- Slow-growing, 4-6 weeks.
- Loewenstein- Jensen medium (potato, glycerin, eggs, malachite green).

Loewenstein- Jensen media

Colonies are cream-coloured



Biochemical properties

- Catalase positive
- Niacinase positive
- Urease positive
- Proteolytic activity positive

Antigens

- Proteins
- Lipids
- Cord factor

Resistance

- M. tuberculosis are relatively resistant to chemical disinfectants.
- M. tuberculosis can be killed at 60 C in 20 min.
- M. tuberculosis in sputum may remain alive for 2-3 days,
- - in soil – 6 months,
- -in gastric juice – 6 hours.

The cell wall is more complex than in any other bacterium. It is characterized by a very high content of lipids, many of which have important biological functions. Surface peptidoglycolipids (mycosides) determine cultural properties and interaction with bacteriophage and serotype. Some glycolipids, especially cord-factor (trehalose dimycolate) and sulpholipids, are toxic. These lipids, together with peptidoglycan, are powerful adjuvants involved in granuloma formation. Lipoarabinomannan interferes with the processing of antigen and its presentation to T cells and may therefore suppress protective immune responses. It also triggers the release of the tumour necrosis factor from activated macrophages.

Virulence factors

- **Cord factor** is glycolipid that inhibits migration of leukocytes and elicits granuloma formation.
- **Sulfatides and glycolipids** inhibit phagocytosis.
- The cell wall (**tuberculoprotein**) causes delayed hypersensitivity.
- **Lipids** cause the accumulation of macrophages and neutrophils.
- **Phosphatides** induce the formation of tubercules consisting of epithelial cells.

Cord Factor

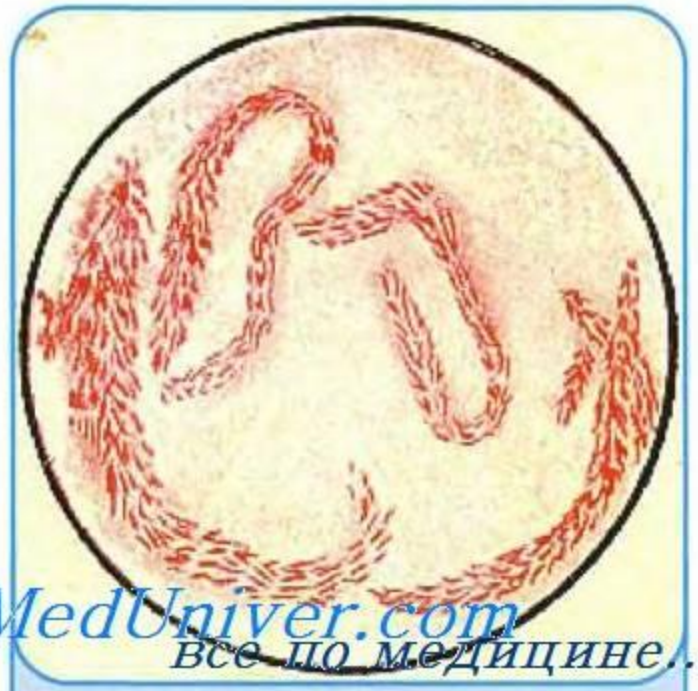


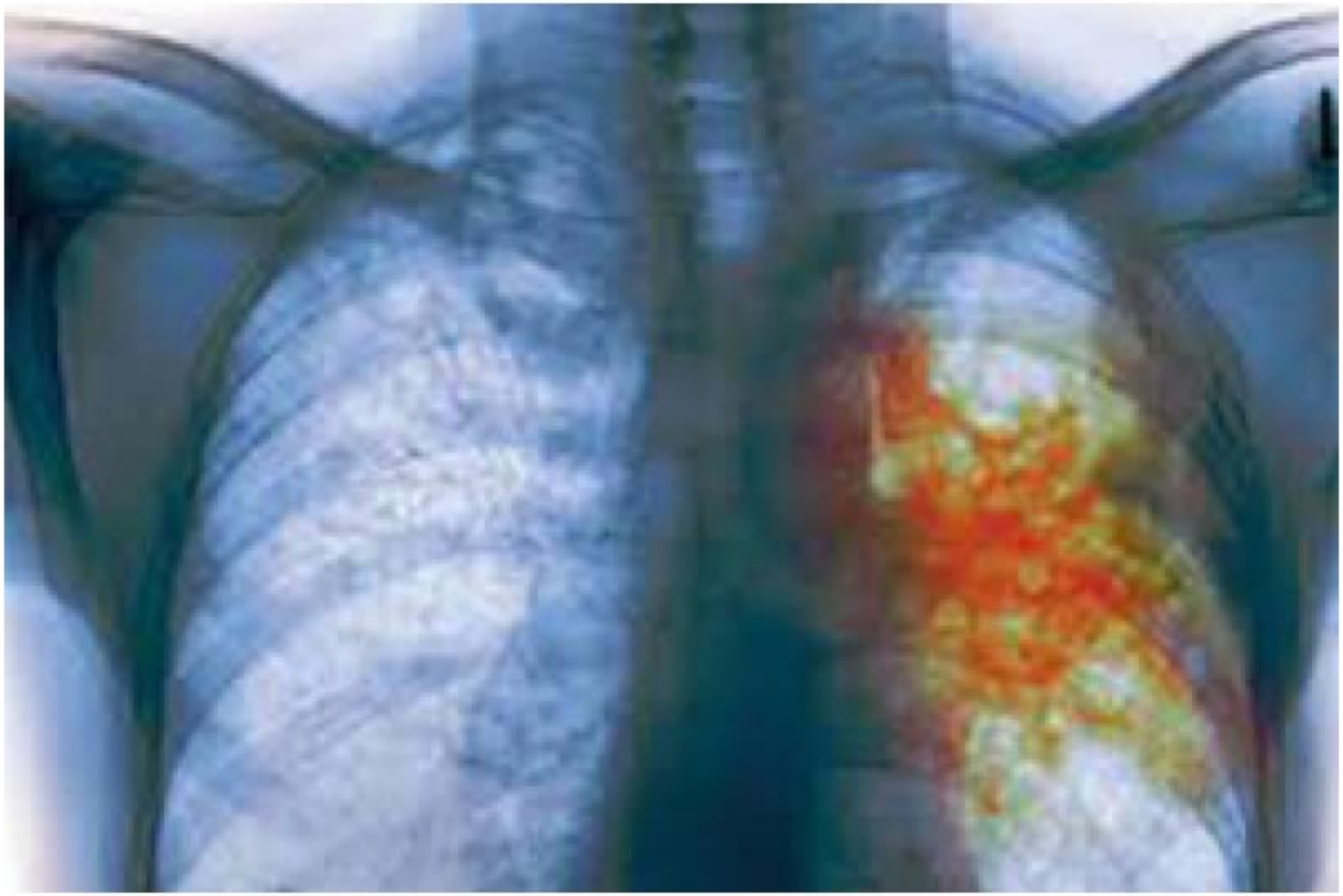
Рис. 3.92. Микроколонии (корд-фактор) M. tuberculosis: палочки, расположены в виде «косы», жгутов

Pathogenesis and clinical findings

- Tuberculosis is transmitted by airborne inhalation.
- Inhaled M.tuberculosis are phagocytized by macrophages, replicate and form the initial lesion.
- Dissemination of microbes occurs through the lymphatic vessels and the bloodstream.
- Any organ can be infected (kidney,bone marrow, brain).
- Tubercules are formed by cell-mediated hypersensitivity response 2-6 weeks after infection. The tubercle has a central area of epithelial cells with a surrounding area of lymphocytic infiltration.

clinical findings

- Tuberculosis is transmitted from an infected person to a susceptible person in airborne particles, called droplet nuclei.
- Typical symptoms of pulmonary tuberculosis include:
- continued cough,
- presence blood in the sputum,
- weight loss and fatigue and loss of appetite,
- fever for a long duration.



Tuberculosis. Clinical Manifestations

The initial lesion of tuberculosis occurs at the site of implantation of the bacillus (the lung, skin, or alimentary tract). Bacteria are ingested by phagocytes, migrate to the draining lymph nodes where secondary lesions develop. The initial pulmonary lesion, the Ghon focus, together with the lymphadenopathy, forms the primary complex . The characteristic lesion of tuberculosis is the granuloma. The primary complex often heals with calcification. Some bacilli are disseminated through lymphatics and blood, leading in some cases to non-respiratory tuberculosis: lymph-node tuberculosis, genitourinary tuberculosis, bone and joint disease, tuberculous meningitis, abdominal tuberculosis, and tuberculous pericarditis.

Tuberculosis. Microbiological Diagnosis

SPECIMENS. Sputum should be collected into sterile wide-mouthed, screw-capped glass or plastic pots. At least three sputum samples, preferably early-morning samples, should be collected. Bronchoscopy enables specimens to be obtained from abnormal areas of the lung by brushing, bronchoalveolar washing, and by bronchial or transbronchial biopsy. In cases of non-respiratory location of the tuberculosis process, other specimens could be obtained.

Tuberculosis. Microbiological Diagnosis

PCR. The polymerase chain reaction should provide an extremely specific, sensitive, and rapid diagnosis. Mycobacteria are detectable in clinical specimens by PCR and by the demonstration of tuberculostearic acid by mass spectroscopy. However, these techniques are not yet widely available.

BACTERIOSCOPICAL EXAMINATION. After drying and heat fixing, the smear is stained by the Ziehl-Neelsen method (Acid-Fast staining). Smears may be stained and examined by fluorescence microscopy. Both methods depend on the acid-fastness of mycobacteria. Microscopy is also used to detect acid-fast bacilli in the urine, pleural, peritoneal, and cerebrospinal fluid after centrifugation, and in homogenates or histological sections of tissue.

Tuberculosis. Microbiological Diagnosis

BACTERIOLOGICAL EXAMINATION. As tubercle bacilli grow very slowly, they are readily overgrown by fungi or other bacteria in the specimen. This may be avoided by treating the specimen with an agent, usually an acid or alkali that will preferentially kill organisms other than mycobacteria. The Lowenstein-Jensen medium is the most widely used. It is solid and contains eggs, glycerol, and mineral salts. Specimens are incubated at 35oC-40oC for 4-8 weeks. The following tests allow division of the tubercle bacilli into individual species: reduction of nitrate to nitrite (nitratase test), oxygen preference (aerobic strains grow on the surface of semisolid agar media, while micro-aerophilic strains form a band deep in the medium), susceptibility to different chemicals. On glycerol-containing media, colonies of M. tuberculosis are usually large and heaped up and produce nicotinamide while those of M. bovis are small and flat.

Tuberculosis. Microbiological Diagnosis

PRICE METHOD. This is the rapid technique of the bacteriological diagnosis of tuberculosis. Samples of sputum, pus, urine, lavage waters, etc. are spread in a thick layer on the sterile slide glass. After the preparation is dried and manipulated with sulfuric acid, it is placed into a vial with citrate blood. After 3 to 4 days of incubation, the preparation is retrieved, fixed, and then stained with the Ziehl-Neelsen stain. Microcolonies in the preparation appear as rope-like microcolonies termed cords. Cord formation is regarded as a marker of the presence of a specific “cord-factor” in the microcapsule of the pathogen.

BIOLOGICAL EXAMINATION. M. tuberculosis causes disease in a wide range of mammals. This microbe is virulent for the guinea-pig. M. bovis causes infection in cattle and badgers and, less frequently, in deer and other wild or feral mammals. Experimentally, M. bovis is highly virulent to rabbits.

Tuberculosis. Microbiological Diagnosis

ALLERGIC SKIN TEST (the Mantoux test). In the Mantoux test, tuberculin, the solution containing a known number of international units of mycobacterial antigen - purified protein derivative (PPD) - is injected intradermally. The diameter of the induration is read 48 to 72 h later. The induration of up to 10 mm-15 mm is usually regarded as positive and may indicate previous exposure to mycobacterial antigens through infection with one of the tubercle bacilli or to BCG vaccination. (That is because of type IV hypersensitivity which develops three to eight weeks after the primary infection). Tuberculin reactivity does not correlate with protective immunity.

Tuberculosis. Microbiological Diagnosis

SEROLOGICAL EXAMINATION. The immune response in tuberculosis is predominantly cell-mediated, and detection of antibody rising is not of great importance in diagnosis. Thus, tuberculin conversion usually occurs 3 to 8 weeks from the time of infection.

The three key first-line drugs used for previously untreated patients are isoniazid, rifampicin, and pyrazinamide. Ethambutol and streptomycin are valuable additional drugs. Reserve drugs, which may be used when first-line treatment has failed, are ethionamide or prothionamide, kanamycin, etc. All M. tuberculosis strains contain drug-resistant mutants.

Treatment

- Rifampicin
- Streptomycin
- Ethionamide
- PAS (para-aminosalicyclic acid)
- Isoniazid

Immunoprophylaxis

- **BCG** (1920)
- Bacillus Calmette-Guérin (BCG) vaccination can protect against tuberculosis. The BCG vaccine is given to all infants.

Mass BCG vaccination of neonates is valuable. The vaccine strain, Bacillus Calmette-Guerrin (BCG), was supposedly derived from a strain of M. bovis. It is now prepared as a freeze-dried live vaccine for intradermal injection. The booster vaccination should be performed only in those negative on tuberculin testing (the Mantoux test).