CHLAMYDIA

They are in the genus Chlamydia, family Chlamydiaceae, order Chlamydiales. This family contains a single genus Chlamydia and three species: **C. trachomatis; C. psittaci; C. pneumonia.**

Chlamydiae are obligate intracellular parasites, because they lack mechanisms for the production of metabolic energy and cannot synthesize ATP. This defect restricts them to an intracellular existence, where the host cell furnishes energy-rich intermediates. The organisms are characterized by low metabolic activity and are cultivated at 33-41º C in yolk sac of a chicken embryo. They are small in size and measured 0.2-1.5µm in diameter. Examination of Chlamydiae indicates that the outer cell wall resembles the cell wall of gram-negative bacteria. It has relatively high lipid content. It is rigid but does not contain a typical bacterial peptidoglycan. Chlamydia contains both RNA and DNA to that in bacteria. Chlamydiae have a replicative cycle different from that of all other bacteria(picture1). The extracellular forms of Chlamydiae are named **elementary bodies -EB(metabolically less active),** which are about 0.3µm in diameter attached to the susceptible host cell. The type and range of susceptible host cell vary with the species and biovar. The host cell phagocytizes the elementary body, housing it in a vacuole. Chlamydia dependent modification of the endocytic membrane prevents lysosomal fusion and thus escapes degradation. This elementary body is reorganized into initial bodies (vegetative forms), after transformation of them to the large, metabolically active **reticulate body- RB)**. The reticulate body grows in size and divides repeatedly by binary fission, producing multiple reticulate bodies. After it the reticulate bodies begin to convert back into elementary bodies. The new- formed elementary bodies may be librated from the host cell (by rupture) to infect new cells. Within cells, the site of replication appears as an inclusion body, which can be stained (by Romanovsky- Giemsa method) and visualized microscopically. These inclusions are useful for the identification of these organisms in the clinical laboratory. Growth, reproduction and maturation of Chlamydia organisms are completed in 40-72 hours.

Antigenic structure: They contain 1.Thermo-stable, lipopolysaccharide antigen, which is genus specific and common to all Chlamydia. This antigen is present in all stages of the development cycle and can be identified by the CFR. 2. Species- specific protein, thermo-labile antigen present at the envelope surface. These are present in all strains of a Chlamydial species. They help in classifying Chlamydia into the species (trachomatis, psittaci, etc.). 3. Outer membrane proteins by which Chlamydiae have been classified into many serological variants.

General characters:

1. They are small, obligate intracellular, gram negative bacteria
2. They possess both RNA and DNA, ribosomes and cell wall similar to that of gram negative bacteria. However, they differ from most true bacteria in that they do not have peptidoglycan.
3. they lack the ability to produce their own ATP, therefore, they use host's ATP.
4. They multiply by binary fission
5. They are non-motile and stain poorly with Gram, but readily with Romanovsky – Giemsa. They are gram negative. Giemsa staining is preferable for staining inclusions in cell culture. Inclusion bodies of Chlamydia are basophilic in nature and look like beef eye.
6. They can also be demonstrated by direct immunofluorescence staining
7. They multiply in the cytoplasm of the host cell forming microcolonies or inclusion bodies which drape around the nucleus like a cloak or mantle (chlamys means mantle)
8. Like gram negative bacteria, the outer membrane of various Chlamydia possess several proteins of which major outer membrane protein has species-specific epitopes
9. They possess a genus–specific lipopolysaccharide-protein complex antigen
10. They infect a wide spectrum of vertebrate hosts including birds, mammals and humans
11. They are susceptible to a wide range of antibiotics such as tetracyclines, erythromycin,

macrolides and rifampicin.

Pathogenic factors: The pathogenic properties depend on surface antigens, which suppress the response mechanisms of human organism. In pathogenicity Endotoxins and Exotoxins participate.

The Endotoxin is lipopolysaccharide of the cell wall, which is similar to gram-negative bacteria's LPS toxins.

Exotoxin is thermolabile protein substance.

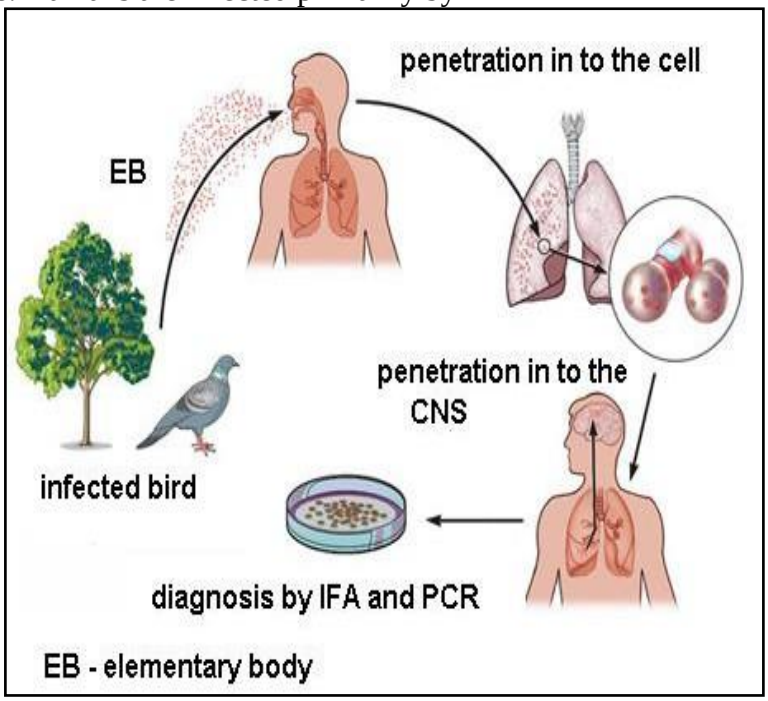
Chlamydia can blockade formation of phagolysosoms (development of **incomplete phagocytosis**).

On the basis of antigenic structure Chlamydiae are subdivided into: **Chlamydia trachomatis** causative agent of trachoma, conjunctivitis and lymphogranuloma venereum, urethritis. **Chlamydia psittaci** is the causative agent of ornithosis, pneumonia, meningoencephalitis and gastroenteritis.

Chlamydia psittaci (the discoverer was K. Meyer, 1933) is the causative agent of ornithosis, which is an acute infection with intoxication and defeated of lungs, CNS and with hepatolienal syndrome.

Ornithosis (GK. ornis bird) is disease of many birds.

Chlamydia psittaci infects birds (parrots, pigeons, chickens and many other species of birds) and many mammals. Humans are infected primarily by inhaling organisms in dry bird faeces (they may also contract the disease cutting poultry, cleaning cages, and taking care of birds).



The agent enters the human body through the respiratory tract. The adhesion is on the epithelium of bronchus, bronchiolus where the reproduction occurs. Then, the aetiological agent invades the blood and produces bacteraemia, which lasts for a week or even longer. As a result of various cycles, which undergo within the tissues and organs, disturbances of metabolism appear and intoxication and allergy develop.

Incubation period is 6-17 days. The onset is sudden, with fever 39□-40º C, severe headache, myalgia, nausea, vomiting, cough, anorexia and sore throat. The clinical picture often resembles that of influenza, pneumonia, or typhoid fever (clinically there are 3 forms: pneumonia, influenza, typhoid).

Immunity: During infection immunoglobulins are produced but they don't have high protective properties and immunity following the disease is relative and of a short duration. Repeated infections occur.

Prophylaxis and treatment: For treatment antibiotics are used (Tetracyclines (doxycycline) and macrolides), especially tetracyclines are the drugs of choice. The prophylaxis comprises sanitary-veterinary measures: early diagnosis, isolation and hospitalization of patients. Sick birds are killed and their nesting places are disinfected. In view of a high ornithosis incidence among pigeons, it is necessary to double veterinary control measures and restrict or ban pigeon breeding in towns or near poultry farms.

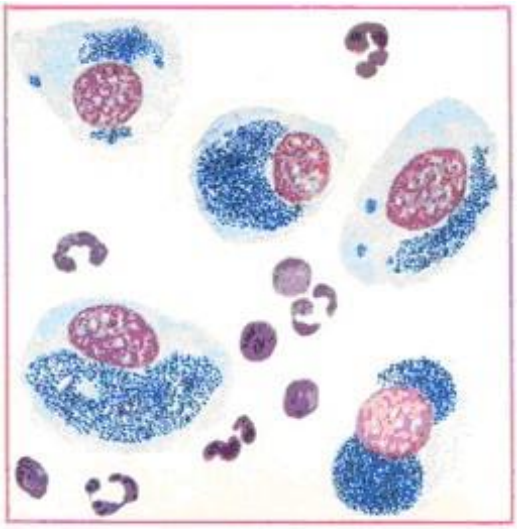
CAUSATIVEA GENT OF TRACHOMA

By biological properties they are similar to Chlamydia genus. They grow in embryonated eggs at 35□ C and in some cell cultures. Chlamydia trachomatis is subdivided

into 15 serotypes: L1; L2; L3 are causative agents of venereal lymphogranulomatosis. A; Ba; B and C are the causative agents of trachoma. D-K serotypes cause urethritis and eye infections.

Trachoma is an anthroponose infection. The reservoir of the causative agent is human. Trachoma spreads by contact, by using common towels, washing in communal basin, and by dirty hands and flies. This is a family infection.

Trachoma (trachys-GK, means rough) is chronic infection with chronic inflammation of the conjunctiva and cornea with hyperplasia of adenoid tissue and hypertrophy of the follicles, which resemble transparent granules. Clinically there are four phases: 1. The inflammation of eye conjunctiva, and mucopurulent secretion. 2.



Prowazekii intranuclear inclusions in Trachoma

Development of inflammatory processes and cicatrix formation 3. The cicatrix formation process is high. 4. Ending of cicatrix formation. It may lead to blindness. Cytoplasm of the affected cells contains microcolonies of large primary and transitory bodies of Chlamydia organisms surrounded by a vesicular membrane. These bodies resemble inclusions, which L. Halberstaedter and S. Prowazek described in 1907, and they have diagnostic meaning.

Treatment: Antibiotics such as Tetracyclines and Sulphonamides are used.

Immunity: Acquired immunity is of a short duration.

Prophylaxis: Prophylaxis comprises timely recognition and proper treatment of patients, a dispensary service in disease foci, observance of hygiene in working and living conditions, and improvement of the welfare and cultural level of the population.

Chlamydia trachomatis is the causative agent of **conjunctivitis in newborns,** or inclusion blennorrhoea, which is marked by infiltration of the conjunctiva, particularly that of the lower eyelid. The sources of infection are mothers in whose genitourinary system the causative agent is preserved, it is transmitted to the infant during delivery. Adults are infected when they go swimming in small ponds and non-chlorinated swimming pools. The disease in them follows the course of acute follicular conjunctivitis. The incubation period is 10-12 days.

Treatment: with sulphanilamides and antibiotics (the administration of silver nitrate for the prophylaxis of gonorrhoeal blennorrhoea does not prevent the development of inclusion blennorrhoea).

CAUSATIVE AGENT OF VENEREALLY MORPHORANULOMATOSIS

Chlamydia trachomatis organism is responsible for **venereal lymphogranulomatosis** in human. Genital routes transmit the infection. Chlamydia trachomatis causes non-gonococcal urethritis and occasionally, epididymitis, prostatitis, or proctitis in men. In women, C. trachomatis causes urethritis, cervicitis, and pelvic inflammatory disease, which can lead to sterility and predispose to ectopic pregnancy. Patients with genital tract infections caused by C. trachomatis have a high incidence of **Reiter's syndrome,** which is characterized by **urethritis, arthritis, and uveitis.** Reiter's syndrome is **an autoimmune disease** caused by antibodies formed against C. trachomatis cross-reacting with antigens on the cells of the

urethra, joints, and uveal tract.

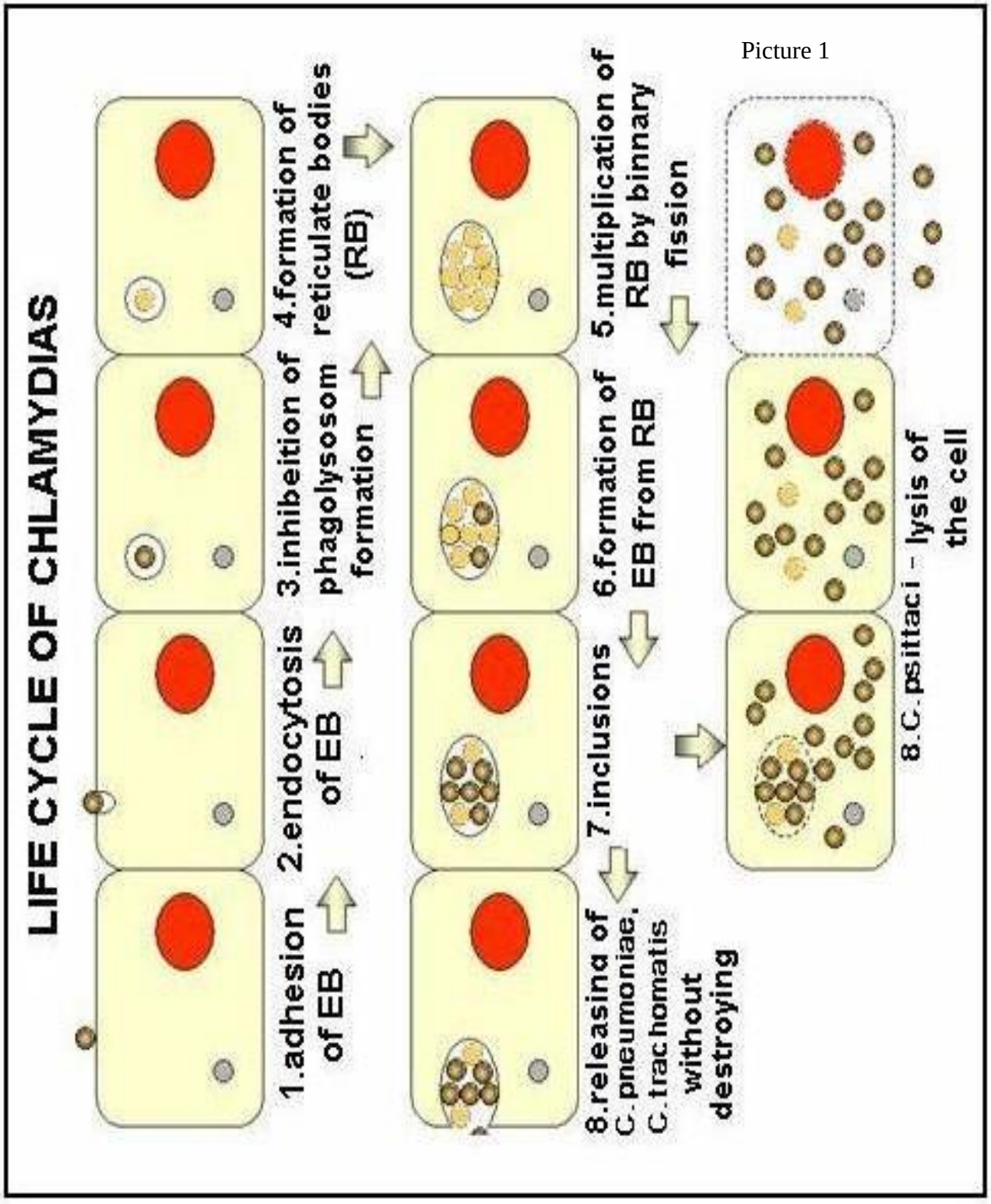
C. trachomatis L1-L3 immune types cause lymphogranuloma venereum, a sexually transmitted disease with lesions on genital and in lymph nodes.

Infection by C. trachomatis leads to formation of antibodies and cell-mediated reactions but not to resistance to reinfection or elimination of organisms.

Treatment –treatment is by the new macrolide antibiotics(clarithromycin, azithromycin).

Laboratory diagnosis – CFR, IF, ELISA

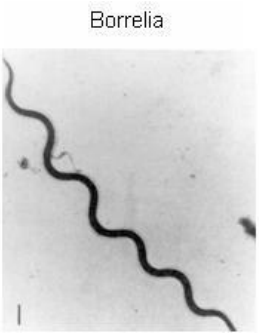
Picture 1

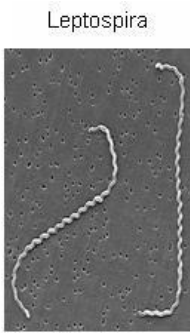


SYPHILIS
(Genus Treponema)

Syphilis is a venereal infection, cyclic infection and infects various organs and systems. **Treponema pallidum** is causative agent of Syphilis, was discovered by Schaudinn and Hoffman in 1905.

Morphology: this organism is thin, delicate about 0.1- 0.2μm in width and 5-15μm in length (trepin-turn, nema thread). It has 8-12 regular spirals at a distance of 1μm from one another. These organisms are actively motile, rotating steadily around their axis even after attaching to cells by tapered ends. They exhibit backward and forward movements, flexion of the whole body. During motion, secondary curves appear and disappear in succession but the primary spirals are unchanged. They do not stain well with aniline dyes. They are stained well by Romanovsky-Giemsa stain and are stained pale-pink (the name pallidum refers to its pale staining). Besides the typical form, Treponema may be seen as granules, L-forms and other structures especially under the action of antibiotics. Under the unfavourable conditions they form **cyst** and can survive in this case for a long time.





Cultivation: They are **strictly anaerobic** organisms. Pathogenic Treponemes do not grow on ordinary nutrient media. They require special nutrient media (they need 11 aminoacids, salts, vitamins, serum albumin) and reproduce on special artificial media. Pure culture isolation of the Treponema organisms is extremely difficult. On prolonged (3-5days) cultivation (generation time is 30hours) the organisms lose their virulence. T. pallidum can be maintained by serial passage in rabbit testes. They haven't oxidase and catalase activity.

Biochemical activity is not clear. Some strains can ferment sugars; proteins with indole and H_2S formation; can liquefy gelatine.

Antigenic structure: The antigenic structure of T. pallidum is complex, and contains polysaccharides, lipids and proteins of the cell wall. A group of lipoproteins are abundant and have unknown functions, though they appear to be important in the immune response. Cardiolipin is an important component of the treponemal antigens. The serotypes and serogroups are not defined.

Ecology: T. pallidum is very delicate. It can be inactivated by drying or by heating (in one hour at 45-48□ C). The organisms are sensitive to acids and other disinfectants (soap, arsenicals, bismuth) and to desiccation. Hence fomites are of little importance in transmission of infection. It is killed in 1-3 days at 0□-4□ C, so that transfusion syphilis can be prevented by storing blood for at least four days in the refrigerator before transfusion.

Pathogenicity: Syphilis is an anthroponose infection. Naturally infection with T. pallidum occurs only in humans. Experimentally, monkeys may be infected and primary syphiloma develops. The source of infection is the patient. The disease is transmitted by sexual contact, in the low per cent cases it may be transmitted by fomites. Syphilis may also be transmitted through the placenta (congenital syphilis).

The Spirochaetes enter the body through minute abrasions on the skin or mucosa. Infectivity of patients to the sexual partner is maximum during the first two years of the disease- the primary, secondary and early latent stages. After five years, the risk is considered minimal. The infective dose is small, as few as 60 treponemes being capable of infecting 50 per cent of human volunteers. The causative agent localizes primarily in the

mucous membranes of the genital organs and mouth and in the skin. At the site of penetration it multiplies and produces proliferative and destructive changes. Clinical disease sets in after an incubation period.

Incubation period is about 3-4 weeks. During the incubation period Spirochaetes multiply locally at the site of entry, and some spread to nearby lymph nodes. In this system the spirochaetes multiply intensively as the concentration of the oxygen in lymph nodes is low, which ensures for them normal conditions for growth. Then they rich the bloodstream and spread through the organs and systems. During the incubation period the Treponema can be distinguished in perineural lymph area. It explains the entering of Spirochaeta into the CNS. In spite of the spread of causative agent during the incubation period into the internal organs there aren't visible pathogenic changes in these organs. So at the end of the incubation period the Spirochaetes are far from the site of entering although they aren't distinguished either clinically or in laboratory tests. After the incubation period three periods of infection are distinguished:

Primary syphilis (ulcus durum, hard chancre), which lasts 6-7 weeks. It occurs at the site of entering. In general the chancre is genital; other common sites are mouth and nipples. Hard chancre is a hard infiltrate with an erosion or ulceration on its surface, at the point where the Treponema enters the body. The floor and edges of the ulcer are of a cartilaginous consistency (for this reason the lesion is known as ulcus durum, primary sclerosis, or hard chancre). The chancre is painless. The size of the ulcer is 10-20 mm. Primary syphiloma is accompanied by the development of regional adenitis manifested by enlarged and hard lymph nodes. During this phase the quantity of immunoglobulins increases and after 2-4 weeks the serologic reactions such as Wasserman complement fixation, precipitation reactions become positive. And this period is subdivided into sero-negative (first 3weeks) and sero–positive (4-7weeks). During this period there are high amounts of Treponema in the tissue liquid of the hard chancre as well as in the lymph nodes and during all the period microscopic method of investigation can be used for diagnosis The chancre invariably heals in about 10-40days, even without treatment leaving a thin scar.

Secondary syphilis (Syphilis secundaria) sets in 2-2,5 months after the primary lesion heals. The secondary lesions are due to widespread multiplication of the spirochetes and their dissemination through blood. Secondary syphilis is manifested by eruptions (roseolar or papular skin rashes) on the skin and mucous membranes (anywhere on the body, including the hands and feet), and moist, pale papules (condylomas) in the anogenital region, axillas and mouth and development of specific lesions in the internal organs, bones, and peripheral and central nervous systems. There may also be syphilitic meningitis, hepatitis, nephritis (immune complex type) or periostitis. This period may vary from 2-3 to several years. Usually in this period regional adenitis and polyadenitis are investigated. In this period the transmission from active form to the latent is described. The relapse rash is rare, not bright, and during each relapse the quantity of rash becomes less. During this period usually no symptoms occur. Both primary and secondary lesions are rich in spirochetes, and the patients are highly infectious.

Syphilis tertiary (Third period) is characterized by the development of granulomatous lesions (gummas) in skin, bones, and liver; degenerative changes in the central nervous system (meningovascular syphilis, paresis, tabes); or cardiovascular lesions (aortitis, aortic aneurysm, aortic valve insufficiency). In all tertiary lesions, treponemas are very rare, and the exaggerated tissue response must be attributed to hypersensitivity to the organisms. However, treponemas can occasionally be found in the eye or central nervous system in late syphilis.

A pregnant syphilitic woman can transmit T. pallidum to the foetus through the placenta beginning in the 10^{th} to15th weeks of gestation. Some of the infected foetuses die, and miscarriages result; others are born live but develop the signs of **congenital syphilis** in childhood: interstitial keratitis, Hutchinson's teeth, saddle-nose, periostitis, and variety of

central nervous system anomalies. Adequate treatment of the mother during the first month of pregnancy prevents congenital syphilis.

Immunity: The diseases produce no insusceptibility. Individuals who have recovered from the disease may be re-infected. Immunity in syphilis is infectious (non-sterile), and characterized by cellular defence reactions (the lymphocytes produce lipolytic enzymes which cause lysis of treponemas). The presence of antibodies does not indicate of body resistance.

A state of infectious allergy, a peculiar manifestation of body reactivity, is a characteristic feature of syphilis. During primary syphilis (hard chancre), the reactivity is lower. An increase in reactivity occurs most frequently during the later periods and is accompanied by deep changes in the tissues and organs.

The state of allergy may be revealed by the intracutaneous luetin reaction. Luetin is prepared from T. pallidum cultures or ill tissues.

Treatment: Penicillin G is the antibiotic agent of choice. Dosage and duration of therapy depend on the stage of the disease and the galenic formulation of the penicillin used.

Epidemiology and prevention. Syphilis is known all over the world. Annual prevalence levels in Europe and the US are 10–30 cases per 100 000 inhabitants. The primary preventive measure is to avoid any contact with syphilitic efflorescences. When diagnosing a case, the physician must try to determine the first-degree contact person, who must then be examined immediately and provided with penicillin therapy as required. National laws governing venereal disease management in individual countries regulate the measures taken to diagnose, prevent, and heal this disease. There is no vaccine.

Diagnostic laboratory tests: Specimens: Tissue fluid - expressed from early surface lesions for demonstration of spirochetes; blood serum for serologic tests.

Methods (picture 1):

1. **Microscopic:** Dark-field examination. Immunofluorescence (fixed slide stained with a fluorescein-labelled anti-treponemal serum, and examined by means of immunofluorescence microscopy for typical fluorescent spirochetes). Staining by Romanovsky-Giemsa and Morozov's methods.
2. **Serological tests: Wasserman CF reaction.** During this reaction is used non-specific antigen: the purified cardiolipin from beef heart. **Precipitin reactions** are widely used in syphilis diagnosis, i.e. the **Sachs-Witebsky** test (the citochol reaction) and the **Kahn test**.
3. **The T. pallidum immobilization test** has a great value.

Diseases related to Syphilis

These diseases are all caused by Treponemes closely related to T. pallidum. None are sexually transmitted diseases; all are commonly transmitted by direct contact. None of the causative organisms has been cultured on artificial media.

Bejel, caused by T. pallidum, occurs chiefly in Africa, Arabian countries (Endemic Arabian syphilis), particularly among children, and produces highly infectious skin lesions but does not affect the cardiovascular and nervous systems; late visceral complications are rare. Penicillin is the drug of choice.

Frambesia (Yaws)-causative agent is T. pertenue. It occurs in hot tropical countries (Africa, Ceylon, South America, Central America, India, Indonesia China). Yaws is endemic, particularly among children. The primary lesion, an ulcerating papule, occurs usually on the arms or legs. Transmission is by person-to person contact in children under 15; transplacental, congenital infection does not occur. Scar formation of skin lesions and bone destruction are common, but visceral or nervous system complications are very rare. It has been debated whether yaws represented by nonsexual means is not climates. There appears to be cross-immunity between yaws and syphilis. Diagnostic procedures and therapy are similar to those for syphilis.

Pinta is a disease caused by T. carateum and occurs epidemically in all age groups in

Mexico, Central and South America, the Philippines. The disease appears to be restricted to dark – skinned races. The primary lesion, a non – ulcerating papule, occurs on exposed areas. Some months later, flat, hyper – pigmented lesions appear on the skin; depigmentation and hyperkeratosis take place years afterward. Late cardiovascular and nervous system involvement occurs very rarely. Pinta is transmitted by non-sexual means, either by direct contact or through the agency of flies or gnats. Diagnosis and treatment are the same as for syphilis.