МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ УКРАИНЫ
Харьковский национальный медицинский университет

CHLAMYDIA. MYCOPLASMAS

Methodical instructions on the subject «Microbiology, virology and immunology» for the II and III year English media students of medical and dentistry faculties

ХЛАМИДИИ. МИКОПЛАЗМЫ

Методические указания по дисциплине «Микробиология, вирусология и иммунология» для студентов II и III курсов медицинского и стоматологического факультетов с английским языком преподавания

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Theme: Microbiological diagnosis of diseases caused by chlamydia and mycoplasmas.

Actuality of the theme.

The family Chlamydiaceae consists of one genus Chlamydia with three species that cause human disease: C. trachomatis, which can cause urogenital infections, trachoma, conjunctivitis, pneumonia and lymphogranuloma venereum (LGV). C. pneumoniae, which can cause bronchitis, sinusitis, pneumonia and possibly atherosclerosis. C. psittaci, which can cause pneumonia (psittacosis).

Mycoplasma species are the smallest free-living organisms. These organisms are unique among prokaryotes in that they lack a cell wall, a feature largely responsible for their biologic properties such as their lack of a reaction to Gram stain and their lack of susceptibility to many commonly prescribed antimicrobial agents, including beta-lactams. Mycoplasmal organisms are usually associated with mucosal surfaces, residing extracellularly in the respiratory and urogenital tracts. They rarely penetrate the submucosa, except in the case of immunosuppression or instrumentation, when they may invade the bloodstream and disseminate to different organs and tissues throughout the body.

Although scientists have isolated at least 17 species of Mycoplasma from humans, 7 types of organisms are responsible for most clinically significant infections that may come to the attention of practicing physicians. These species are Mycoplasma pneumoniae, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Mycoplasma fermentans, Mycoplasma pirum, and Mycoplasma penetrans.

Goal: Studying of laboratory diagnosis of diseases caused by chlamydia and mycoplasma.

Concrete goals:

1. Study of classification and morphology of chlamydia and mycoplasmas.
2. Study life cycles of chlamydia.
3. Study pathogenesis and clinical manifestations of chalamydial and mycoplasmas diseases.
4. Study of the methods of laboratory diagnosis of chalamydiosis and mycoplasmosis.
5. Interpret the results of the bacteriological examination of parasites.
6. Study treatment and prophylaxis of mycoplasmosis and chlamydiosis.

Students should be able to:

1. Interpret the results of microscopical examination.
2. Interpret the results of serological examination.

Equipment: slides, immersion microscope, tables, atlas.

CHLAMYDIA

The chlamydia are a small group of nonmotile coccoid bacteria that are obligate intracellular parasites of eukaryotic cells. Chlamydial cells are unable to carry out energy metabolism and lack many biosynthetic pathways; therefore they are entirely dependent on the host cell to supply them with ATP and other intermediates. Because of their dependence on host biosynthetic machinery, the chlamydiae were originally thought to be viruses; however, they have a cell wall and contain DNA, RNA, and ribosomes and therefore are now classified as bacteria. The group consists of two genera, Chlamydia and Chlamydophila (order Chlamydiales, family Chlamydiaceae). These are three species Chlamydia trachomatis, Chlamydophila psittaci, and Chlamydophila pneumoniae. All three species cause disease in humans. C. psittaci infects a wide variety of birds and a number of mammals, whereas C. trachomatis is limited largely to humans. C. pneumoniae has been found only in humans.

Structure. The chlamydia exist in nature in two forms: (1) a nonreplicating, infectious particle called the elementary body (EB), 0.25 to 0.3 μm in diameter, that is released from ruptured infected cells and can be transmitted from one individual to another (C. trachomatis, C .pneumoniae) or from infected birds to humans (C. psittaci), and (2) an intracytoplasmic form called the reticulate body (RB), 0.5 to 0.6 μm in diameter, that engages in replication and growth (Fig. 1). The elementary body, which is covered by a rigid cell wall, contains a DNA genome. A cryptic DNA plasmid (7,498 base pairs) is also found. It contains an open reading frame for a gene involved in DNA replication. In addition, the elementary body contains an RNA polymerase responsible for the transcription of the DNA genome after entry into the host cell cytoplasm and the initiation of the growth cycle. Ribosomes and ribosomal subunits are present in the elementary bodies. Throughout the developmental cycle, the DNA genome, proteins, and ribosomes are retained in the membrane-bound prokaryotic cell (reticulate body).

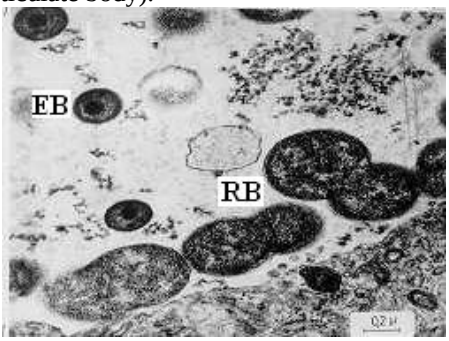

Fig. 1. Electron micrograph of elementary and reticulate bodies.

Because of their rigid outer membrane the elementary bodies are resistant to harsh environmental conditions encountered when the chlamydia are outside of their eukaryotic host cells. The elementary bodies bind to receptors on host cells and initiate infection. Most chlamydia infect columnar epithelial cells but some can also infect macrophages.

Reticulate bodies are the non-infectious intracellular form of the chlamydia. They are the metabolically active replicating form of the chlamydia.

A complex series of events occurs during the developmental cycle of chlamydia (Fig. 2). The infectious elementary body develops into a noninfectious reticulate body (RB) within a cytoplasmic vacuole in the infected cell. There is an eclipse phase of about 20 hours after entry of the elementary body into the infected cell, during which the infectious particle develops into a reticulate body. In these structures the chlamydial genome is transcribed into RNA, proteins are synthesized, and the DNA is replicated. The reticulate body divides by binary fission to form particles which, after synthesis of the outer cell wall, develop into new infectious elementary body progeny. The yield of chlamydial elementary bodies is maximal 36 to 72 hours after infection.

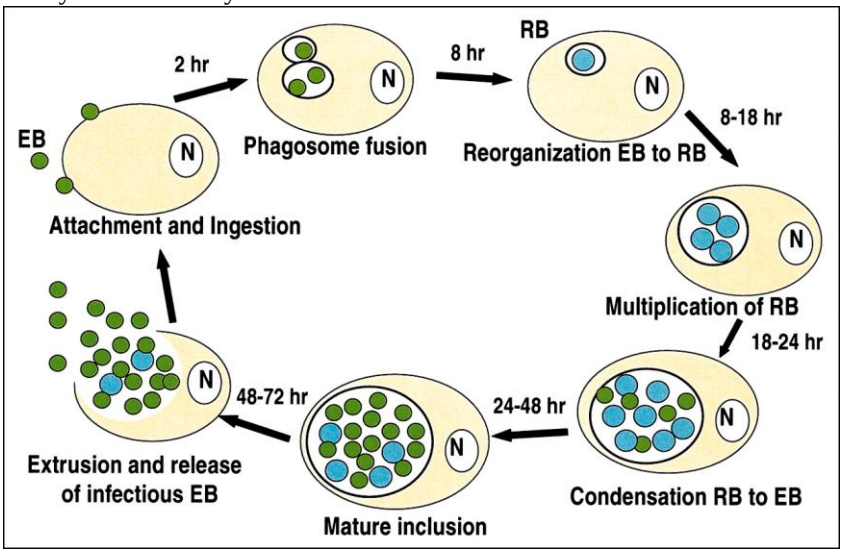

Fig. 2. Infectious life cycle of Chlamydia.

Antigenic Types. Several distinct antigenic components have been recognized in C. trachomatis and C. psittaci, some group specific and others species specific. C. pneumoniae is serologically unique and differs from C. trachomatis species and all C. psittaci strains tested.

The outer chlamydial cell wall contains several antigenic proteins, including a major outer membrane protein (MOMP), cysteine-rich proteins, a species-specific protein, and eukaryotic cell-binding proteins, which share the same primary sequence.

There are 15 human C. trachomatis serotypes, establishing the species specificity of the cysteine-rich membrane proteins antigen.

Virulence Factors. There are numerous factors that contribute to the pathogenicity of C. trachomatis. Colonization of Chlamydia begins with attachment to sialic acid receptors on the eye, throat, or genitalia. It persists at body sites that are inaccessible to phagocytes, T-cells, and B-cells.

Its unique cell wall structure is another virulence factor. Studies reveal that Chlamydia, because of its cell wall, is able to inhibit phagolysosome fusion in phagocytes. The cell wall is proposed to be gram-negative in that it contains an outer lipopolysaccharide membrane, but it lacks peptidoglycan in its cell wall. This lack of peptidoglycan is shown by the inability to detect muramic acid and antibodies directed against it. It may, however, contain a carboxylated sugar other than muramic acid. The proposed structure consists of a major outer membrane protein cross-linked with disulfide bonds. It also contain cysteine-rich proteins (CRP) that may be the functional equivalent to peptidoglycan. This unique structure allows for intracellular division and extracellular survival.

The surface of chlamydia does not contain proteins that are distinctive enough to induce a full immune response. The cell wall does contain an exoglycolipid antigen that induces a weak immune response (for reasons unknown, the immune response is weaker to carbohydrate antigens).

Epidemiology. Trachoma is still prevalent in Africa and Asia (more than 500 million people are estimated to have the disease), and sporadic cases occur all over the world. The disease flourishes in hot, dry areas where there is a shortage of water and where standards of hygiene are low. The agent is spread to the eyes by flies, dirty towels, fingers, or cosmetic eye pencils. The initial infection usually occurs in childhood, and the active disease eventually appears (mostly by 10 to 15 years of age). Trachoma may leave a residuum of permanent lesions that can lead to blindness. C. trachomatis also resides in the genital tract, and urethra of adults, and genital infection is spread sexually.

C. psittaci, the cause of psittacosis in birds and occasionally in humans, is carried by wild and domestic birds, including poultry.

C. pneumoniae spreads in human populations by respiratory tract infections. It is the agent of atypical pneumonia in hospitalized patients as well as in young individuals with an acute respiratory disease.

Spread of Agents. Human diseases caused by chlamydia can be divided into two types: (1) chlamydial agents transmitted by direct contact (C. trachomatis genital and ocular infections) and (2) chlamydial agents that are transmitted by the respiratory route (C. psittaci and C. pneumoniae.)

The spread of C. trachomatis from person to person may cause trachoma, inclusion conjunctivitis, or lymphogranuloma venereum. Transmission of C. trachomatis from the urogenital tract to the eyes and vice versa occurs via contaminated fingers, towels, or other fomites and, in neonates, by passage through an infected birth canal. These diseases appear in an epidemic form in populations with low standards of hygiene. C. trachomatis genital infections are sexually transmitted.

C. pneumoniae spreads from infected individuals by respiratory tract infections.

C. psittaci is transmitted from infected birds or animals to humans through the respiratory tract. It can also be transmitted via feathers and eggs or direct contact of contaminated materials, and is typically either inhaled or ingested.

Pathogenesis. Chlamydial agents are intracytoplasmic obligate parasites of mammalian cells and can damage infected cells in tissues. The elementary bodies are infectious particles that can be transmitted from the infected tissues to uninfected tissues in the same person (transfer of C. trachomatis elementary bodies from an infected genital tract to the eyes and vice versa) or from a person with atypical pneumonia (caused by C. psittaci or C. pneumoniae) to healthy individuals (respiratory release of elementary bodies). In the infected individuals the chlamydial agent causes tissue damage and induction of interleukin-1α, interleukin-1ß, and tumor necrosis factor alpha, which are cytokines involved in the inflammation process. Ocular infections by C. trachomatis strains cause acute purulent conjunctivitis either due to infection of the neonate during passage through the birth canal or due to subsequent infections leading to scarring of the conjunctiva and to blindness subsequent to mucopurulent follicular conjunctivitis. The genital tract infection serves as a source of infectious elementary bodies for the eyes.

If a Chlamydia infection causes the fallopian tubes to swell it can cause a build-up of scarring that blocks the tubes, making it even more difficult for sperm to swim up them to reach the egg. It's also more difficult for the egg to move down the fallopian tubes to meet the sperm. Chlamydia breaks the hairs in the fallopian tube which are needed to move the eggs from ovaries to the womb. If left untreated, it can cause scarring, adhesion and finally complete blockage.

The respiratory tract is the main portal of entry of C.psittaci. From the lungs the organisms enter the blood stream and are transported to the liver and spleen. The bacteria replicate at these sties where they produce focal areas of necrosis. Hematogenous seeding of the lungs and other organs then occurs. A lymphocytic inflammatory response in the alveoli and interstitial spaces leads to edema, infiltration of macrophages, necrosis and sometimes hemorrhage. Mucus plugs may develop in the alveoli causing cyanosis and anoxia.

Clinical Manifestations. The diseases caused by chlamydia are summarized in Table 1.

Table 1. Human diseases caused by chlamydia

Species	Serotypes	Diseases
C.trachomatis	A, B, Ba, C	Trachoma
	D, E, F, G, H, I, J, K	Inclusion conjunctivitis, nongonococcal urethritis, genital infections, pneumonia of newborns
	L-1, L-2, L-3	Lymphogranuloma vebereum
C.pneunoniae		Pneumonia, upper respiratory infections, bronchitis
C.psittaci		Pneumonia (psittacosis)

C. trachomatis

Trachoma, a C. trachomatis infection of the conjunctival epithelial cells, results in subepithelial infiltration of lymphocytes, leading to the development of follicles. The infected epithelial cells contain cytoplasmic inclusion bodies. As a result of damage to the epithelial cells, fibroblasts and blood vessels invade the infected area, a pannus forms, and the cornea becomes vascularized and clouded. The eyelids become scarred and malformed, causing trichiasis, an abnormal inward growth of the eyelashes. Continual scraping of the cornea by the eyelashes leads to corneal opacification and blindness.

C. trachomatis also causes inclusion conjunctivitis, an eye disease of children and adults that is milder than trachoma. It consists of purulent conjunctivitis that heals spontaneously without scarring.

C. trachomatis also causes sexually transmitted genital and rectal infections. The frequency of C. trachomatis infections in men may equal or exceed the frequency of gonorrhea. Nongonoccocal urethritis, epididymitis, and proctitis in men can result from infection with C. trachomatis. Superinfection of gonorrhea patients with C. trachomatis also occurs. Reiter syndrome (arthritis, urethritis, and bilateral conjunctivitis) also can occur. Acute salpingitis and cervicitis in young women can be caused by a C. trachomatis infection ascending from the cervix. A high rate genital tract coinfection by C. trachomatis in women with gonorrhea has been reported. C. trachomatis was isolated from the fallopian tubes of infected women. In one report C. trachomatis elementary bodies attached to spermatozoa were recovered from the peritoneal cavity of patients with salpingitis. Vaginitis in prepubertal girls; urethritis, cervicitis, endometritis, salpingitis, and perihepatitis (Fitz-Hugh-Curtis syndrome) in postpubertal females. Infection can persist for months to years. Reinfection is common.

Chronic chlamydial infections lead to the development of female and male infertility due to obstruction of the fallopian tubes in women, chronic prostatitis, accompanied by pain in the perineum, frequent urging and painful

urination, to the development of various pathologies of pregnancy (non developing pregnancy, miscarriage, premature birth, fetal abnormality), intrauterine infection of the fetus.

Neonates exposed to C. trachomatis in an infected birth canal may develop acute conjunctivitis within 5 to 14 days. The disease is characterized by marked conjunctival erythema, lymphoreticular proliferation, and purulent discharge. Untreated infections can develop into pneumonitis; this type of pneumonitis occurs only during the first 4 to 6 months of life.

Recently, C. trachomatis has been suspected of causing lower respiratory tract infections in adults, and several cases of C. trachomatis pneumonia have been reported in immunocompromised patients from whom the pathogen was isolated. Evidence also indicates that C. trachomatis may cause pneumonia or bronchopulmonary infections in immunocompetent persons.

Lymphogranuloma venereum is a human venereal disease caused by C.trachomatis strains L1-3. The disease usually occurs in men and involves inguinal lymphadenopathy. Signs of lymphogranuloma venereum appear a few days after venereal exposure. The initial lesions, or vesicles, appear in the urogenital tract in men and women. If the disease does not heal spontaneously, regional lymph nodes become involved. Anorectal infection is associated with anal intercourse and can cause hemorrhagic proctocolitis or stricture among women and men who engage in anal intercourse. The proctocolitis can be moderate to severe and can resemble inflammatory bowel disease.

C. pneumoniae

C. pneumoniae is a common cause of pneumonia around the world; it is typically acquired by otherwise healthy people and is a form of community-acquired pneumonia. Because its treatment and diagnosis are different from historically recognized causes, such as Streptococcus pneumoniae, pneumonia caused by C. pneumoniae is categorized as an "atypical pneumonia".

In addition to pneumonia, C. pneumoniae less commonly causes several other illnesses. Among these are meningoencephalitis (infection and inflammation of the brain and meninges), arthritis, myocarditis.

Multiple studies have evaluated prior C. pneumoniae infection and a possible connection to lung cancer. One meta-analysis of serological data comparing prior C. pneumoniae infection in patients with and without lung cancer found results suggesting prior infection was associated with a slightly increased risk of developing lung cancer.

In research into the association between C. pneumoniae infection and atherosclerosis and coronary artery disease, serological testing, direct pathologic analysis of plaques and in vitro testing suggest chronic infection with C. pneumoniae may be a risk factor for development of atherosclerotic plaques. C. pneumoniae infection increases adherence of macrophages to endothelial cells in vitro and aortas ex vivo.

C. pneumoniae has been found in the cerebrospinal fluid of some patients diagnosed with multiple sclerosis.

Serological evidence for possible chronic C. pneumoniae infection was first associated with wheezing, asthmatic bronchitis and adult-onset asthma in 1991. Subsequent studies of bronchoalveolar lavage fluid from pediatric patients with severe chronic respiratory illnesses including asthma have demonstrated that over half had evidence of C. pneumoniae by direct organism identification.

C. psittaci

In medicine (pulmonology), psittacosis — also known as parrot disease, parrot fever, and ornithosis — is a zoonotic infectious disease caused by a bacterium called Chlamydophila psittaci (formerly Chlamydia psittaci) and contracted from parrots, such as macaws, cockatiels and budgerigars, and pigeons, sparrows, ducks, hens, gulls and many other species of bird. The incidence of infection in canaries and finches is believed to be lower than in psittacine birds.

In certain contexts, the word "psittacosis" is used when the disease is carried by any species of bird belonging to the Psittacidae family, whereas "ornithosis" is used when other birds carry the disease.

In humans, after an incubation period of 5–14 days, the symptoms of the disease range from inapparent illness to systemic illness with severe pneumonia. It presents chiefly as an atypical pneumonia. Splenomegaly is frequent toward the end of first week. Headache can be so severe that suggests meningitis and some nuchal rigidity is not unusual. Towards the end of first week stupor or even coma can result in severe cases.

The second week is more akin to acute bacteraemic pneumococcal pneumonia with continuous high fevers, cough and dyspnoea.

Complications in the form of endocarditis, hepatitis, myocarditis, arthritis, keratoconjunctivitis, and neurologic complications (encephalitis) may occasionally occur. Fatal cases have been reported (less than 1% of cases).

Host Defenses

Nonspecific Responses. Infections with chlamydial agents evoke responses from the blood vessels (ocular trachoma), connective tissue (scars in C.trachomatis infections), and lymphocyte infiltration (pannus). Chlamydial infections are characterized by chronic inflammation. Chlamydiae induce the production of interleukin-1, an important mediator of inflammation and scarring. Interleukin-1α and interleukin-1ß can be induced in human monocytes by C. trachomatis lipopolysaccharide. Release of angiogenesis factors from infected cells may cause proliferation of blood vessels in the infected eye. Fever accompanies C. psittaci pneumonitis.

Cultured chlamydiae are sensitive to interferon, which is produced by cultured cells infected with chlamydiae.

Immune Response. All chlamydial infections induce IgM, IgG, IgA, and IgE antibodies, but these antibodies do not prevent reinfection. Although

secretions from trachomatous eyes contain specific antitrachoma IgG and IgA antibodies, these antibodies do not impede the infection. Moreover, antibodies that bind to C. trachomatis elementary bodies do not impair their infectivity in cell cultures. Monoclonal antibodies to proteins in the outer elementary body envelope were reported to neutralize elementary body infectivity. Most patients with C trachomatis infections have antibodies that react with the C. trachomatis cell wall proteins. Sera from individuals with genital infections caused by C. trachomatis also reacted with the cysteine-rich proteins of all the C. trachomatis serotypes.

Diagnosis. There are several laboratory tests for diagnosis of C. trachomatis but the sensitivity of the tests will depend on the nature of the disease, the site of specimen collection and the quality of the specimen. Since chlamydia are intracellular parasites, swabs of the involved sites rather than exudate must be submitted for analysis. It is estimated that as many as 30% of the specimens submitted for analysis are inappropriate.

1. *Cytology* - Examination of stained cell scrapings for the presence of inclusion bodies has been used for diagnosis but this method is not as sensitive as other methods. C. trachomatis can be identified microscopically in scrapings from the eyes or the urogenital tract. Inclusion bodies in scraped tissue cells are identified by iodine staining of glycogen present in the cytoplasmic vacuoles in infected cells.

2. *Culture* - Culture is the most specific method for diagnosis of C. trachomatis infections. Specimens are added to cultures of susceptible cells (e.g., irradiated McCoy cells) and the infected cells are examined for the presence of iodine-staining inclusion bodies. Iodine stains glycogen in the inclusion bodies. The presence of iodine-staining inclusion bodies is specific for C. trachomatis since the inclusion bodies of the other species of chlamydia do not contain glycogen and stain with iodine. Each chlamydial agent can also be identified by using specific immunofluorescent. Homogenates or exudates of infected tissues also have been used to isolate the agent in the yolk sac of embryonated eggs.

3. *Antigen detection* - Direct immunofluorescence and ELISA kits that detect the group specific LPS or strain-specific outer membrane proteins are available for diagnosis. Fluorescent monoclonal antibodies are used to stain C. trachomatis elementary bodies in urethral and cervical exudates. Neither is as good as culture, particularly with samples containing few organisms (e.g. asymptomatic patients).

4. *Serology* - Serological tests for diagnosis are of limited value in adults, since the tests do not distinguish between current and past infections. Detection of high titer IgM antibodies is indicative of a recent infection. Detection of IgM antibodies in neonatal infection is useful.

Sera and tears from infected humans are used to detect anti-Chlamydia antibodies by the complement fixation or microimmunofluorescence tests. The latter is useful for identifying specific serotypes of C. trachomatis.

5. *Nucleic acid probes* – Three new tests based on nucleic acid probes are available. These tests are sensitive and specific and may replace culture as the method of choice.

It is possible to diagnose C. trachomatis in tissue biopsy specimens by in situ DNA hybridization with cloned C. trachomatis DNA probes.

DNA from C. trachomatis isolates can be examined by restriction endonuclease analysis. The DNA cleavage pattern of C. trachomatis isolates differs greatly from that of DNA from C. psittaci isolates. DNAs of the agents of trachoma and lymphogranuloma venereum differ in their cleavage patterns, and this allows identification of the biovars.

PCR tests are more sensitive than cell culture, direct fluorescent antibody (DFA) tests, or enzyme immunoassay (EIA), although specificity is variable compared with culture.

C. pneumoniae DNA has 10 percent homology with C. trachomatis or C.psittaci; C. pneumoniae isolates have 100 percent homology. C.pneumoniae isolates can be diagnosed by PCR or hybridization with a specific DNA probe that does not hybridize to other chlamydiae. Additional serologic tests are in use: the microimmunofluorescence test or ELISA with C. pneumoniae-specific elementary body antigen, and the CFT, which measures Chlamydia antibodies.

Diagnosis of psittacosis involves microbiological cultures from respiratory secretions of patients or serologically with a fourfold or greater increase in antibody titers against C. psittaci in blood samples combined with the probable course of the disease. Typical inclusions called "Leventhal-Cole-Lillie bodies" can be seen within macrophages in BAL (Bronchial Alveolar Lavage) fluid. Culture of C.psittaci is hazardous and should only be carried out in biosafety laboratories.

Control. Attempts to use C. trachomatis vaccines for prophylaxis and treatment of trachoma have failed. The course of trachoma is more severe in immunized than in nonimmunized individuals. Specific anti-Chlamydia antibodies fail to neutralize chlamydial elementary bodies in vivo.

Tetracycline and erythromycin are the antibiotics commonly used to treat chlamydial infections in humans. Penicillin is not effective. Patients with trachoma have been treated effectively with erythromycin, rifampin, sulfonamides, chloramphenicol, and tetracyclines. Repeated treatment cycles of long-acting sulfonamides also have been used in local or systemic treatment of trachoma infections. In trachoma patients with trichiasis, corrective surgery is necessary. Patients with inclusion conjunctivitis usually are not treated, because the infection is self-limiting and relatively mild.

Tetracyclines or sulfonamides sometimes are effective in patients with lymphogranuloma venereum, but treatment does not always improve the condition. Tetracyclines and chloramphenicol are the drugs of choice for treating patients with psittacosis. Erythromycin probably is the best alternative agent for persons for whom tetracycline is contraindicated (e.g., children aged less than 9 years and pregnant women).

MYCOPLASMAS

Structure and Antigenic Types. Mycoplasmas are the smallest and simplest self-replicating bacteria. The mycoplasma cell contains the minimum set of organelles essential for growth and replication: a plasma membrane, ribosomes, and a genome consisting of a double-stranded circular DNA molecule (Fig. 3). There is an attachment organelle at the tip of filamentous M pneumoniae, M genitalium, and several other pathogenic mycoplasmas. Unlike all other prokaryotes, the mycoplasmas have no cell walls, and they are consequently placed in a separate class Mollicutes (mollis, soft; cutis, skin). The trivial term mollicutes is frequently used as a general term to describe any member of the class, replacing in this respect the older term mycoplasmas.

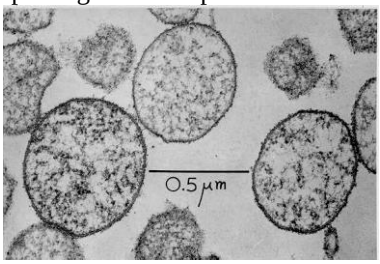

Fig. 3. Electron micrograph of thin-sectioned mycoplasma cells.

Mycoplasmas are spherical to filamentous cells with no cell walls. The coccus is the basic form of all mycoplasmas in culture. The diameter of the smallest coccus capable of reproduction is about 300 nm. In most mycoplasma cultures, elongated or filamentous forms (up to 100 μm long and about 0.4 μm thick) also occur. The filaments tend to produce truly branched mycelioid structures, hence the name mycoplasma (myces, a fungus; plasma, a form). Mycoplasmas reproduce by binary fission, but cytoplasmic division frequently may lag behind genome replication, resulting in formation of multinuclear filaments and multiple cell divisions to form coccoid cells (Fig. 4).

The lack of cell walls and intracytoplasmic membranes facilitates isolation of the mycoplasma membrane in a relatively pure form. The isolated mycoplasma membrane resembles that of other prokaryotes in being composed of approximately two-thirds protein and one-third lipid. The mycoplasma lipids

resemble those of other bacteria, apart from the large quantities of cholesterol in the sterol-requiring mycoplasmas.

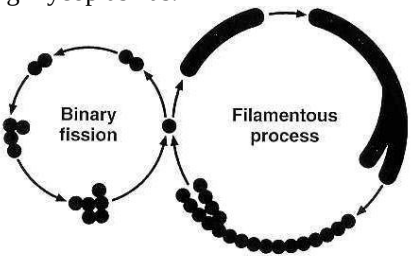

Fig. 4. Schematic presentation of the mode of mycoplasma reproduction.

Mycoplasmas have surface antigens such as membrane proteins, lipoproteins, glycolipids, and lipoglycans. Some of the membrane proteins undergo spontaneous antigenic variation. Antisera containing antibodies to these components inhibit growth and metabolism of the mycoplasmas and, in the presence of complement, cause lysis of the organisms. These properties are used in various serologic tests that differentiate between mycoplasma species and serotypes and detect antibodies to mycoplasmas in sera of patients.

Culture properties. The dependence of mycoplasmas on their host for many nutrients explains the great difficulty of cultivation in the laboratory. The complex media for mycoplasma culture contain serum, which provides fatty acids and cholesterol for mycoplasma membrane synthesis. The requirement of most mycoplasmas for cholesterol is unique among prokaryotes. The consensus is that only a small fraction of mycoplasmas existing in nature have been cultivated so far. Some of the cultivable mycoplasmas, including the human pathogen M. pneumoniae, grow very slowly, particularly on primary isolation. U. urealyticum, a pathogen of the human urogenital tract, grows very poorly in vitro, reaching maximal titers of 10^7 organisms/ml of culture. M.genitalium, another human pathogen, grows so poorly in vitro that only a few successful isolations have been achieved.

Glucose and other metabolizable carbohydrates can be used as energy sources by the fermentative mycoplasmas possessing the Embden-Meyerhof-Parnas glycolytic pathway. All mycoplasmas examined thus far possess a truncated, flavin-terminated respiratory system, which rules out oxidative phosphorylation as an ATP-generating mechanism. Breakdown of arginine by the arginine dihydrolase pathway has been proposed as a major source of ATP in nonfermentative mycoplasmas. Ureaplasmas have a requirement, unique among living organisms, for urea. Because they are non-glycolytic and lack the arginine dihydrolase pathway, it has been suggested, and later proven experimentally, that ATP is generated through an electrochemical gradient

produced by ammonia liberated during the intracellular hydrolysis of urea by the organism's urease.

One of the most useful distinguishing features of mycoplasmas is their peculiar fried-egg colony shape, consisting of a central zone of growth embedded in the agar and a peripheral one on the agar surface that is lighter in color (Fig. 5). Due to the slow growth of mycoplasmas, the colonies may take up to 3 weeks to develop and are usually very small. The colonies of Ureaplasma are extremely small and thus Ureaplasma are also called T-strains (tiny strains). The mycoplasmas require sterols for growth and for membrane synthesis. The three species can be differentiated by their ability to metabolize glucose (M. pneumoniae), arginine (M. hominis) or urea (U. urealyticum). The fourth species M. genitalium is extremely difficult to culture.

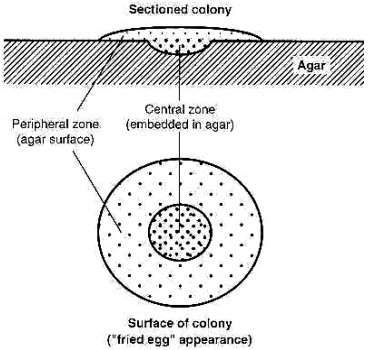

Fig. 5. Morphology of a typical "fried-egg" mycoplasma colony.

Virulence factors:

- Adherence factors are one of the major virulence factors. Adhesin localizes at tips of the bacterial cells and binds to sialic acid residues on host epithelial cells. Some mycoplasmas possess unique attachment organelles, which are shaped as a tapered tip in M. pneumoniae and M. genitalium. M. pneumoniae is a pathogen of the respiratory tract, adhering to the respiratory epithelium, primarily through the attachment organelle. Colonization of the respiratory tract by M. pneumoniae results in the cessation of ciliary movement. Interestingly, these two human mycoplasmas exhibit gliding motility on liquid-covered surfaces.
- Toxic Metabolic Products: generation of hydrogen peroxide and superoxide radicals by adhering mycoplasmas that oxidize host lipids in infected tissues, induce oxidative stress, including host cell membrane damage. Furthermore, the mycoplasmas have been shown to inhibit host cell catalase, thereby increasing the peroxide concentrations.

- Competition for and depletion of nutrients or biosynthetic precursors by mycoplasmas: disrupts host cell maintenance and function.
- Existence of capsule-like material and electron-dense surface layers or structures: provides increased integrity to the mycoplasma surface and confers immunoregulatory activites.
- Antigenic variation: results in surface diversity and possible avoidance of protective host immune defenses.
- Secretion or introduction of mycoplasmal enzymes: such as phospholipases, ATPases, hemolysins, proteases, and nucleases into the host cell milieu which leads to localized tissue disruption and disorganization and chromosomal aberrations.
- Intracellular residence: sequesters mycoplasmas, establishes latent or chronic states, and circumvents mycoplasmicidal immune mechanisms and selective drug therapies.
- Immunopathogenesis: Mycoplasmas can activate macrophages and stimulate cytokine production and lymphocyte activation (M. pneumoniae has a superantigen).

Epidemiology. Infection with M. pneumoniae occurs worldwide all year round but shows a predilection for the colder months, apparently because of the greater opportunity for transmission by droplet infection. M. pneumoniae appears to require close personal contact to spread; successful spreading usually occurs in families, schools, and institutions.

U. urealyticum, M.genitalium, M. hominis are spread primarily through sexual contact. Colonization has been linked to the frequency of sexual intercourse and the number of sexual partners. Women may be asymptomatic reservoirs of infection.

Pathogenesis. All mycoplasmas cultivated and identified thus far are parasites of humans, animals, plants, or arthropods. The primary habitats of human and animal mycoplasmas are the mucous surfaces of the respiratory and urogenital tracts and the joints in some animals. Although some mycoplasmas belong to the normal flora, many species are pathogens, causing various diseases that tend to run a chronic course.

Most mycoplasmas that infect humans and other animals are surface parasites, adhering to the epithelial linings of the respiratory and urogenital tracts. Adherence is firm enough to prevent the elimination of the parasites by mucous secretions or urine. The intimate association between the adhering mycoplasmas and their host cells provides an environment in which local concentrations of toxic metabolites excreted by the parasite build up and cause tissue damage. Moreover, because mycoplasmas lack cell walls, fusion between the membranes of the parasite and host has been suggested, and some experimental evidence for it has recently been obtained. Membrane fusion would alter the composition and permeability of the host cell

membrane and enable the introduction of the parasite's hydrolytic enzymes into the host cell, events expected to cause serious damage. Recent studies have indicated the presence in mycoplasmas of antigenic variability systems. These systems, some of which are already defined in molecular genetic terms, are responsible for rapid changes in major surface protein antigens. The change in the antigenic coat of the parasite helps it to escape recognition by the immune mechanisms of the host.

Toxins are rarely found in mycoplasmas. Consequently, researchers considered whether the end products of mycoplasma metabolism were responsible for tissue damage. Hydrogen peroxide, the end product of respiration in mycoplasmas, has been implicated as a major pathogenic factor ever since it was shown to be responsible for the lysis of erythrocytes by mycoplasmas in vitro; however, the production of H_2O_2 alone does not determine pathogenicity, as the loss of virulence in M. pneumoniae is not accompanied by a decrease in H_2O_2 production. For the H_2O_2 to exert its toxic effect, the mycoplasmas must adhere closely enough to the host cell surface to maintain a toxic, steady-state concentration of H_2O_2 sufficient to cause direct damage, such as lipid peroxidation, to the cell membrane. The accumulation of malonyldialdehyde, an oxidation product of membrane lipids, in cells exposed to M. pneumoniae supports this notion. Moreover, M. pneumoniae inhibits host cell catalase by excreting superoxide radicals (O^{2-}). This would be expected to further increase the accumulation of H_2O_2 at the site of parasite-host cell contact (Fig. 6).

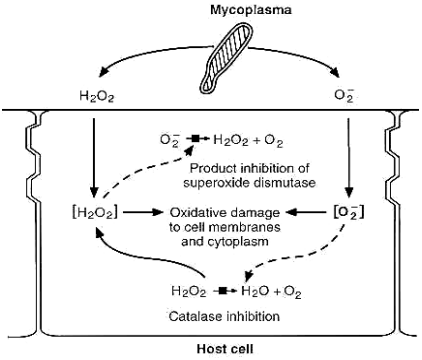

Fig. 6. Proposed mechanism of oxidative damage to host cells by adhering M. pneumoniae.

Recent research has found a receptor on the surface of M.pneumoniae thought to be integral in the attachment to the host cell surface. This receptor can attach to a number of different cell types such as respiratory tract epithelia and red blood cells. At high concentrations, M. pneumoniae can inhibit ciliary action within the respiratory tract, induce local inflammation that consists primarily of perivascular and peribronchial infiltration of mononuclear leukocytes, and as well as cause cell necrosis. This damage may be mediated by

liberation of peroxides from M. pneumoniae as well as indirectly from the host immune response. The organism also has the ability to exist intracellularly.

There is evidence that both organism-related and host-related factors are involved in the pathogenesis of mycoplasma infections. Mycoplasmas activate macrophages, and induce cytokine production and lymphocyte proliferation. Thus, in the case of M pneumoniae, the host may be largely responsible for the pneumonia by mounting a local immune response to the parasite. Epidemiologic data also suggest that repeated infections in humans are required before symptomatic disease occurs: serum antibodies to M. pneumoniae can be found in most children 2 to 5 years of age, although the illness occurs with greatest frequency in individuals 5 to 15 years of age.

An immunopathologic mechanism also may explain the complications affecting organs distant from the respiratory tract in some patients infected with M. pneumoniae. Various autoantibodies have been detected in the sera of many of these patients, including cold agglutinins reacting with the erythrocyte I antigen, and antibodies reacting with lymphocytes, smooth muscle cells, and brain and lung antigens. Serologic cross-reactions between M. pneumoniae and brain and lung antigens have been demonstrated, and these antigens are probably related to the glycolipids of M. pneumoniae membranes.

Clinical Presentation

M. pneumoniae remains an important cause of pneumonia and other airway disorders such as tracheobronchitis and pharyngitis. Approximately one third of infected persons will develop pneumonia which is usually mild but of long duration. Pneumonia caused by this agent has been referred to a 'primary atypical pneumonia' and 'walking pneumonia'. Generally, the incubation period is 2-3 weeks. The organism may persist in the respiratory tract for several months, and sometimes for years in patients who are immunosuppressed, after initial infection.

The term primary atypical pneumonia was coined in the early 1940s to describe pneumonias different from the typical lobar pneumonia caused by pneumococci. Several common respiratory viruses, including influenza virus and adenovirus, were shown to be responsible for a significant number of these pneumonias. From other cases, many of which developed antibodies agglutinating red blood cells in the cold (cold agglutinins), an unidentified filterable agent was isolated by Eaton and associates and was called Eaton agent. This agent was identified as a new Mycoplasma species after its successful cultivation on cell-free media in 1962. Named M. pneumoniae, it was the first clearly documented mycoplasma pathogenic for humans.

This organism is also associated with extrapulmonary manifestations such as hematopoietic, joint, central nervous system, liver, pancreas and cardiovascular syndromes.

M. genitalium could be involved in nongonococcal urethritis, genital infections, pelvic inflammatory disease. A DNA probe hybridization assay has

indicated that M.genitalium was present in urogenital specimens collected from 60% of male homosexual patients with recurrent or persistent nongonococcal urethritis and 22% of heterosexual men with recurrent urethritis, compared with 9% of men without urethritis.

U. urealyticum has been found at a colonization rate of 40 to 80% of sexually-active women. In some colonized pregnant women, ureaplasmas have been considered to be a cause of chorioamnionitis and premature delivery. They are frequently transmitted from mothers to their infants, and this may cause various diseases which include pneumonia, persistent pulmonary hypertension, chronic infection of the central nervous system and bronchopulmonary dysplasia.

M. fermentans, M. pirum, M. hominis, and M. penetrans have been proposed as human pathogens and possible cofactors in HIV infection. These organisms may contribute to the variation in the time from infection with HIV to the development of AIDS symptoms.

M. hominis is associated with pyelonephritis, pelvic inflammatory diseases such as tubo-ovarian abscess or salpingitis and post-partum fevers.

M. fermentans is considered to be a commensal in the human mucosal tissues and has often been found in saliva and oropharyngeal of 45% of healthy adults. Also, M. fermentans has been isolated from the human urogenital tract and are suspected of invading host tissues from a site of mucosal colonization.

Host Defenses. Infection with M. pneumoniae induces the development of serum antibodies that fix complement, inhibit growth of the organism and lyse the organism in the presence of complement. Generally, the first antibodies produced are of the IgM class, whereas later in convalescence the predominant antibody is IgG. Secretory IgA also develop and appear to be important in host resistance. The first infection in infancy usually is asymptomatic and generates a brief serum antibody response. Recurrent infections generate a more prolonged systemic antibody response and increasing numbers of circulating antigen-responsive lymphocytes. By late childhood, clinically apparent lower respiratory disease, including pneumonia, becomes more common. Therefore, mycoplasma respiratory disease manifestations appear to vary, depending on the state of local and systemic immunity at the time of reinfection. One hypothesis is that local immunity mediates resistance to infection and that systemic immunity contributes substantially to the pulmonary and systemic reaction characteristic of M. pneumoniae pneumonia. The development of delayed type hypersensitivity, however, is associated with the severity of the disease, which supports the suggestion that pathogenesis is at least, in part, immunopathogenesis.

Laboratory diagnosis.

Respiratory tract specimens suitable for culture include throat swabs, sputum, tracheal aspirates, bronchial lavage fluid, pleural fluid, or lung biopsy tissue, depending on the patient's clinical condition.

Urogenital tract specimen: scrapings from the urethra and genital organs.

Culture. A routine mycoplasma medium consists of heart infusion, peptone, yeast extract, salts, glucose or arginine, and horse serum (5 to 20 percent). Fetal or newborn calf serum is preferable to horse serum. To prevent the overgrowth of the fast-growing bacteria that usually accompany mycoplasmas in clinical materials, penicillin, thallium acetate or both are added as selective agents. For Ureaplasma culture, the medium is supplemented with urea and its pH is brought to 6.0. Ureaplasm and M. genitalium are relatively sensitive to thallium, which is, therefore, omitted from their culture media. For M.pneumoniae isolation, nasopharyngeal secretions are inoculated into a selective diphasic medium (pH 7.8) made of mycoplasma broth and agar and supplemented with glucose and phenol red. When M. pneumoniae grows in this medium, it produces acid, causing the color of the medium to change from purple to yellow. Broth from the diphasic medium is subcultured to mycoplasma agar when a color change occurs, or at weekly intervals for a minimum of 8 weeks.

Identification. Colonies appearing on the plates can be identified as M.pneumoniae by staining directly on agar with homologous fluorescein-conjugated antibody or by demonstrating that a specific antiserum to M.pneumoniae inhibits their growth on agar. Colonies of ureaplasmas are usually minute (less than 100 μm in diameter); because of urea hydrolysis and ammonia liberation, the medium becomes alkaline. When manganous sulfate is added to the medium, the ureaplasma colonies stain dark brown. Isolates can be characterized in more detail by a variety of biochemical and serologic tests. More sophisticated tests, including electrophoretic analysis of cell proteins, DNA-DNA hybridization tests, and PCR tests employing species-specific primers for amplification, may be performed in a research laboratory.

It may take 2 -3 weeks to get a positive identification. Culture is essential for a definitive diagnosis.

Serodiagnosis consists of examining serum samples for antibodies that inhibit the growth and metabolism of the organism or fix mycoplasmal antigens. A fourfold or greater rise in IgG titer is considered indicative of recent infection, whereas a sustained high antibody titer may not be significant, because a relatively high level of antibody may persist for at least 1 year after infection.

a. *Complement fixation test* has good sensitivity and specificity. However, the titers do not peak until 4-6 weeks after infection. A fourfold rise in titer is indicative of a recent infection. Since antibodies may persist for up to 1 year, a sustained high titer does not necessarily indicate a current infection.

b. *Cold agglutinins* – Approximately 34%-68% of patients with M.pneumoniae infection develop cold agglutinins. Cold agglutinins are antibodies that agglutinate human erythrocytes at 4^0C but not at 37^0C. These antibodies arise before the complement fixing antibodies and they decline faster. Cold agglutinins are not specific for M. pneumoniae infections, they can

also appear in other infections and in other diseases (e.g. Infectious mononucleosis, influenza infections, cold agglutinin disease, leukemia). However, if present in a patient with clinical signs of M. pneumoniae infection, a presumptive diagnosis can be made.

c. *ELISA* – There is a new ELISA for IgM that has been used for diagnosis of acute infection. It is sensitive and specific.

Definitive diagnosis requires seroconversion documented by paired specimens obtained 2-4 weeks apart. Although researchers purport that single-titer IgM or IgA assays reveal current infection, data regarding how long IgM persists after acute infection are not clear, and as many as 50% of adults may not mount a detectable IgM response. Conversely, some children may not mount a measurable IgG response, and the IgG response in adults may be delayed for several days. Therefore, relying on a single serological test can be clinically misleading, and experts recommend basing diagnosis of acute infection on seroconversion measured simultaneously in assays for both IgM and IgG.

Molecular analysis. PCR for detection of mycoplasma genomes is still the gold standard. However, confirmation of PCR should be done by southern blot and molecular probes in order to decrease the rate of false positivity and improve false negativity. Antibodies (IgG, IgM and IgA) against peptide-specific mycoplasma should be performed simultaneously.

Carriage of mycoplasmas in the upper respiratory tract for variable periods following prior infection may confound the interpretation of a single positive polymerase chain reaction assay result. Furthermore, a polymerase chain reaction assay may reveal very small numbers of organisms that may not be of etiologic significance. A specific threshold of quantity of mycoplasmas in the respiratory tract that can differentiate colonization from infection has not been established, so a highly sensitive detection method such as the polymerase chain reaction performed in a nonquantitative manner may overestimate the clinical importance of M. pneumoniae as a pathogen since it often cocirculates with other bacterial and viral respiratory pathogens. For these reasons, molecular-based assays should be accompanied by serological assays for maximum diagnostic accuracy unless testing a normally sterile body fluid in which the presence of any number of mycoplasmas would be considered evidence of disease.

As no diagnostic tool is 100% accurate, one suggests that PCR, molecular probe, and IgG, IgM, and IgA antibodies should all be performed to gain the most accurate result.

Prevention. Chemoprophylaxis of mycoplasma infections is not recommended, and no vaccine is available. Prior natural infection appears to provide the most effective resistance; however, evidence shows that M.pneumoniae infections recur at intervals of several years. These observations suggest that immunity to a single natural infection is relatively short-term.

Treatment. The mycoplasmas are sensitive to tetracyclines, macrolides, and the newer quinolones, but are resistant to antibiotics that specifically inhibit

bacterial cell wall synthesis. Tetracycline or erythromycin is recommended for treatment of M. pneumoniae pneumonia, although effective treatment of the symptoms usually is not accompanied by eradication of the organism from the infected host. To prevent recurrence of nongonococcal urethritis caused by U.urealyticum, sexual partners should be treated simultaneously with tetracycline. The incidence of tetracycline-resistant strains of U. urealyticum and M. hominis is on the rise.

Practical tasks, being carried out during practical classes:

1. Studying of microslides: C.trachomatis (stained after Giemsa), M.pneumoniae (stained after Giemsa).
2. Studying of the scheme of laboratory diagnosis of chlamydiosis and mycoplasmosis.

Therminology: Chlamydiaceae, Chlamydia trachomatis, Chlamydophila psittaci, Chlamydophila pneumoniae, trachoma, nongonoccocal urethritis, lymphogranuloma venereum, psittacosis, Mycoplasmataceae, M. pneumoniae, M.genitalium, M. fermentans, M. hominis, M. penetrans, U. urealyticum.

Theoretical questions for control:

1. Classification and characteristic of Chlamydia and mycoplasmas.
2. Morphology of chlamydia. Life cycles.
3. Routes of transmission and pathogenesis of chlamydiosis and mycoplasmosis.
4. Laboratory diagnosis of chlamydiosis and mycoplasmosis.
5. Immunity against chlamydiae and mycoplasmas.
6. Therapy and prophylaxis of chlamydiosis and mycoplasmosis.

Test tasks for control:

1. Mycoplasmas differ from chlamydiae by which of the following characteristics?

A. Susceptibility to penicillin.
B. Elementary body evolves in reticular body.
C. Ability to cause urinary tract infection.
D. Lack of a true bacterial cell wall.
E. All of the above.

2. Which of the following members of the mycoplasma group have been implicated as a cause of nongonococcal urethritis (NGU)?

A. M.hominis. *C. M.fermentans.* *E. M.mycoides.*
B. C.psittacci. *D. U.urealyticum.*

3. Which of the following microorganisms is the causative agent of nongonococcal urethritis?

A. Giardia lamblia. *C. C.trachomatis D-K.* *E. C. psittacci.*
B. C. trachomatis L_{1-3}. *D. M. pneumoniae.*

4. A patient was diagnosed Reiter's syndrome. Which of the following diseases is characterized with this syndrome?

A. S. aureus. *C. C. trachomatis.* *E. C. albicans.*
B. M. tuberculosis. *D. C. diphtheriae.*

5. Which of the following test-systems is used to cultivate Chlamydia?

A. Blood agar. *C. Medium 199.* *E. Serum broth.*
B. Tissue culture. *D. Serum agar.*

6. A man with chills, fever, and headache is thought to have "atypical" pneumonia. History reveals that he raises chickens and that approximately 2 weeks ago he lost a large number of them to an undiagnosed disease. The most likely diagnosis of this man's condition is:

A. Anthrax. *C. Q fever.* *E. Psittacosis.*
B. Relapsing fever. *D. Leptospirosis.*

7. The mycoplasmas are distinguished from true bacteria by their lack of:

A. A cell wall. *C. ATP synthesis.* *E. A capsule.*
B. Flagella. *D. Lipopolysaccharide.*

8. Which host defense system is markedly impaired by Mycoplasma pneumoniae?

A. Neutrophil chemotaxis. *D. Ciliary function.*
B. Phagocytosis. *E. Intracellular killing.*
C. Secretory antibody.

9. Mycoplasmas are bacterial cells that:

A. Reproduce on artificial media. *D. Stain well with Giemsa's stain.*
B. Have not a rigid cell wall. *E. All of the above.*
C. Are resistant to penicillin.

Answers to test: 1 – B; **2** – D; **3** – C; **4** – C; **5** – B; **6** – E; **7** – A; **8** – D; **9** – E.

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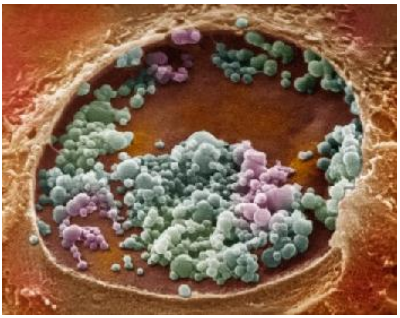

CHLAMYDIA. MYCOPLASMAS

Methodical instructions on subject "Microbiology, virology and immunology" for the II and III year English media students of medical and dentistry faculties

ХЛАМИДИИ. МИКОПЛАЗМЫ

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