МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ УКРАИНЫ
Харьковский национальный медицинский университет

PATHOGENIC CLOSTRIDIA

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)

ПАТОГЕННЫЕ КЛОСТРИДИИ

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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Составитель Н. И. Коваленко

Learning guide is related to the program of Ministry of Health of Ukraine and is recommended to students of medical and dentistry faculties of high medical schools of III-IV level accreditation.

Learning guide includes sections of morphology, culture, biochemical properties and toxin production of pathogenic representatives of Clostridia. The most modern information on pathogenesis, methods of laboratory diagnosis and specific prophylaxis is represented.

Theme: Microbiological diagnosis of diseases caused by clostridia

Actuality of the theme.

Goal: Studying of laboratory diagnosis of clostidial infections.

Concrete goals:

1. Study of biological properties and classification of clostridia.
2. Study pathogenesis and clinical manifestations of tetanus, gas gangrene, botulism, C. perfringens food poisoning, pseudomembranous enterocolitis.
3. Study of the methods of laboratory diagnosis of clostridial infections.
4. Study of specific prophylaxis and therapy of tetanus and botulism.

Students should be able to:

1. Differentiate of pathogenic clostridia on biochemical and antigenic properties.
2. Isolate pure cultures of clostridia and examine growth of clostridia on Kitt-Tarrozzi medium and blood agar.
3. Identify of isolated pure culture of clostridia for morphology, culture and biochemical properties, antigenic structure.
4. Perform neutralization test to diagnosis of tetanus and botulism.

Equipment: slides, immersion microscope, culture of clostridia on Kitt-Tarrozzi medium and blood agar, anaerobic jar, basic dyes, inoculating loops, biological preparations for laboratory diagnosis, specific prophylaxis and therapy of anaerobic infection, tables, atlas.

GENERAL CHARACTERISTICS OF ANAEROBIOSIS

The clinical importance of the anaerobic organisms, especially the toxicogenic Clostridia and some of the nonsporulating anaerobes, has been recognized for some time. Only within the last 20 years, however, owing to improved methodology, Gram-negative anaerobic bacilli, anaerobic cocci, and streptococci have been commonly recognized and encountered in clinical infections. Today, anaerobic organisms are common isolates from infections involving intra-abdominal sites, the female genital tract, soft tissue, and oral areas and from major infections involving the lung, brain, and head and neck. Because anaerobes are prevalent normal flora of the body, almost all anaerobic infections are of endogenous origin. Many of these anaerobes are opportunists; given the appropriate set of conditions, they will penetrate tissue and cause infection. Many have been associated with wound infection subsequent to bowel surgery or trauma, tubo-ovarian abscess, perirectal abscess, subphrenic abscess, postabortal sepsis, appendicitis, and many other infectious conditions.

The broad classification of bacteria as anaerobic, aerobic, or facultative is based on the types of reactions they employ to generate energy for growth and other activities. In their metabolism of energy-containing compounds, aerobes require molecular oxygen as a terminal electron acceptor and cannot grow in its

absence. Anaerobes, on the other hand, cannot grow in the presence of oxygen. Oxygen is toxic for them, and they must therefore depend on other substances as electron acceptors. Their metabolism frequently is a fermentative type in which they reduce available organic compounds to various end products such as organic acids and alcohols. Understanding the general characteristics of anaerobiosis provides insight into how anaerobic bacteria can proliferate in damaged tissue and why special care is needed in processing clinical specimens that may contain them.

Oxygen toxicity. Several studies indicate that aerobes can survive in the presence of oxygen only by virtue of an elaborate system of defenses. Without these defenses, key enzyme systems in the organisms fail to function and the organisms die. Obligate anaerobes, which live only in the absence of oxygen, do not possess the defenses that make aerobic life possible and therefore cannot survive in air.

During growth and metabolism, oxygen reduction products are generated within microorganisms and secreted into the surrounding medium. The superoxide anion O_2, one oxygen reduction product, is produced by univalent reduction of oxygen.

It is generated during the interaction of molecular oxygen with various cellular constituents, including reduced flavins, flavoproteins, quinones, thiols, and iron-sulfur proteins. The exact process by which it causes intracellular damage is not known; however, it is capable of participating in a number of destructive reactions potentially lethal to the cell. Moreover, products of secondary reactions may amplify toxicity. For example, one hypothesis holds that the superoxide anion reacts with hydrogen peroxide in the cell:

$O_2^- + H_2O_2 \rightarrow OH^- + OH^- + O_2$

This reaction, known as the Haber-Weiss reaction, generates a free hydroxyl radical (OH), which is the most potent biologic oxidant known. It can attack virtually any organic substance in the cell. A subsequent reaction between the superoxide anion and the hydroxyl radical produces singlet oxygen (O_2^*), which is also damaging to the cell: $O_2^- + OH^- \rightarrow OH^- + O_2^{*\cdot}$

The excited singlet oxygen molecule is very reactive. Therefore, superoxide must be removed for the cells to survive in the presence of oxygen.

Most facultative and aerobic organisms contain a high concentration of an enzyme called superoxide dismutase. This enzyme converts the superoxide anion into ground-state oxygen and hydrogen peroxide, thus ridding the cell of destructive superoxide anions: $2O_2 + 2H^+ \underline{\text{Superoxide Dismutase}} \rightarrow O_2 + H_2O_2$

The hydrogen peroxide generated in this reaction is an oxidizing agent, but it does not damage the cell as much as the superoxide anion and tends to diffuse out of the cell. Many organisms possess catalase or peroxidase or both to eliminate the H_2O_2. Catalase uses H_2O_2 as an oxidant (electron acceptor) and a

reductant (electron donor) to convert peroxide into water and ground-state oxygen: $H_2O_2 + H_2O_2 \underline{\text{Catalase}} \rightarrow 2H_2O + O_2$

Peroxidase uses a reductant other than H_2O_2: $H_2O_2 + H_2R \underline{\text{Peroxidase}} \rightarrow 2H_2O + R$

The most oxygen-sensitive anaerobes as a rule contained little or no superoxide dismutase. In addition to the activity of superoxide dismutase, the rate at which an organism takes up and reduces oxygen was determined to be a factor in oxygen tolerance. Very sensitive anaerobes, which reduced relatively large quantities of oxygen and exhibited no superoxide dismutase activity, were killed after short exposure to oxygen. More tolerant organisms reduced very little oxygen or else demonstrated high levels of superoxide dismutase activity.

The continuous spectrum of oxygen tolerance among bacteria appears to be due partly to the activities of superoxide dismutase, catalase, and peroxidase in the cell and partly to the rate at which the cell takes up oxygen (*Fig. 1*).

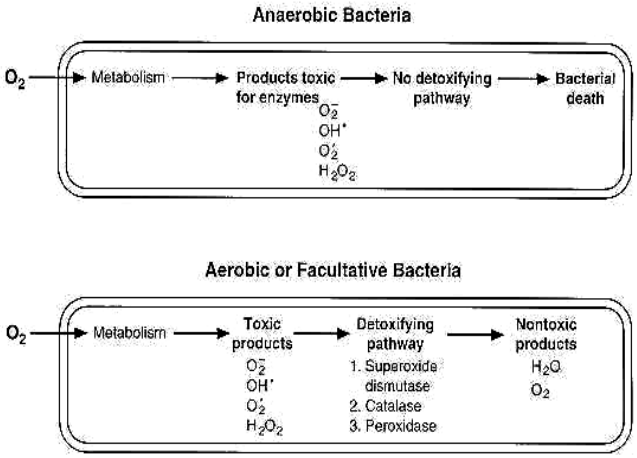

Fig. 1. Effects of oxygen on aerobic, anaerobic, and facultative anaerobic bacteria

Processing of Clinical Specimens. When collecting specimens from patients for isolation and identification of anaerobic bacteria associated with infections, precautions must be taken to exclude air (*Fig. 2*). Materials for anaerobic culture are best obtained with a needle and syringe. Unless the specimen can be sent to the laboratory immediately, it is placed in an anaerobic transport tube containing oxygen-free carbon dioxide or nitrogen. The specimen is injected through the rubber stopper in the transport tube and remains in the anaerobic environment of the tube until processed in the bacteriology laboratory. If the specimen is collected with a swab, only a special commercially available anaerobic swab transport system is used.

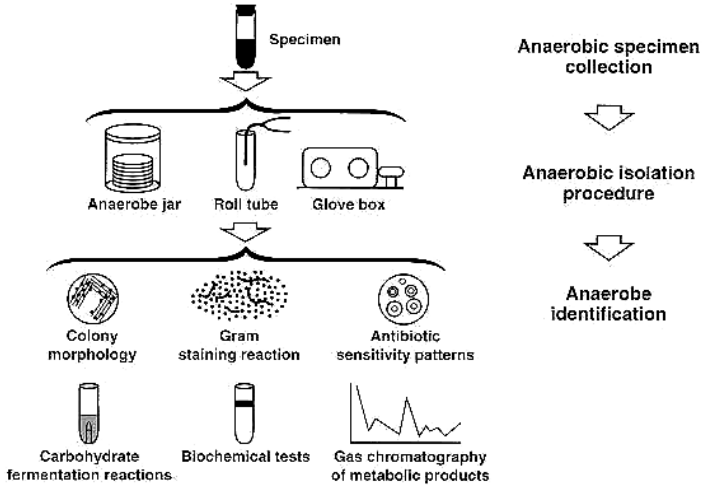

Fig. 2. Isolation and identification of anaerobes

Specimens should be free of contaminating bacteria. Material from sites that are normally sterile, such as blood, spinal fluid, or pleural fluid, poses no problem provided the usual precautions are taken to decontaminate the skin properly before puncturing it to obtain the specimen. Fecal specimens, sputum specimens, or vaginal secretions cannot be cultured routinely for pathogenic anaerobes because they normally contain other anaerobic organisms. Aspirates from abscesses or the specific sites of infections must be obtained in these cases to avoid undue contamination with indigenous flora components.

Although several techniques are available for maintaining an oxygen-free environment during the processing of specimens for anaerobic culture, the anaerobic jar is the most common (*Fig. 3*). It is a medium-sized glass or plastic jar with a tightly fitting lid containing palladium-coated alumina particles, which serve as a catalyst. It can be set up by two methods. The easiest uses a commercially available hydrogen and carbon dioxide generator envelope (GasPak) that is placed in the jar along with the culture plates. The generator is activated with water. Oxygen within the jar and the hydrogen that is generated are converted to water in the presence of the catalyst, thus producing anaerobic conditions. Carbon dioxide, which is also generated, is required for growth by some anaerobes and stimulates the growth of others. An alternative method for achieving anaerobiosis in the jar consists of evacuation and replacement. Air is evacuated from the sealed jar containing the culture plates and is replaced with an oxygen-free mixture of 80 % nitrogen, 10 % hydrogen, and 10 % carbon dioxide.

Fig. 3. Anaerobic jar

The anaerobic glove box isolator is innovation developed for isolating anaerobic bacteria (*Fig. 4*). It is essentially a large clear-vinyl chamber, with attached gloves, containing a mixture of 80 % nitrogen, 10 % hydrogen, and 10 % carbon dioxide. A lock at one end of the chamber is fitted with two hatches, one leading to the outside and the other to the inside of the chamber. Specimens are placed in the lock, the outside hatch is closed, and the air in the lock is evacuated and replaced with the gas mixture. The inside hatch is then opened to introduce the specimen into the chamber. Conventional bacteriologic procedures are employed to process the specimen in the oxygen-free atmosphere.

Fig. 4. Anaerobic glove box

A number of methods are described for achieving anaerobiosis on the basis of following principles:

- exclusion of oxygen;
- production of vacuum;
- displacement of oxygen with other gases (H2);
- absorption of oxygen by chemical or biological means;
- reduction of oxygen.

Absorption of oxygen by chemical or biological means:

- Pyrogallic acid and sodium hydroxide.
- Chromium and sulfuric acid.

Reduction of oxygen in medium is achieved by using various reducing agents: 1 % glucose; 0.1 % thyoglycollate; 0.1 % ascorbic acid; 0.05 % cyctine; broth containing fresh animal tissue e.g. rabbit kidney, spleen (Kitt-Tarozzi medium) + glucose.

There are two methods of isolations of anaerobes in pure culture: Zeissler's and Weinsberg's methods. In accordance to Zeissler's method specimens are cultured on Kitt–Tarozzi medium then transfer in blood agar (Petri dishes with blood agar are placed into anaerobic jar) to obtain isolated colonies (examine them) and seed desired colony in Kitt-Tarozzi medium. In accordance to Weinsberg's method isolated colonies are obtained by seeding in special test tubes (Wenyal-Wiyonn). Culturing anaerobes in deep agar or in special tubes filled with meat-peptone agar and sealed at the ends. The air is removed by boiling prior to seeding and to inhibit the subsequent entry of air, the medium is covered with a layer of oil 0.5–1 cm thick.

Procedures for cultivation and identification of anaerobic bacteria are well established (*Fig. 2*). A variety of selective and nonselective media is available for cultivation of anaerobes. Usual bacteriologic procedures are used to identify anaerobes. These are based on Gram-staining reactions, cellular and colony morphology, antibiotic sensitivity patterns, carbohydrate fermentation reactions, and other biochemical tests. Analysis of metabolic end products, especially organic acids, provides additional information useful in classifying these organisms.

Clostridia: Sporeforming Anaerobic Bacilli

Clostridia are strictly anaerobic to aerotolerant sporeforming bacilli found in soil as well as in normal intestinal flora of man and animals. Exotoxin(s) play an important role in disease pathogenesis.

Of the anaerobes that infect humans, the clostridia are the most widely studied. They are involved in a variety of human diseases, the most important of which are gas gangrene, tetanus, botulism, pseudomembranous colitis and food poisoning. In most cases, clostridia are opportunistic pathogens; that is, one or more species establishes a nidus of infection in a particular site in a compromised host. All pathogenic clostridial species produce protein exotoxins that play an important role in pathogenesis.

Many clostridia are transient or permanent members of the normal flora of the human skin and the gastrointestinal tracts of humans and animals. Unlike typical members of the human bacterial flora, most clostridia can also be found worldwide in the soil.

Gas Gangrene

Structure. The clostridia that cause gas gangrene are anaerobic, spore-forming bacilli. C perfringens, C novyi, C histolyticum, C septicum are pleomorphic, plump, Gram-positive bacilli with straight, parallel sides, rounded or truncated

ends about 4 μ×6 μm. They may occur singly or in chains. Spores are central or subterminal. Spores are wider than bacillary bodies. C novyi, C histolyticum and C septicum are motile with peritrichous flagella, c perfringens is not motile, but it produces capcule.

Classification and Antigenic Types. Clostridial wound infections usually are polymicrobic because the source of wound contamination (feces, soil) is polymicrobic. In gas gangrene and anaerobic cellulitis, the primary pathogen can be any one of various clostridial species including *C perfringens* (80 %), *C novyi* (40 %), *C septicum* (20 %), and, occasionally, *C bifermentans, C histolyticum*, or *C fallax*. Other bacterial isolates may be any of a wide number and variety of organisms (for example, *Proteus, Bacillus, Escherichia, Bacteroides, Staphylococcus*).

The most frequently isolated pathogen, *C perfringens*, has five types, designated A, B, C, D, and E. Each of these types produces a semi-unique spectrum of protein toxins. Alpha-toxin (a lecithinase, also called phospholipase-C) and theta-toxin (oxygen-labile cytolysin) are both considered important in the disease pathology. Alpha-toxin is lethal and necrotizing; it lyses cell membrane lecithins, disrupting cell membranes and causing cell death. Theta-toxin also contributes to rapid tissue destruction by several mechanisms. At the site of infection, theta-toxin acts as a cytolysin, promoting direct vascular injury; lower toxin concentrations activate polymorphonuclear leukocytes and endothelial cells, promoting distal vascular injury by stimulating leukocyte adherence to the endothelium. The result is leukostasis, thrombosis, decreased perfusion, and tissue hypoxia. Theta-toxin also mediates the production of shock through induction of inflammatory mediators such as platelet activating factor, tumor necrosis factor, interleukin 1 and interleukin 6.

Epidemiology. Clostridial spores are ubiquitous in the soil, on human skin, and in the gastrointestinal tracts of humans and animals. Thus, the causative agents of clostridial wound infections are not environmentally restricted. Even operating theaters can be habitats for infecting clostridial organisms and spores.

All clostridial wound infections occur in an anaerobic tissue environment caused by an impaired blood supply secondary to trauma, surgery, foreign bodies, or malignancy. Contamination of the wound by clostridia from the external environment or from the host's normal flora produces the infection.

Pathogenesis. Gas gangrene is an acute disease with a poor prognosis and often fatal outcome (*Fig. 5*). Initial trauma to host tissue damages muscle and impairs blood supply. This lack of oxygenation causes the oxidation-reduction potential to decrease and allows the growth of anaerobic clostridia. Initial symptoms are generalized fever and pain in the infected tissue. As the clostridia multiply, various exotoxins (including hemolysins, collagenases, proteases, and lipases) are liberated into the surrounding tissue, causing more local tissue necrosis and systemic toxemia. Infected muscle is discolored (purple mottling)

and edematous and produces a foul-smelling exudate; gas bubbles form from the products of anaerobic fermentation. As capillary permeability increases, the accumulation of fluid increases, and venous return eventually is curtailed. As more tissue becomes involved, the clostridia multiply within the increasing area of dead tissue, releasing more toxins into the local tissue and the systemic circulation. Because ischemia plays a significant role in the pathogenesis of gas gangrene, the muscle groups most frequently involved are those in the extremities served by one or two major blood vessels.

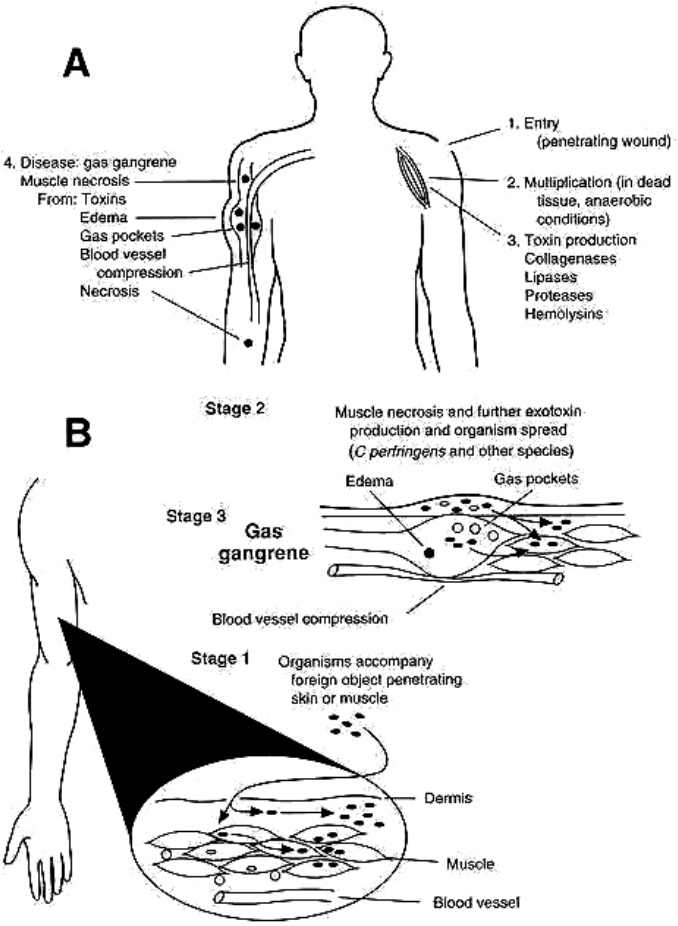

Fig. 5. Pathogenesis of gas gangrene caused by C perfringens.
A = macroscopic, B = microscopic

Clinical Manifestations. Clostridial wound infections may be divided into three categories: gas gangrene or clostridial myonecrosis, anaerobic cellulitis, and superficial contamination. Gas gangrene can have a rapidly fatal outcome and requires prompt, often severe, treatment. The more common clostridial wound infections are much less acute and require much less radical treatment; however, they may share some characteristics with gas gangrene and must be included in the differential diagnosis.

Clostridial septicemia, although rare, may occur in the late stages of the disease. Severe shock with massive hemolysis and renal failure is usually the ultimate cause of death. The incubation period, from the time of wounding until the establishing of gas gangrene, varies with the infecting clostridial species from 1 to 6 days, but it may be as long as 6 weeks. Average incubation times for the three most prevalent infecting organisms are as follows: *C perfringens*, 10–48 hours; *C septicum*, 2–3 days; and *C novyi*, 5–6 days. Because the organisms need time to establish a nidus of infection, the time lag between wounding and the appropriate medical treatment is a significant factor in the initiation of gas gangrene.

Like gas gangrene, clostridial cellulitis is an infection of muscle tissue, but here the infecting organisms invade only tissue that is already dead; the infection does not spread to healthy, undamaged tissue. Clostridial cellulitis has a more gradual onset than gas gangrene and does not include the systemic toxemia associated with gas gangrene. Pain is minimal, and although only dead tissue is infected, the disease can spread along the planes between muscle groups, causing the surrounding tissue to appear more affected than it actually is. Anaerobic cellulitis may cause formation of many gas bubbles, producing infected tissue that looks similar to the gaseous tissue of gas gangrene. Some tissue necrosis does occur, but it is caused by decreased blood supply and not invasion by the infecting organism. With adequate treatment, anaerobic cellulitis has a good prognosis.

Superficial contamination, the least serious of the clostridial wound infections, involves infection of only necrotic tissue. Usually, the patient experiences little pain, and the process of wound healing proceeds normally; however, occasionally an exudate may form and the infection may interfere with wound healing. Superficial wound contamination caused by clostridia usually involves *C perfringens*, with staphylococci or streptococci, or both, as frequent co-isolates.

Host defenses against gas gangrene and other clostridial wound infections are mostly ineffective. Even repeated episodes of clostridial wound infection do not seem to produce effective immunity.

Diagnosis of clostridial wound infections is based on clinical symptoms coupled with Gram stains and bacterial culture of clinical specimens. Gas gangrene, once initiated, may spread and cause death within hours. By the time the typical lesions of gas gangrene are evident, the disease usually is firmly

established and the physician must treat the patient on a clinical basis without waiting for laboratory confirmation. Characteristic lesions and the presence of large numbers of Gram-positive bacilli (with or without spores) in a wound exudate provide strong presumptive evidence. Spores are rare in cultures of C perfringens, the most common etiologic agent of these diseases. A commonly used laboratory test for presumptive identification of C perfringens is the Nagler reaction which detects the presence of alpha-toxin (phospholipase-C). Nagler reaction is demonstrated on egg-yolk agar. C. perfringens phospholipase causes turbidity around the colonies on egg-yolk medium which may be inhibited by specific antiserum.

Control. Correction of the anaerobic conditions combined with antibiotic treatment form the basis for therapy. Penicillin is the drug of choice for all clostridial wound infections; chloramphenicol is a second-choice antibiotic. Treatment of gas gangrene includes radical surgical debridement coupled with high doses of antibiotics. Blood transfusions and supportive therapy for shock and renal failure also may be indicated.

The usefulness of gas gangrene antitoxin is currently a disputed matter. Some physicians maintain that the efficacy of this polyvalent antitoxin has been proved in the past, but better medical care now may have eliminated the need for its use. Others believe that because of insufficient data, antitoxin should be administered systemically as early as possible after diagnosis, and that the antitoxin should be injected locally into tissue that cannot be excised.

Obviously, prevention of wound contamination is the single most important factor in controlling clostridial wound infections.

Tetanus

Structure and Classification. *C tetani* is an anaerobic Gram-positive rod that forms spherical terminal and bulging spore, giving it a characteristic drumstick appearance (*Fig. 6*). C. tetani is slender, long slightly curved, 4.8 μ×0.5 μm and occurring singly or chain. Some strains do not sporulate readily, and spores may not appear until the third or fourth day of culture. Most strains are motile with peritrichous flagella; colonies often swarm on agar plates.

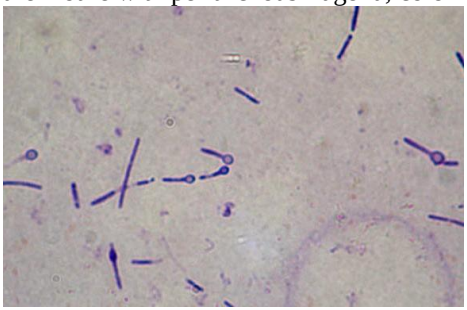

Fig. 6. Morphology of C tetani

The presence of *C tetani* should be suspected on isolation of a swarming rod that produces indole and has terminal spherical spores, but does not produce acid from glucose. Toxigenic *C tetani* contains a plasmid that produces a toxin called tetanospasmin, but nontoxigenic strains also exist. Tetanospasmin is responsible for the infamous toxemia called tetanus. The two animal species most susceptible to this toxemia are horses and humans.

Epidemiology. *C tetani* can be isolated from the soil in almost every environment throughout the world. The organism can be found in the gastrointestinal flora of humans, horses, and other animals. Isolation of *C tetani* from the intestinal flora of horses, coupled with the high frequency of equine tetanus, led to the erroneous assumption that the horse was the animal reservoir of *C tetani*.

Generalized outbreaks of tetanus do not occur, but certain populations can be considered at risk. Umbilical tetanus (tetanus neonatorum) usually is a generalized, fulminating, fatal disease that occurs with the neonates of unimmunized mothers who have given birth under unsanitary conditions. One million cases of tetanus occur annually in the world. In some less developed countries, tetanus is still one of the ten leading causes of death, and neonatal tetanus accounts for approximately one-half of the cases worldwide. In less developed countries, approximate mortality rates remain 85 % for neonatal tetanus and 50 % for nonneonatal tetanus. This is an unfortunate situation because with adequate immunization, tetanus is a completely preventable disease.

Pathogenesis. As with all clostridial wound infections, the initial event in tetanus is trauma to host tissue, followed by accidental contamination of the wound with *C tetani* (*Fig. 7*). Tissue damage is needed to lower the oxidation-reduction potential and provide an environment suitable for anaerobic growth. Once growth is initiated, the organism itself is not invasive and remains confined to the necrotic tissue, where the vegetative cells of *C tetani* elaborate the lethal toxin. The incubation period from the time of wounding to the appearance of symptoms varies from a few days to several weeks, depending on the infectious dose and the site of the wound (the more peripheral the wound, the longer the incubation time).

Tetanus can be initiated in two different ways, resulting in either generalized or local tetanus. In generalized tetanus (also called descending tetanus), all of the toxin cannot be absorbed by local nerve endings; therefore, it passes into the blood and lymph with subsequent absorption by motor nerves. The clinical pattern of generalized tetanus consists of severe painful spasms and rigidity of the voluntary muscles. The characteristic symptom of "lockjaw" involves spasms of the masseter muscle. It is an early symptom which is followed by progressive rigidity and violent spasms of the trunk and limb muscles. Spasms of the pharyngeal muscles cause difficulty in swallowing.

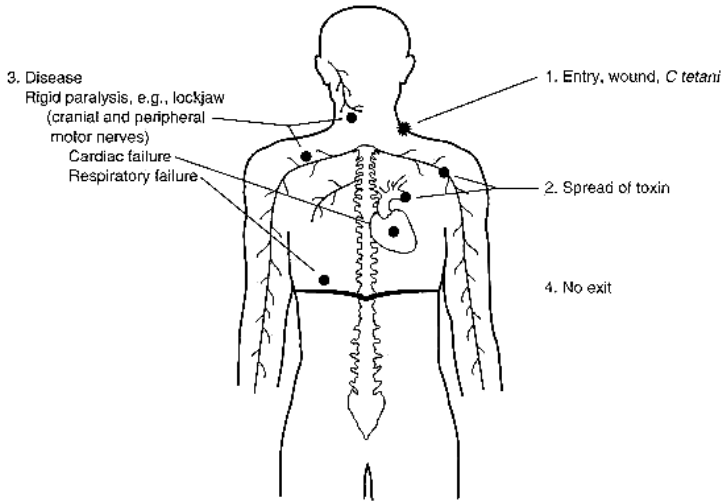

Fig. 7. Pathogenesis of tetanus caused by C tetani

As the disease progresses, the spasms increase in severity, becoming very painful and exhausting. During spasms, the upper airway can become obstructed, resulting in respiratory failure. Spasms often are initiated by environmental stimuli that may be as insignificant as the flash of a light or the sound of a footstep. In the localized form of tetanus (also called ascending tetanus), toxins travel along the neural route (peripheral nerves), causing a disease confined to the extremities and seen most often in inadequately immunized persons. Localized tetanus may last for months but usually resolves spontaneously. Another unusual form of tetanus is called cephalic tetanus which results from head wounds and affects the face, most commonly the muscles innervated by lower cranial nerves. Neonatal tetanus is seen in newborns when the mother lacks immunity and the umbilical stump becomes contaminated with *C tetani* spores.

Toxin action. *C tetani* actually produces two toxins: tetanolysin, a hemolysin that is inactivated by cholesterol and has no role in pathogenesis, and tetanospasmin, a spasmogenic toxin responsible for the classical symptoms of the disease.

Tetanus toxin is one of the three most poisonous substances known, the other two being the toxins of botulism and diphtheria. The toxin is produced by growing cells and released only on cell lysis. Cells lyse naturally during germination the outgrowth of spores, as well as during vegetative growth. After inoculation of a wound with C. tetani spores, only a minimal amount of spore germination and vegetative cell growth are required until the toxin is produced. The toxin is heat labile, being destroyed at 56 °C in 5 minutes, and is O_2 labile. The purified toxin rapidly converts to toxoid at 0^0C in the presence of formalin.

Because the toxin has a specific affinity for nervous tissue, it is referred to as a neurotoxin. The actions of tetanospasmin are complex and involve three components of the nervous system: central motor control, autonomic function, and the neuromuscular junction. Toxin enters the nervous system primarily through the neuromuscular junction of alpha motor neurons. The toxin is then transported to the other neurons, most importantly presynaptic inhibitory cells, where it is no longer accessible to be neutralized by antitoxin. The toxin also spreads hematogenously, but it still must enter the central nervous system via retrograde transport from peripheral neuronal processes. Once the toxin gains access to inhibitory neurons, it blocks the release of the neurotransmitters glycine and gamma-aminobutyric acid. The absence of this inhibition permits the simultaneous spasms of both agonist and antagonist muscles, producing muscle rigidity and convulsions. Tetanospasmin also acts on the autonomic nervous system and is associated with elevated plasma catacholamine levels; respiratory failure is a frequent complication of the disease. Peripherally, there is a failure of transmission at the neuromuscular junction, involving defective release of acetylcholine. Tetanospasmin may be as potent as the toxin of *C botulinum*; as little as 130 μg constitutes a lethal dose for humans. In untreated tetanus, the fatality rate is 90 % for the newborn and 40% for adults. However, with aggressive hospital care, these fatality rates can be substantially reduced. The ultimate cause of death is usually pulmonary or cardiac failure.

Clinical Manifestations. Tetanus is a severe disease caused by the toxin of *C tetani*. This organism grows in a wound and secretes a toxin that invades systemically and causes muscle spasms. The initial symptom is cramping and twitching of muscles around a wound. The patient usually has no fever but sweats profusely and begins to experience pain, especially in the area of the wound and around the neck and jaw muscles (trismus) (*Fig. 8*). Portions of the body may become extremely rigid, and opisthotonos (a spasm in which the head and heels are bent backward and the body bowed forward) is common (*Fig. 9*). Complications include fractures, bowel impaction, intramuscular hematoma, muscle ruptures, and pulmonary, renal, and cardiac problems.

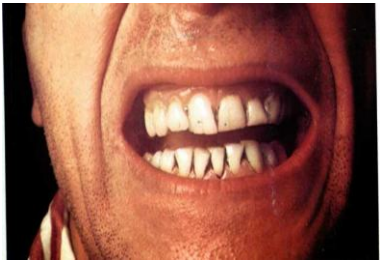

Fig. 8. Trismus or lockjaw

Fig. 9. Opisthotonos

Immunity. Host defenses are essentially absent. There is little, if any, innate immunity and the disease does not produce immunity in the patient. In addition, one or more episodes of tetanus do not produce immunity to future attacks. The reason for the lack of immune response may be twofold: the toxin is potent, and the amount released may be too small to trigger immune mechanisms but still be enough to cause symptoms and, because the toxin binds firmly to neural tissue, it may not interact effectively with the immune system.

Prophylactic immunization is accomplished with tetanus toxoid, as part of the DPT (DTP) vaccine or the DT (TD) vaccine. Three injections are given in the first year of life, and a booster is given about a year later, and again on the entrance into elementary school.

Whenever a previously-immunized individual sustains a potentially dangerous wound, a booster of toxoid should be injected. Currently, booster doses are recommended only every 10 years by the CDC.

Laboratory diagnosis only required in suspicion of iatrogenic infections e.g. infection of umbilical cord stump, post-partum infections, etc. In most cases diagnosis relies on clinical aspect and history (tetanigenic circumstances e.g. wounds contaminated with dirt, faeces, soil; puncture wounds; animal bites, burns). Diagnosis of tetanus is obvious in advanced cases; however, successful treatment depends on early diagnosis before a lethal amount of toxin becomes fixed to neural tissue. The patient should be treated on a clinical basis without waiting for laboratory data. *C tetani* can be recovered from the wound in only about one-third of the cases, and a wound is not even evident in 10-20% of cases. It is important for the clinician to be aware that toxigenic strains of *C tetani* can grow actively in the wound of an immunized person, but the presence of antitoxin antibodies prevents initiation of tetanus.

Numerous syndromes, including rabies and meningitis, have symptoms similar to those of tetanus and must be considered in the differential diagnosis. Ingestion of strychnine (found in rat poison) can cause symptoms that closely resemble those of generalized tetanus. Trismus can occur in encephalitis, phenothiazine reactions, and diseases involving the jaw.

Control. Treatment of diagnosed tetanus has a number of aspects. The offending organism must be removed by local debridement, after the patient's spasms are controlled by benzodiazepines. Penicillin or metronidazole is usually administered to kill the bacteria, but may not be a necessary adjunct in therapy. Immunoglobulin is injected intramuscularly: dosage recommendations vary from 500 IU in a single intramuscular injection to 3 000–6 000 IU injected intramuscularly in several sites.

In cases of clean, minor wounds, tetanus toxoid should be administered only if the patient has not had a booster dose within the past 10 years. For more serious wounds, toxoid should be administered if the patient has not had a booster dose within the past 5 years. All patients who have a reasonable potential for contracting tetanus should receive injections of tetanus immunoglobulin, including those recovering from diagnosed cases of tetanus.

Botulism

C. botulinum is widely distributed in soil, sediments of lakes and ponds, and decaying vegetation. Hence, the intestinal tracts of birds, mammals and fish may occasionally contain the organism as a transient.

Structure, classification and antigenic types. C botulinum is anaerobic rod, Gram-positive, motile by peritrichous flagella. Oval, subterminal and building spores that resemble tennis racket (*Fig. 10*) are produced in extremely variable numbers, depending on the particular isolate and on the culture medium. Seven toxigenic types of the organism exist, each producing an immunologically distinct form of botulinum toxin. The toxins are designated A, B, C1, D, E, F, and G. Cultural reactions vary greatly, and the species includes highly proteolytic and nonproteolytic strains as well as saccharolytic and nonsaccharolytic strains.

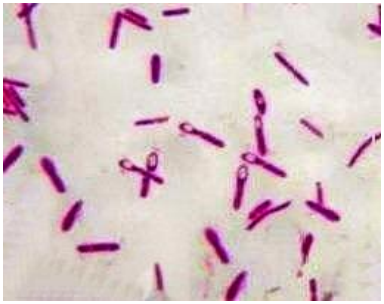

Fig. 10. Morphology of C botulinum

Of the seven serologically distinct neurotoxins produced by *C botulinum* (A, B, C, D, E, F, and G), humans are most susceptible to types A, B, E, and F. Types C and D are most toxic for animals. Type G is rare, with only a few reported human cases. The toxins often are released from the bacteria as

inactive proteins that must be cleaved by a protease to expose the active site. These proteases may be produced by the cell itself or may be in the body fluids of the infected host. Type A toxin is the most potent poison known; ingestion of only 10^{-8} grams of this toxin can kill a human.

Epidemiology. *C botulinum* spores are found worldwide in the soil (including in sea sediments) and in low numbers in the gastrointestinal tracts of some birds, fish, and mammals. Originally, botulism food poisoning was thought to be associated only with contaminated meat, especially sausage; however, it is now known that *C botulinum* can grow equally well in many types of food including vegetables, fish, fruits, and condiments. Home canning using inadequate sterilization techniques has been responsible for most cases of botulism during this century. The spores are heat resistant and can survive 100 °C for hours, but the toxin is relatively heat labile. The toxin is usually produced at pH 4.8–8.5. However, even acid foods such as canned tomatoes have been responsible for several recent cases of botulism food poisoning. In general, germination of botulinum spores is favored in food kept at warm temperatures under anaerobic conditions for a long period of time.

Pathogenesis. The pathogenicity of *C botulinum* depends entirely on neurotoxin production (*Fig. 11*). In humans, these toxins cause disease in three ways: the well-known form of food poisoning results from ingestion of toxin in improperly preserved food; wound botulism, a rare disease, results from *C botulinum* growing in the necrotic tissue of a wound; and infant botulism is caused when the organism grows and produces toxin in the intestines of infants.

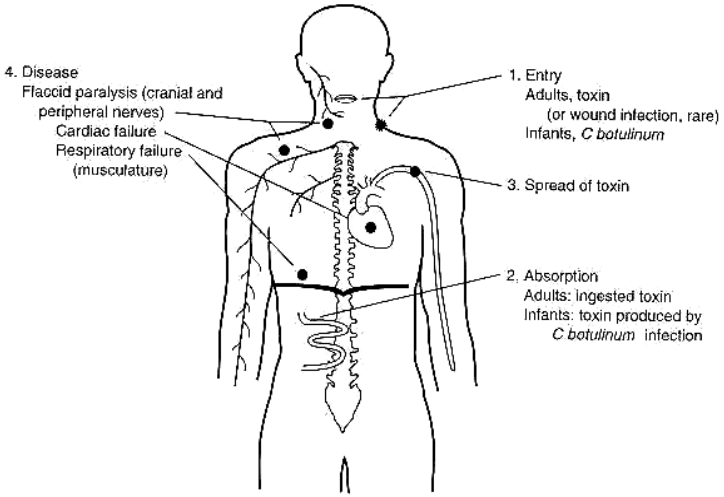

Fig. 11. Pathogenesis of botulism

The Botulinum Toxins are very similar in structure and function to the tetanus toxin, but differ dramatically in their clinical effects because they target different cells in the nervous system. Botulinum neurotoxins predominantly affect the peripheral nervous system reflecting a preference of the toxin for stimulatory motor neurons at a neuromuscular junction. Toxin is ingested, absorbed in the intestine, enters bloodstream and is internalized by cholinergic nerve cells where it cleaves synaptobrevins involved in acetylcholine release. As a result it blocks nerve transmission (*Fig. 12*). The primary symptom is weakness or flaccid paralysis. Tetanus toxin can affect the same system, but the tetanospasmin shows a tropism for inhibitory motor neurons of the central nervous system, and its effects are primarily rigidity and spastic paralysis.

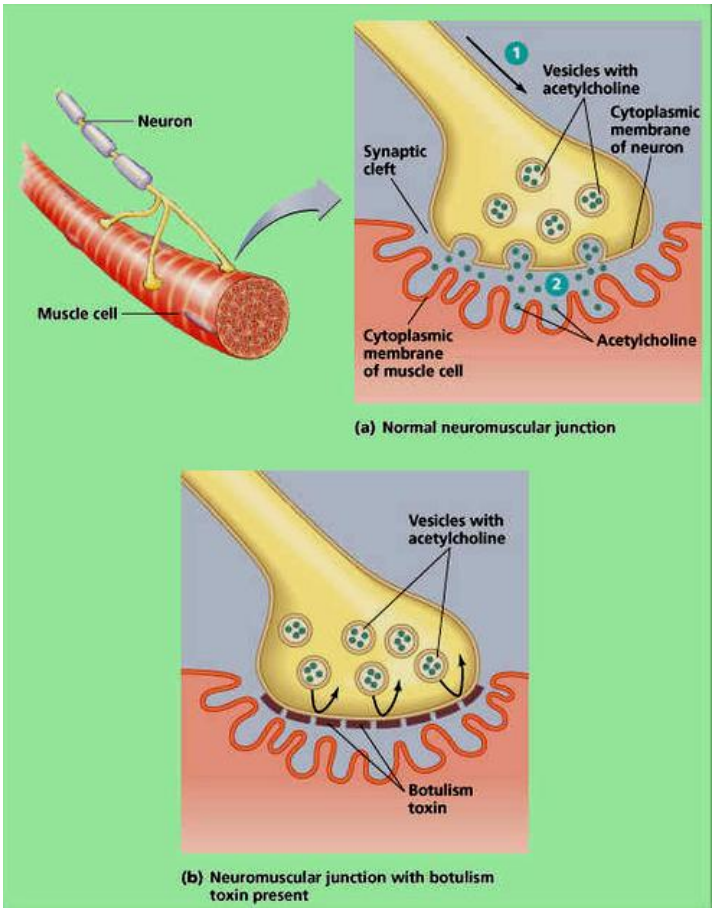

Fig. 12. Action of botulinum toxin in neuromuscular junction

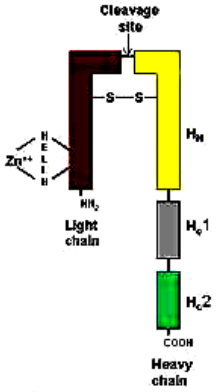

Fig. 13. Structure of botulinim toxin

Botulinum toxin is synthesized as a single polypeptide chain with a molecular weight around 150 kDa. In this form the toxin has a relatively low potency. The

toxin is nicked by a bacterial protease (or possibly by gastric proteases) to produce two chains: a light chain with a molecular weight of 50 kDa; and a heavy chain, with a weight of 100 kDa (*Fig. 13*). As with tetanospasmin the chains remain connected by a disulfide bond. The light chain of the nicked toxin, on a molecular weight basis, becomes the most potent toxin found in nature.

Food-borne Botulism is not an infection but an intoxication since it results from the ingestion of foods that contain the preformed clostridial toxin. In this respect it resembles staphylococcal food poisoning. Botulism results from eating uncooked foods in which contaminating spores have germinated and produced the toxin. C. botulinum spores are relatively heat resistant and may survive the sterilizing process of improper canning procedures. The anaerobic environment produced by the canning process may further encourage the outgrowth of spores. The organisms grow best in neutral or “low acid” vegetables (>pH 4.5).

In food-borne botulism the botulinum toxin is ingested with food in which spores have germinated and the organism has grown. The toxin is absorbed by the upper part of the GI tract in the duodenum and jejunum, and passes into the blood stream by which it reaches the peripheral neuromuscular synapses. It then becomes fixed to cranial and peripheral nerves, but exerts almost all of its action on the peripheral nervous system. The toxin binds to the presynaptic stimulatory terminals and blocks the release of the neurotransmitter acetylcholine which is required for a nerve to simulate the muscle. The result is flaccid muscular paralysis.

The cranial nerves are affected first, followed by a descending, symmetric paralysis of motor nerves. The early involvement of cranial nerves causes problems with eyesight, hearing, and speech. Double or blurred vision, dilated pupils, and slurred speech are common symptoms. Decreased saliva production causes a dryness of the mouth and throat, and swallowing may be painful. An overall weakness ensues, followed by descending paralysis with critical involvement of the respiratory tree. Death usually is caused by respiratory failure, but cardiac failure also can be the primary cause. Mortality is highest for type A, followed by type E, and then type B, possibly reflecting the affinities of the toxins for neural tissue: type A binds most firmly, followed by type E, then type B. Fatality rates are directly proportional to the infectious dose and inversely proportional to the incubation time of the disease.

Wound botulism is a rare disease. The initial event is contamination of a wound by *C botulinum*. The organisms are not invasive and are confined to the necrotic tissue, where they replicate and elaborate the lethal neurotoxin. The incubation time varies from a few days to as long as 2 weeks. The only differences in the symptoms of wound botulism and food poisoning (in addition to a possibly longer incubation time) are that wound botulism lacks gastrointestinal symptoms, and a wound exudate or a fever, or both may be present. *C botulinum* may be present in a wound but creates no symptoms of botulism. There have been several recent reports of wound botulism in intravenous drug abusers, who are now considered a population at risk.

Infant botulism. In contrast to food poisoning with toxemia caused by ingestion of preformed toxin, infant botulism results from germination of spores in the gastrointestinal tract. Here vegetative cells replicate and release the botulinum toxin. It is unclear as to why spores can germinate and bacteria replicate in the infant intestine, but phenomenon appears to be related to the composition of the intestinal flora of infants. Almost all reported cases have occurred in infants between 2 weeks and 6 months of age, with the median age of onset being 2 to 4 months. Toxins A or B are most frequently implicated. In infant botulism, the usual first indication of illness, constipation, is often overlooked. The infant then becomes lethargic and sleeps more than normally. Suck and gag reflexes diminish, and dysphagia often becomes evident as drooling. Later, head control may be lost, and the infant becomes flaccid. In the most severely affected babies, respiratory arrest can occur. Infant botulism can be lethal and is the likely etiologic agent in 4 to 15 % of the cases of sudden infant death.

Clinical symptoms of food-born botulism begin 18-36 hours after toxin ingestion with weakness, dizziness and dryness of the mouth. Gastrointestinal disturbances are early symptoms of the disease in about one-third of the patients with toxin types A or B, and in almost all of the cases involving type E toxin. These symptoms include nausea, vomiting, and abdominal pain. Diarrhea often is present, but constipation also may occur. Symptoms of toxemia then become apparent. No fever occurs in the absence of complicating infections.

Neurologic features soon develop: blurred vision, inability to swallow, difficulty in speech, descending weakness of skeletal muscles and respiratory paralysis.

Infant botulism is characterized by constipation and weak sucking ability and generalized weakness. *C botulinum* can apparently establish itself in the bowel of infants at a critical age before the establishment of competing intestinal bacteria (normal flora). Production of toxin by bacteria in the GI tract induces symptoms. Almost all known cases of the disease have recovered.

Host defense. As with tetanus, immunity to botulism does not develop, even with severe disease, because the amount of toxin necessary to induce an immune response is toxic. Repeated occurrence of botulism has been reported.

The toxins that cause botulism are specifically neutralized by its antitoxin. Botulinum toxins can be toxoided and make good antigens for inducing protective antibody.

Once the botulinum toxin has bound to nerve endings, its activity is unaffected by antitoxin. Any circulating ("unfixed") toxin can be neutralized by intravenous injection of antitoxin. Individuals known to have ingested food with botulism should be treated immediately with antiserum.

A multivalent toxoid evokes good protective antibody response but its use is unjustified due to the infrequency of the disease.

Prevention. The most important aspect of botulism prevention is proper food handling and preparation. The spores of *C botulinum* can survive boiling (100 degrees at 1 atm) for more than one hour although they are killed by autoclaving. Because the toxin is heat-labile boiling or intense heating (cooking) of contaminated food will inactivate the toxin. Food containers that bulge may contain gas produced by *C botulinum* and should not be opened or tasted. Other foods that appear to be spoiled should not be tasted.

For both food poisoning and wound botulism, antitoxin therapy is most effective if administered early. Unfortunately all antitoxins are equine preparations, so a significant percentage of patients experience reactions typical of anaphylaxis and serum sickness. Thus, before they receive antitoxin, all patients should be tested for sensitivity to horse serum.

All persons suspected of ingesting contaminated food should be closely observed. Antitoxin should be administered both to those with overt symptoms and to those who have definitely ingested contaminated food.

Laboratory diagnosis is performed in reference laboratories. Specimens: blood for serology, vomit, faeces, suspected food. Identification based on: Gram stain: Gram positive bacilli with spores; anaerobic growth; neurotoxin detection: experimental disease in mice; immunoassay; PCR (*Fig. 14*).

Confirmation of the initial diagnosis rests on demonstrating toxin in the patient's feces, serum, or vomitus. In adult botulism, serum samples rarely yield type A toxin because of the strong affinity of this toxin for neural tissue. In infant botulism, circulating toxin can occasionally be found in the serum. Fecal samples are the best specimens for detecting toxin in botulism food poisoning or infant botulism because only a small percentage of ingested or in situ formed toxin is absorbed through the intestinal mucosa. Toxin may be excreted for days or even weeks following botulism food poisoning. Toxin is usually detected by its lethal effect in mice coupled with neutralization of this effect by specific antisera. In infants, the organism can usually be cultured from the stool.

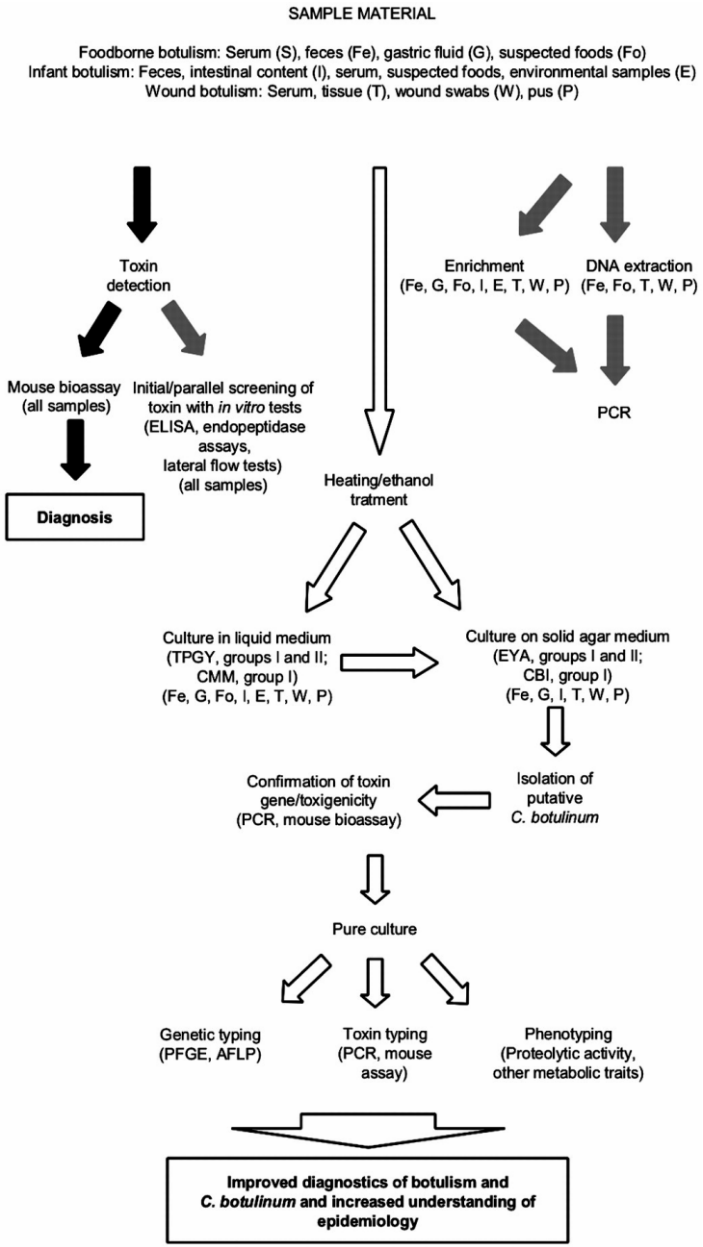

Fig. 14. Scheme of laboratory diagnosis of botulism

Antibiotic-Associated Diarrhea, Pseudomembranous Colitis

Clostridium difficile is a major nosocomial pathogen that causes a spectrum of intestinal disease from uncomplicated antibiotic-associated diarrhea to severe, possibly fatal, antibiotic-associated colitis. Diarrhea has come to be accepted as a natural accompaniment of treatment with many antibiotics. Although this diarrhea usually causes only minor concern, it can evolve into a life-threatening enterocolitis.

Many antibiotics have been associated with diarrhea and with pseudomembranous colitis, including ampicillin, cephalosporins, clindamycin, and amoxicillin. Patients treated with clindamycin have a higher incidence of *C difficile* disease, but most cases are found in patients treated with other antibiotics because of the more widespread use of these agents.

Biological properties of C difficile. *C difficile* is a long, slender, Gram-positive bacillus that produces large, oval, subterminal spores (*Fig. 15*). It is an anaerobe, and some strains are extremely sensitive to oxygen. *C difficile* is nonhemolytic and does not produce lecithinase or lipase reactions on egg yolk agar. It produces various tissue degradative enzymes, including proteases, collagenases, hyaluronidase, heparinase, and chondroitin-4-sulfatase. The products of fermentation are many and complex and include acetic, butyric, isovaleric, valeric, isobutyric, and isocaproic acids; however, only small amounts of each are produced.

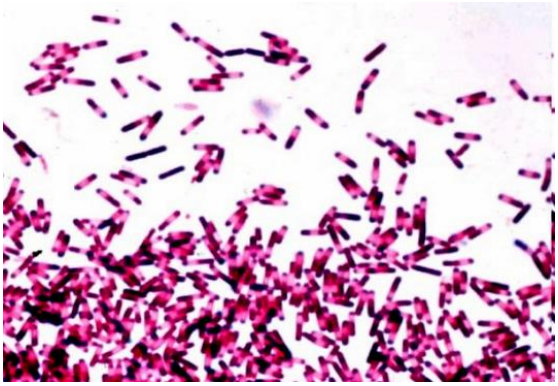

Fig. 15. Cells of *C difficile* with subterminal spores

Epidemiology. *C difficile* is a member of the normal intestinal flora of <3 % of adults. The organism can be acquired as a nosocomial pathogen and a variable incidence of disease is noted in hospitals and nursing homes. This seems to be due in part to environmental contamination with *C difficile* spores, and in part to different patient populations in various institutions. Patients with *C difficile* diarrhea excrete large numbers of *C difficile* spores, and epidemiological studies have shown that the organism can reside on environmental surfaces as well as on the hands of health care workers. Healthy

adults do not carry significant numbers of the organism in their intestinal tracts, but healthy infants may have large numbers of these organisms in their feces. There is circumstantial evidence supporting the theory that infants do not develop disease because they lack specific intestinal receptors for *C difficile* toxins. In recent years, *C difficile* as also emerged as one of the causes of chronic diarrhea in AIDS patients.

Pathogenesis. *C difficile* disease is caused by the overgrowth of the organism in the intestinal tract, primarily in the colon. The organism appears unable to compete successfully in the normal intestinal ecosystem, but can compete when normal flora are disturbed by antibiotics, allowing overgrowth of *C difficile (Fig. 16).*

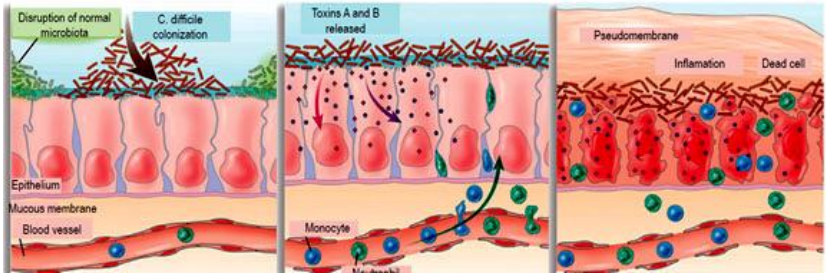

Fig. 16. Pathogenesis of C difficile infection

This organism then replicates and secretes two toxins. Toxin A is an enterotoxin that causes fluid accumulation in the bowel, and it is a weak cytotoxin for most mammalian cells; toxin B is a potent cytotoxin. Nearly all toxigenic strains produce both toxins A and B. Highly toxigenic strains produce high levels of both toxins, while weakly toxigenic strains produce low levels of both toxins. Results from in vitro studies using cultured intestinal epithelial cells have indicated that toxin A causes necrosis, increased intestinal permeability, and inhibition of protein synthesis. Toxin A somehow affects phospholipase A2, resulting in the production of several arachidonic acid metabolites including prostaglandins and leukotrienes. Although the exact mechanism of endocytosis is unclear, both toxins A and B are internalized by host cells, resulting in alterations in the actin-containing cytoskeleton. Toxin A is a chemotactic factor for granulocytes; both toxins A and B have effects on leukocytes that include alterations in actin cytoskeletal microfilaments, and induction of tumor necrosis factor, interleukin 1, and interleukin 6. These latter effects contribute to the inflammatory response associated with *C difficile* disease.

Both toxins A and B kill experimental animals, and both probably are involved in the pathology of disease. Toxin production causes diarrhea that may progress to pseudomembranous colitis, where the characteristic pseudomembranes are largely limited to the colon. Toxin A binds to the apical side of the cell and, after internalization, causes cytoskeletal changes that result

in disruption of tight junctions and loosening of the epithelial barrier, in cell death or in the production of inflammatory mediators that attract neutrophils (*Fig. 17*). Disruption of tight junctions enables both toxin A and toxin B to cross the epithelium. Toxin B binds preferentially to the basolateral cell membrane. Both toxins are cytotoxic and induce the release of various immunomodulatory mediators from epithelial cells, phagocytes and mast cells, resulting in inflammation and the accumulation of neutrophils. In an animal model, toxin was shown to have a tropism for cardiac tissue, which would require that toxin B enter the bloodstream. This tissue damage causes a viscous hemorrhagic fluid response. In contrast, toxin B does not have noticeable enterotoxic activity, but it is lethal when injected into experimental animals. Thus, it seems reasonable to speculate that, in humans, toxin B exerts its pathogenic effect following dissemination through a damaged gut wall to extraintestinal organs. It has been speculated that infants harboring high levels of intestinal toxins A and B are at risk for the systemic toxicity of toxin B if their intestinal barrier is compromised.

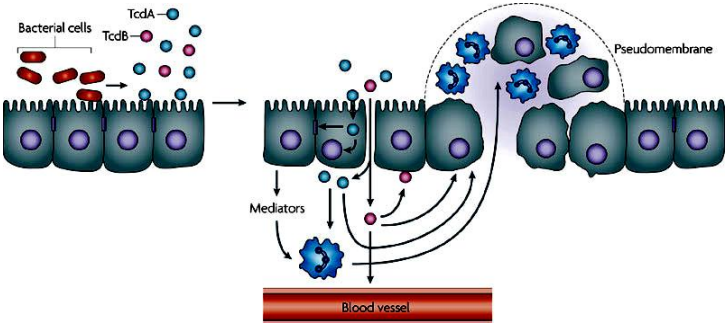

Fig. 17. Pathogenesis of pseudomembranous colitis: in the intestinal tract, toxin A damages villous tips and brush border membranes, and may result complete in erosion of the mucosa

Clinical Manifestations. Clinical symptoms of *C difficile* disease vary widely from mild diarrhea to severe abdominal pain accompanied by fever (typically >101 °F) and severe weakness. Diarrhea is watery and usually nonbloody, but approximately 5 to 10 % of patients have bloody diarrhea. Fecal material typically contains excess mucus, and pus or blood may also be noted. Hypoalbuminemia and leukocytosis are common findings. Pathology involves only the colon where there may be disruption of brush border membranes followed by extensive damage to the mucosa. The disease may progress to a pseudomembranous colitis, possibly including intestinal perforation and toxic megacolon. There is a leukocytic infiltrate into the lamina propria accompanied by elaboration of a mixture of fibrin, mucus, and leukocytes, which can form

gray, white, or yellow patches on the mucosa. These areas are called pseudomembranes; hence the common term pseudomembranous colitis. Pseudomembranes usually develop after 2–10 days of antibiotic treatment, but they may appear 1–2 weeks after all antibiotic therapy has stopped. Mortality varies, but may be as high as 10 % in patients with pseudomembranous colitis. The ultimate cause of death often is difficult to determine, as most patients show a nonspecific deterioration over a period of weeks.

C difficile is now considered a major cause of diarrhea in hospitals and nursing homes. In most instances, once a patient develops antibiotic-associated diarrhea and *C difficile* organisms and/or toxin is detected in the stool, appropriate antimicrobial therapy is begun, and the symptoms are not allowed to progress to the formation of colonic pseudomembranes.

Host defenses for *C difficile* disease are not completely understood, but it seems reasonable to assume that the best host defense against *C difficile* disease is maintenance of the stability of the normal intestinal flora. Production of specific neutralizing antibodies to toxins A and B may participate in host defense, and a specific intestinal secretory IgA response to toxin A is more evident in the colon than the upper intestinal tract, compatible with the colon as the primary site of intestinal disease. The intestinal tract responds to *C difficile* toxins by increased fluid production, by secretory IgA neutralization of toxin, and by mucus production, which may inhibit the attachment of the toxins to their putative receptor sites on intestinal epithelial cells.

Diagnosis. Many cases of severe diarrhea are caused not by *C difficile*, but are caused by other enteric pathogens such as Campylobacter spp, Salmonella spp, Shigella spp, toxigenic strains of Escherichia coli, etc. *C difficile* is likely responsible for 25 % of cases of antibiotic-associated diarrhea and colitis. Diagnosis of pseudomembranous colitis requires demonstration of pseudomembranes by colonoscopy or histopathology showing disclose pseudomembranous colitis, and *C difficile* can be isolated from the stools of almost all patients with this disease.

Toxin detection is also used for diagnosis. Although the most appropriate test for toxin detection remains controversial, a cellular cytotoxicity test remains the "gold standard"; here, filter sterilized fecal extract (or filter-sterilized broth containing a pure culture of *C difficile*) is added to a monolayer of cultured mammalian cells such as Vero cells (African green monkey kidney cells) resulting in a cytopathic effect that is neutralized with specific antiserum. Unfortunately, this test is time-consuming and cumbersome. A rapid latex agglutination test is widely used, but this test is not specific, does not detect toxin A or B, and often results in false negative or false positive reactions. Enzyme-linked immunoassays can be used to detect both toxin A and toxin B, and these tests are useful for diagnosis of *C difficile* disease.

Recent clinical practice guidelines recommend a 2-step approach to diagnosis. The first step is use of an enzyme immunoassay to detection

glutamate dehydrogenase (GDH) which also known as the antigen of C. difficile. Even though these tests can cross-react with other bacterial species such as *C sporogenes, C botulinum* and *Peptostreptococcus anaerobius*, its negative predictive value test is 99 %, so a negative result excludes the diagnosis of *Cl difficile*. If the test is positive for GDH, the second step is confirmation of *C difficile* by culture and/or detection of the toxin through methods such as a cytotoxicity assay or toxigenic culture.

Control. In many cases, symptoms resolve 1–14 days after the offending antibiotic is discontinued, and antibiotic treatment is not needed. Vancomycin or metronidazole are the antibiotics of choice to treat active disease. Oral vancomycin is the "gold standard," and metronidazole is most often used to treat milder infections. *C difficile* is susceptible to both of these antimicrobial agents, but relapses occur in 15 to 20 % of patients. Some patients have had many repeated relapses. Supportive therapy is needed to compensate for the often severe fluid and electrolyte loss. Health care workers caring for patients infected with *C difficile* should wear gloves and strictly adhere to proper hand washing procedures.

Other Pathogenic Clostridia

Food Poisoning and Clostridium perfringens. *C perfringens* is one of the major causes of food poisoning. The disease results from ingestion of a large number of organisms in contaminated food, usually meat or meat products. Food poisoning usually does not occur unless the food contains at least 10^6–10^7 organisms per gram. The spores are ubiquitous and, if present in food, can be triggered to germinate when the food is heated. Some heat-sensitive strains do not need heating to germinate. After germination, the number of organisms quickly increases in warm food because the generation time can be extremely short (minutes) and bacterial multiplication occurs over a wide temperature range. Regardless, food poisoning results from the ingestion food contaminated with enterotoxin-producing *C perfringens*. The enterotoxin directly affects the permeability of the plasma membrane of mammalian cells.

C perfringens type A is the usual causative agent, and serotyping is necessary and available for epidemiologic studies. Incubation time is 8–22 hours after ingestion of contaminated food, with a mean of 14 hours. Symptoms include diarrhea, cramps, and abdominal pain. Fever, nausea, and vomiting are rare, and the disease lasts only about 24 hours. The organism and its enterotoxin usually can be isolated from the feces of infected persons. The mortality rate is essentially zero, but elderly and immunologically compromised patients should be closely supervised.

Necrotizing Enteritis and Clostridium perfringens. In adults, the disease appears to result from ingesting large amounts of food contaminated with *C perfringens*, usually type C. It generally follows ingestion of a large meal,

implicating bowel distention and bacterial stasis as contributing factors. The intestinal pathology varies considerably, and may include sloughing of intestinal mucosa, submucosa, and mesenteric lymph nodes. Intestinal perforations occur frequently. The course of the disease is fulminate, and the mortality rate is high.

Some evidence suggests that acute necrotizing enterocolitis of infants may be caused by a clostridium, but definitive evidence is lacking. The theory is supported by the fact that pneumatosis cystoides intestinalis, a syndrome that can be caused by *C perfringens*, often is present in cases of acute necrotizing enterocolitis of infants.

Bacteremia, endometritis and nonbacteremic infections with Clostridium sordellii. *C sordellii* is part of the normal intestinal flora of humans. The organism produces several exotoxins including toxins serologically related to the toxins of *C difficile*. *C sordellii* has been occasionally implicated in bone and joint infections, in pulmonary infections, in bacteremia, and in fulminate endometritis.

Malignancy and Clostridium septicum. *C septicum* is a spindle-shaped rod that is motile in young cultures. The organism produces toxins designated alpha, beta, gamma, and delta; the alpha toxin is necrotizing and lethal for mice. Whether *C septicum* is a member of the host's normal flora or whether it takes advantage of a compromised host is uncertain. The organism is not strongly invasive, but has been associated with gas gangrene. Fewer than 200 cases of invasive disease have been reported, but the majority have a malignancy somewhere in the body. The most frequent association is with colorectal cancer, but other types of malignancies have been noted, including leukemia, lymphoma, and sarcoma. In one survey of *C septicum* bacteremia, 49 of 59 (83 %) cases had an underlying malignancy and, in 28 of these cases, the portal of entry appeared to be the distal ileum or the colon.

Penicillin is the antibiotic of choice, but chloramphenicol, carbenicillin, and cephalothin also have been used successfully.

Practical tasks, being carried out during practical classes:

1. Studying morphology of clostridia (in atlas and microslides).
2. Studying biological preparations for serological methods (antigens and diagnostic sera).
3. Studying the scheme of laboratory diagnosis of clostridial infections.
4. Studying vaccines and immune sera for specific prophylaxis and therapy of tetanus and botulism.
5. Prepare a microslide from Kitt-Tarrozzi medium. Stain the smear after Gram. Examination of the smear with immersion microscope.
6. Studying of the scheme of laboratory diagnosis of clostridial infections.

Therminology: *Clostridium tetani, Clostridium botulinum, Clostridium perfringens, Clostridium septicum, Clostridium histolyticum*, blood agar, Kitt-Tarrozzi medium, anaerobic jar.

Theoretical questions for control:

1. Genus Clostridia, major characteristics, antigenic structure.
2. Culture properties of clostridia.
3. Routes of transmission and pathogenesis of gas gangrene, tetanus, botulism, food poisoning, pseudomembranous enterocolitis.
4. Laboratory diagnosis of clostridial infections.
5. Treatment and control of clostridial infections.

Test tasks for control:

1. Patient with vomiting, dizziness, sensation of dubble vision, difficult swallowing was admitted to the hospital. Doctor suspects botulism. What diagnostic methods should be used for diagnosis approving?

A. Serological, microscopical
B. Biological test, bacteriological
C. Protozoological, microscopical
D. Bacteriologocal, mycological
E. Allergic test, serological

2. Symptoms of Clostridium botulinum food poisoning include double vision, inability to speak, and respiratory paralysis. These symptoms are consistent with:

A. Endotoxin shock
B. Secretion of an enterotoxin
C. Invasion of the gut epithelium by C. botulinum
D. Ingestion of a neurotoxin
E. Activation of cyclic AMP

3. Since botulism results from the ingestion of foods that contain the preformed clostridial toxin botulism is referred as:

A. Intoxication
B. Reinfection
C. Primary infection
D. Mix-infection
E. Secondary infection

4. The agent for artificial immunization against Clostridium tetani consists of:

A. Purified capsular antigen
B. Purified cell-wall protein
C. A toxic protein treated with formaldehyde
D. An attenuated live organism
E. O and Vi antigens

5. Which of the following bacterium has terminally located spore giving the bacterium a “tennis racket” shape?

A. Bacillus anthracis
B. Clostridium botulinum
C. Clostridium perfringens
D. Bacillus subtilis
E. Clostridium tetani

Answers to test tasks: **1** – B; **2**– D; **3** – A; **4** – C; **5** – B; **6** – D; **7** – E; **8** – A; **9** – E; **10** – C.

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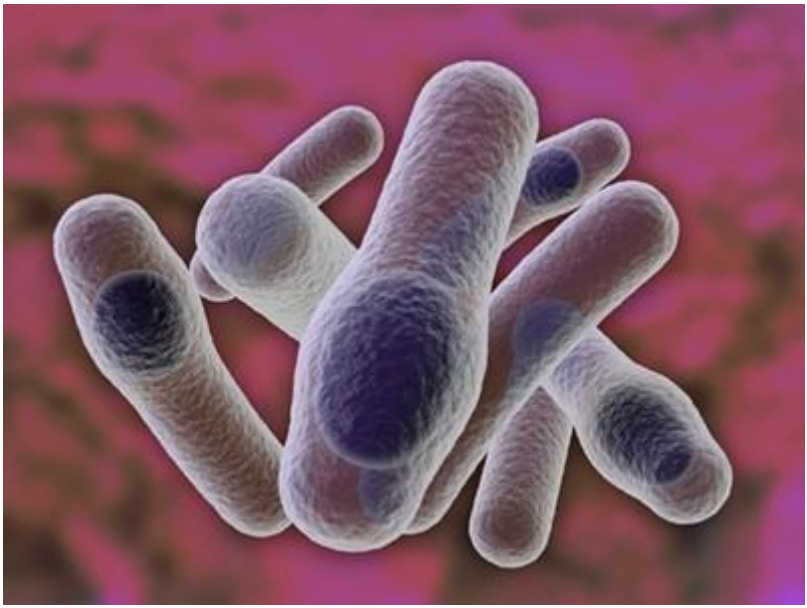

PATHOGENIC CLOSTRIDIA

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)