Introduction to Microbiology

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M.D. Ph.D.

Lecture 15

Endopores

- When faced with harsh environmental conditions, like depletion of any of several nutrients (carbon, nitrogen, or phosphorous). **Some gram positive bacteria** undergo a cycle of differentiation called **sporulation.**

- Sporulation involves the production of many new structures, enzymes, and metabolites along with the disappearance of many vegetative cell components.

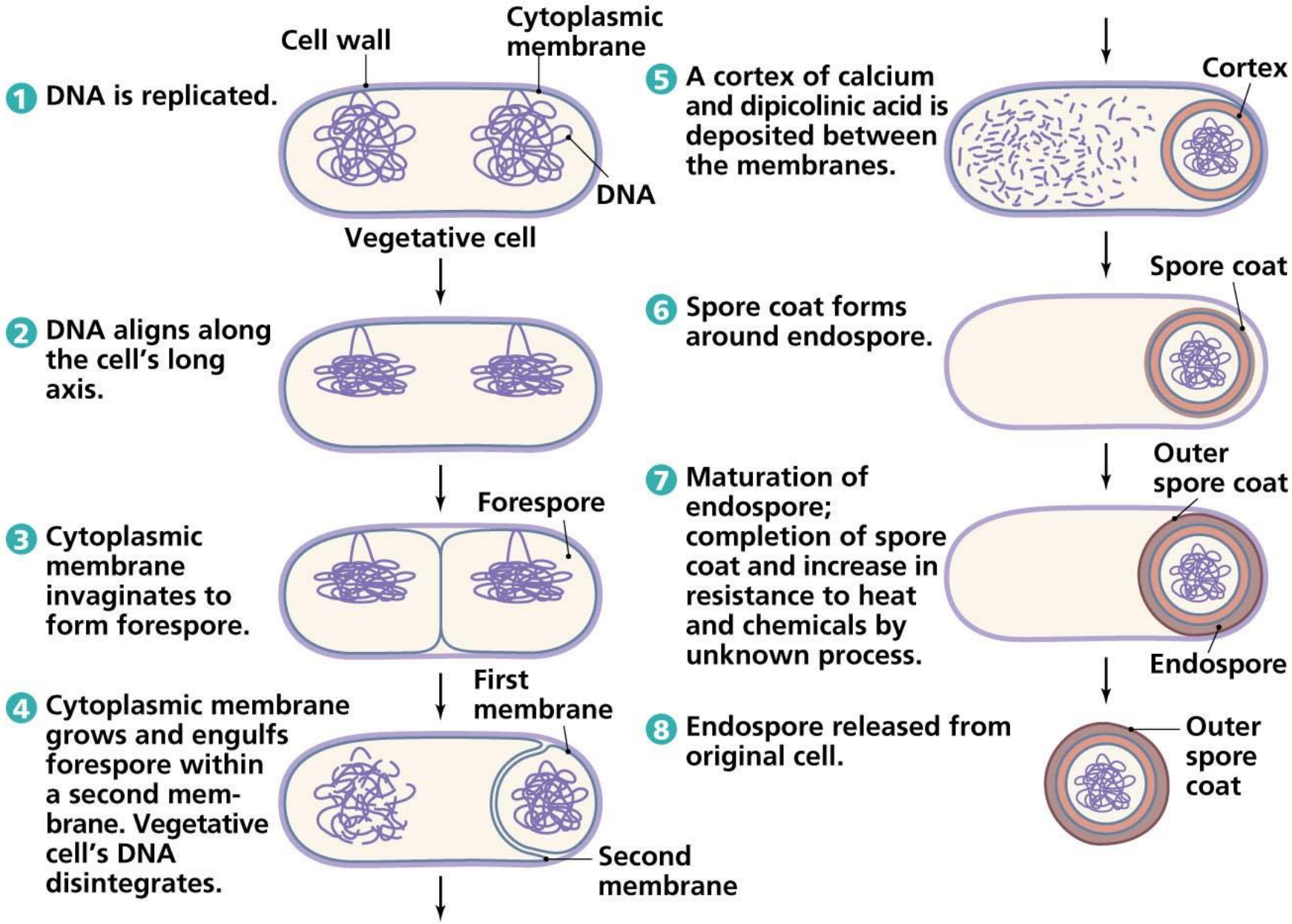
- The spore contains a complete copy of the chromosome, the bare minimum concentrations of essential proteins and ribosomes, and a high concentration of **calcium bound to dipicolinic acid**

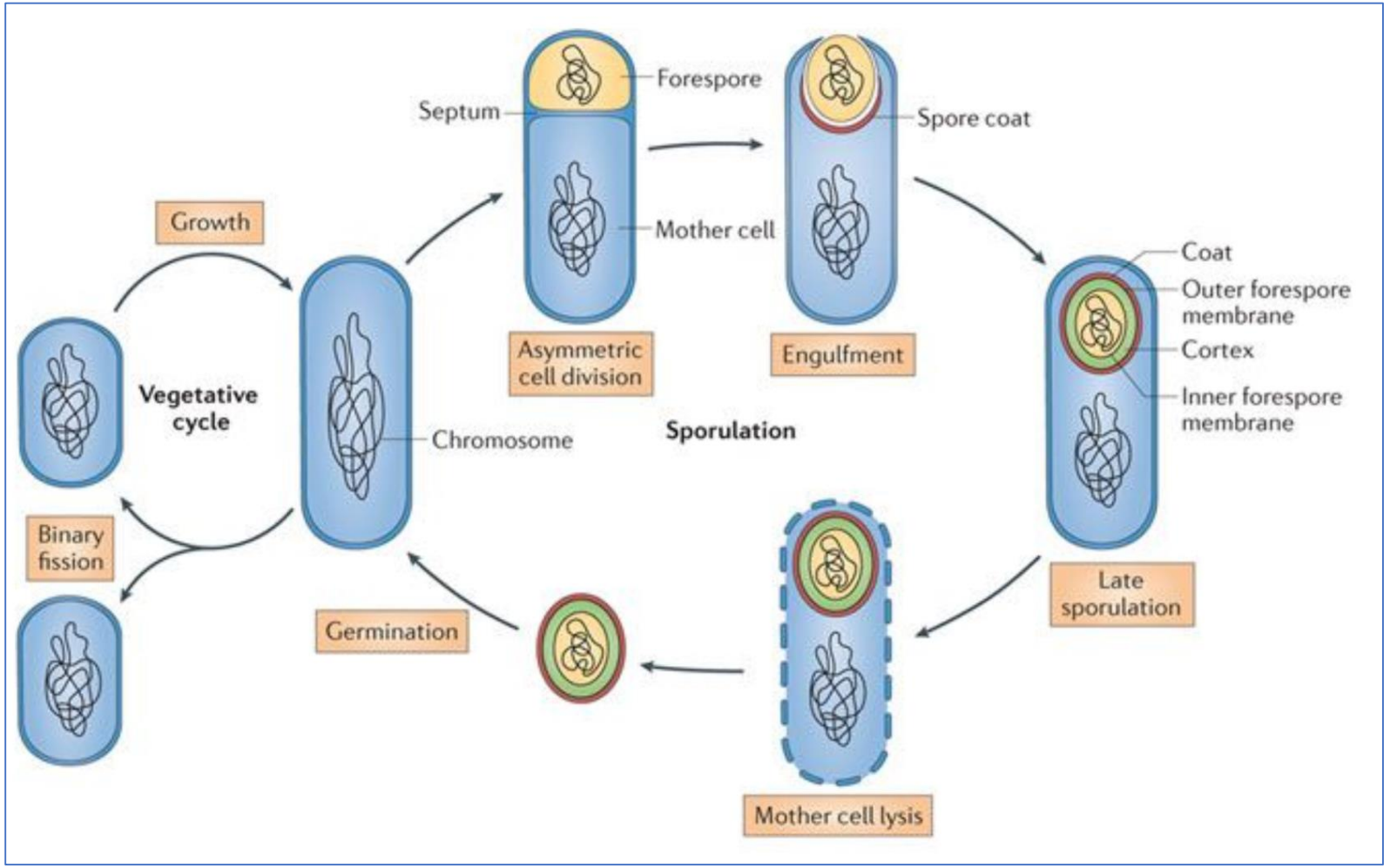
- The **spore** is a resting cell, highly resistant to desiccation, heat, and chemical agents. can exist for centuries as viable spores.

- When returned to favorable nutritional conditions, the spore **germinates** to produce a single vegetative cell.

- The location of the spore within a cell can assist in identification of the bacterium.

- The ultra structure and formation process of spores can vary from one species to another. (<u>exact detailed structure is not exam material</u>).





Sporulation begins when a sporangium divides asymmetrically to produce two compartments: the mother cell and the forespore, which are separated by a septum. Next, the mother cell engulfs the forespore, and following membrane fission at the opposite pole of the sporangium, a double-membrane bound forespore is formed. Coat assembly begins just after the initiation of engulfment and continues throughout sporulation. The peptidoglycan cortex between the inner and outer forespore membranes is assembled during late sporulation. In the final step, the mother cell lyses to release a mature spore into the environment. Spores are capable of quickly germinating and resuming vegetative growth in response to nutrients.

Spore-Forming Gram-Positive rods

- ***Bacillus sepcies:*** The genus *Bacillus* includes large **aerobic**, gram-positive rods occurring in chains.

Major pathogens include ***Bacillus anthracis*** and ***Bacillus cereus.***

- ***Clostridium Species: a*** genus of Gram-positive rods***, are obligate anaerobes.***

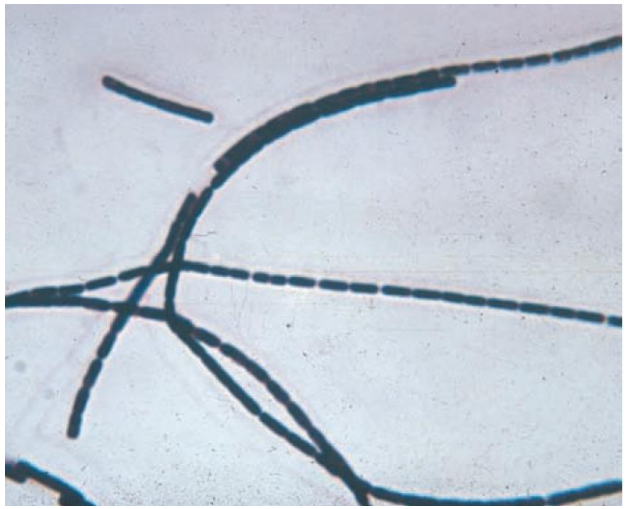
Major pathogens include ***Clostridium difficile. Clostridium perfringens, Clostridium tetani, Clostridium botulinum***

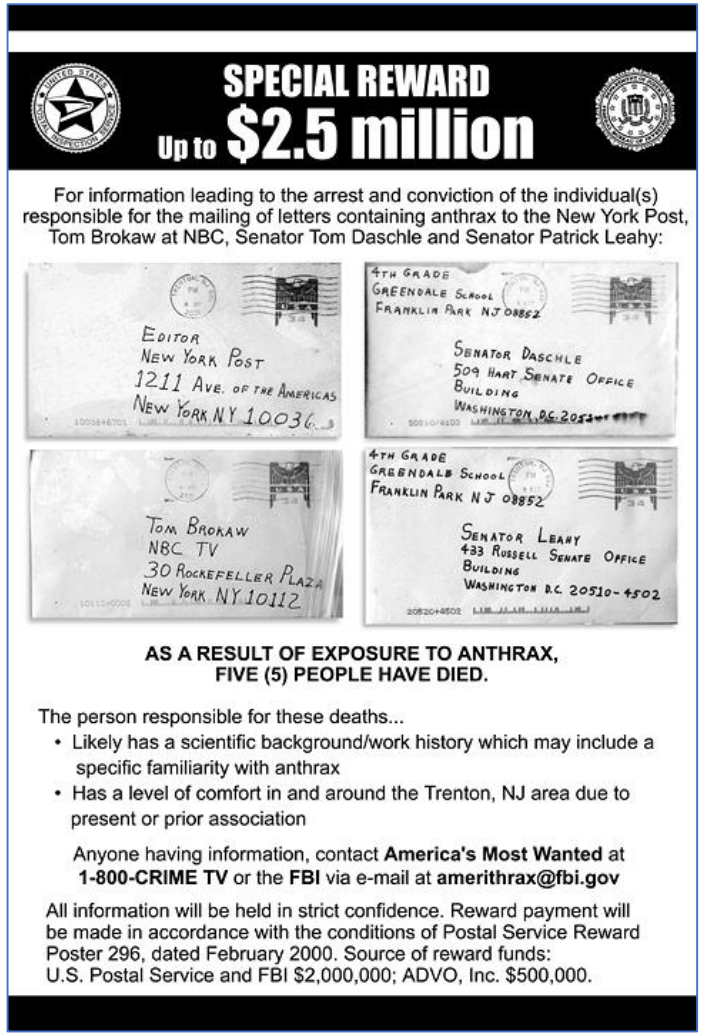
Bacillus anthracis

B. anthracis is a large (1 × 3 to 8 µm) organism arranged as single or paired **Gram positive rods** or as long, serpentine chains. Spores are are not seen in clinical specimens.

Anthrax is primarily a disease of herbivores; humans are infected through exposure to contaminated animals or animal products. Exposure can also be part of **biological warfare**.

Human *B. anthracis* disease is acquired by one of three routes: **inoculation (**Skin infections represent more than95% of cases)**, ingestion,** and **inhalation.**





Bacillus anthracis

protective antigen (PA), edema factor (EF), and **lethal factor (LF),** are nontoxic individually but form important toxins when combined: PA plus EF forms **edema toxin,** and PA plus LF forms **lethal toxin**

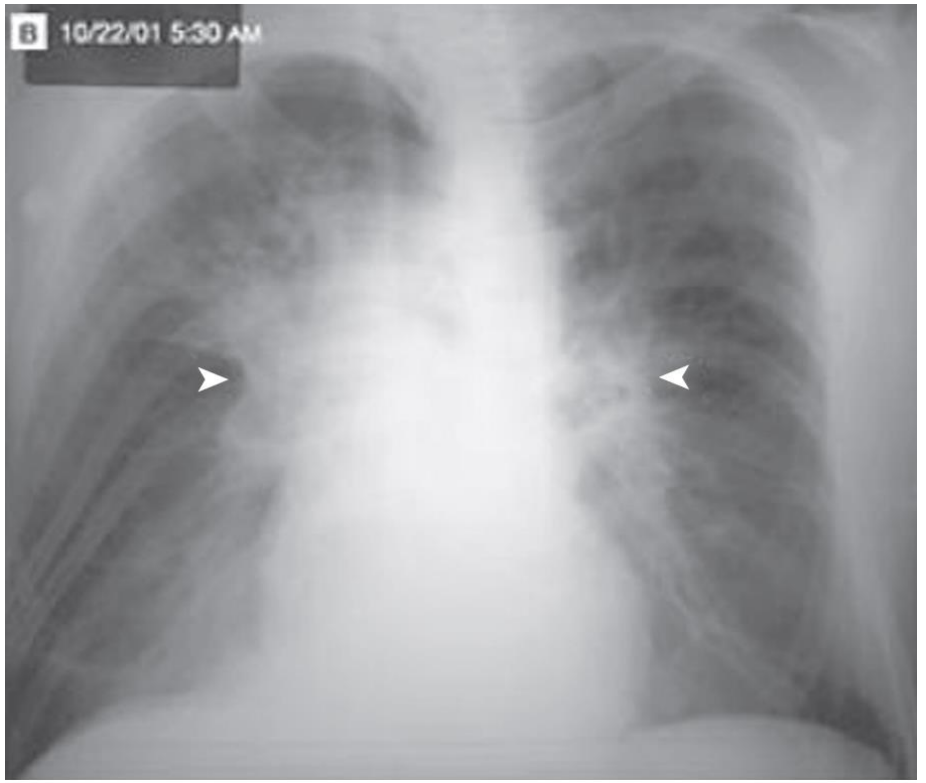
The major factors responsible for the virulence of *B. anthracis* are the **capsule, edema toxin, and lethal toxin**. The capsule made of **poly-d-glutamic acid** inhibits phagocytosis of replicating cells. **Edema toxin** is responsible for the fluid accumulation observed in anthrax. **Lethal toxin is** cytotoxic and stimulates macrophages to release proinflammatory cytokines.

The spores germinate in the tissue at the site of entry, and growth of the vegetative organisms results in formation of a gelatinous edema and congestion.

Almost all cases progress to **shock and death within 3 days of initial symptoms** unless anthrax is suspected and treatment is initiated immediately

Bacillus anthracis

Typically, **cutaneous anthrax** starts with the development of a painless papule at the site of inoculation that rapidly progresses to an ulcer surrounded by vesicles and then to a necrotic eschar.



Inhalation anthrax can be associated with a **prolonged latent period** (2 months or more), during which the infected patient remains asymptomatic. Spores phagocytosed in the lungs; and transported by the lymphatic drainage to the **mediastinal lymph nodes, where germination occurs**. Hemorrhagic **necrosis** and **edema** of the mediastinum are early manifistations, Sepsis occurs and spread to other organs (GI ulcerations, meningitis) can take place.

Bacillus cereus

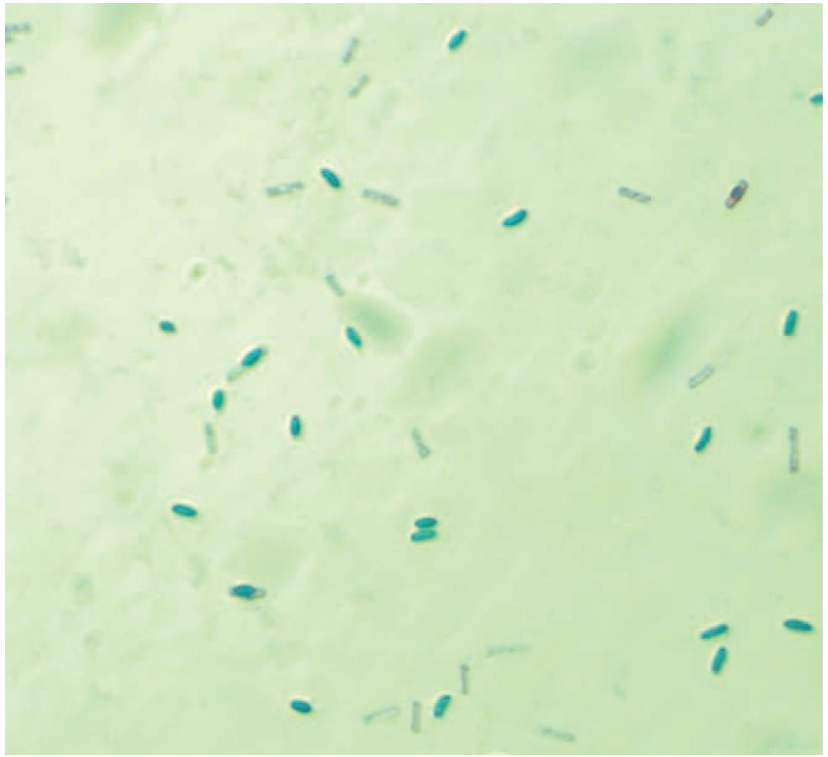
B. cereus and other *Bacillus* species are ubiquitous organisms, present in virtually all environments.

B. cereus is responsible for two forms of food poisoning: **vomiting disease (emetic form)** and **diarrheal disease (diarrheal form).**

The **emetic form** of disease results from consumption of **contaminated rice. An intoxication** caused by ingestion of the enterotoxin, not the bacteria. Thus the incubation period after eating the contaminated rice is **short (1 to 6 hours)**, and the duration of illness is also short (<24 hours).

The **diarrheal form** of *B. cereus* food poisoning is a true infection resulting from ingestion of the bacteria in contaminated meat, vegetables, or sauces. With longer incubation period.

B. cereus **ocular infections** usually occur after traumatic, penetrating injuries of the eye with a soil-contaminated object.



Spores retain the malachite green dye in this special spore stain, and the vegetative cells are gray or colorless.

Clostridium difficile

C. difficile is a large (0.5 to 1.9 by 3.0 to 17 µm) anaerobic rod that freely forms spores in vivo and in culture. The vegetative cells die rapidly when exposed to oxygen.

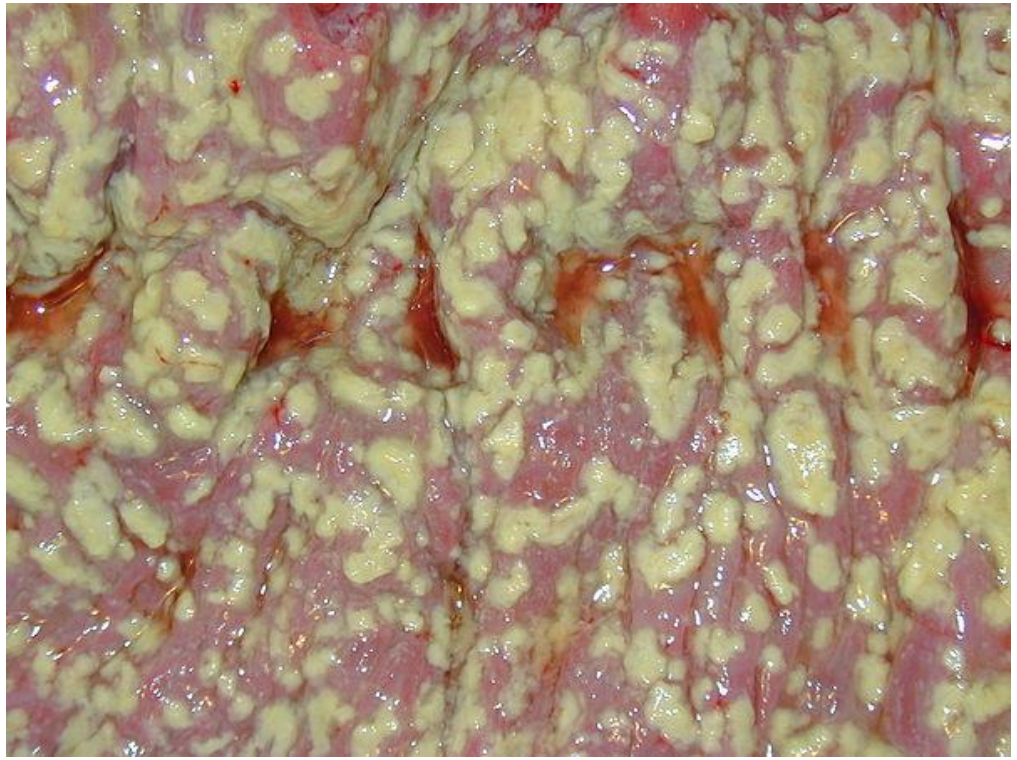
Transmitted feco-orally and commonly a **nosocomial infection**. *C. difficile* is part of the normal intestinal flora in a **small number** of healthy people and hospitalized patients.

C. difficile produces two toxins: an **enterotoxin (toxin A)** and a **cytotoxin (toxin B),** both of which may produce diarrhea and inflammation in infected patients**.** Range from mild diarrhea to severe life-threatening inflammation of the colon (e.g. **Pseudomembranous Colitis**).

The disease develops in people **taking antibiotics**, because the drugs **alter the normal enteric flora**, either **permitting overgrowth** of these relatively resistant organisms or making the patient more **susceptible to exogenous acquisition** of *C. difficile.*

Remarkable success with “fecal transplants” has been demonstrated, illustrating the fact that *C. difficile* does not become established when a healthy enteric population of bacteria is present.

Clostridium difficile

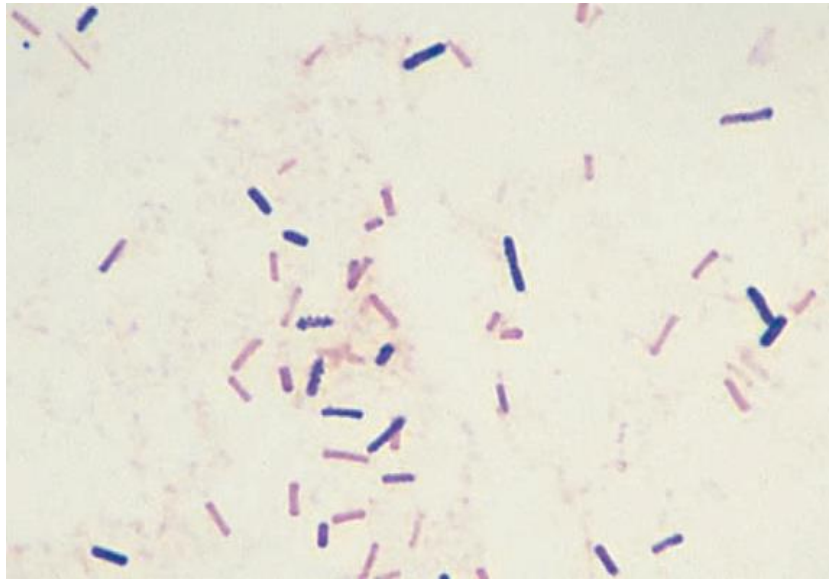


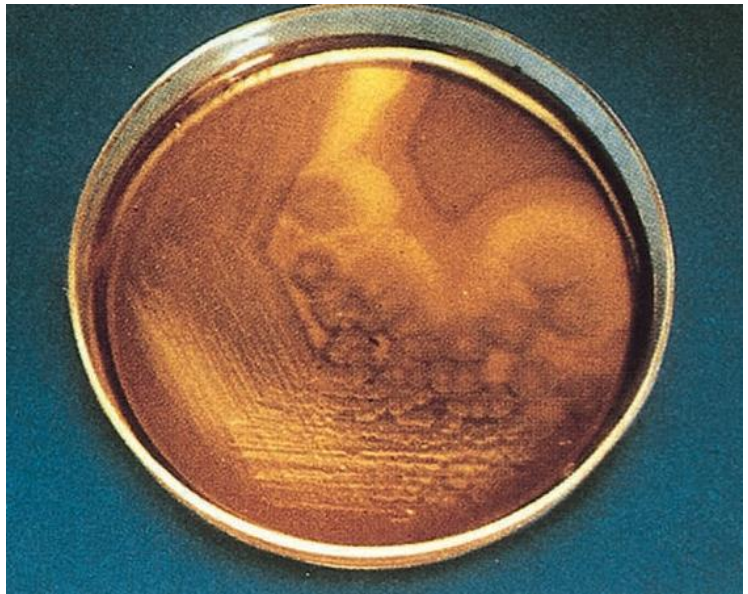
Pseudomembranous colitis is an inflammatory condition of the colon characterized by elevated yellow-white plaques that coalesce to form pseudomembranes on the mucosa.

Clostridium perfringens

C. perfringens is a large (0.6 to 2.4 × 1.3 to 19.0 µm), rectangular, gram-positive rod, with **spores rarely observed** either in vivo or after in vitro cultivation, an important characteristic that differentiates this species from most other clostridia. **Colonies** of *C. perfringens* are also distinctive, with their **rapid, spreading growth.**

Type A *C. perfringens* commonly inhabits the intestinal tract of humans and animals and is **widely distributed in nature**, particularly in soil and water contaminated with feces. Spores are formed under adverse environmental conditions and can survive for prolonged periods. Strains of types B through E do not survive in soil but colonize the intestinal tracts of animals and occasionally humans





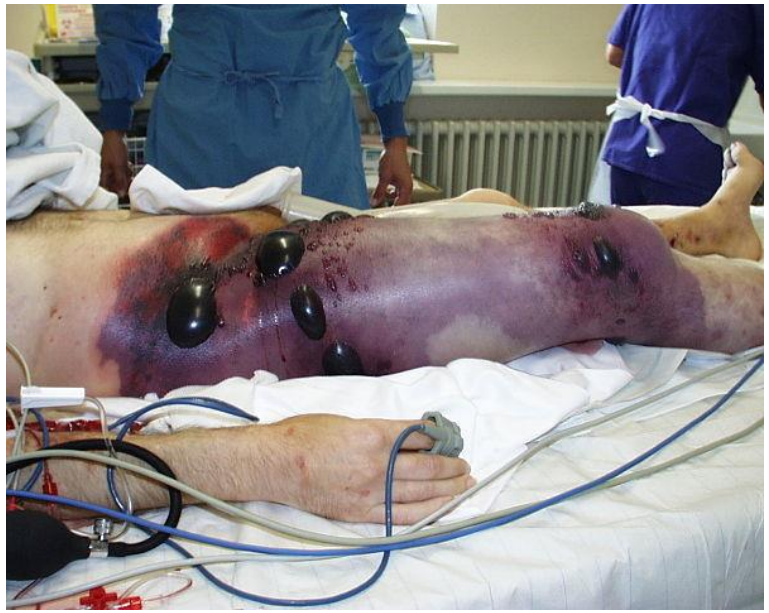
A presumptive identification of *C. perfringens* can be made by detection of a zone of **complete hemolysis** (caused by the theta toxin) and a wider zone of **partial hemolysis** (caused by the alpha toxin), combined with the characteristic microscopic morphology.

Clostridium perfringens

- *C. perfringens* is responsible for a range of soft-tissue infections including **cellulitis,** fasciitis or suppurative **myositis,** and **myonecrosis** with gas formation (caused by the metabolic activity of the rapidly dividing bacteria) in the soft tissue (**gas gangrene**). The toxin involved in gas gangrene is known as **α-toxin**, which inserts into the plasma membrane of cells, producing gaps in the membrane that disrupt normal cellular function

- **Clostridial food poisoning,** an **intoxication** characterized by (1) a short incubation period (8 to 12 hours), (2) a clinical presentation that includes abdominal cramps. (3) a clinical course lasting less than 24 hours.

- *C. perfringens* produces **enterotoxin,** primarily by type A strains, The enterotoxin is produced during the phase transition from vegetative cells to spores and is released in the alkaline environment of the small intestine when the cells undergo the terminal stages of spore formation **(sporulation).**



Treatment is usually **debridement and excision**, with amputation necessary in many cases. Water-soluble antibiotics (such as penicillin) alone are not effective because they **do not penetrate ischaemic muscles** sufficiently to be effective.

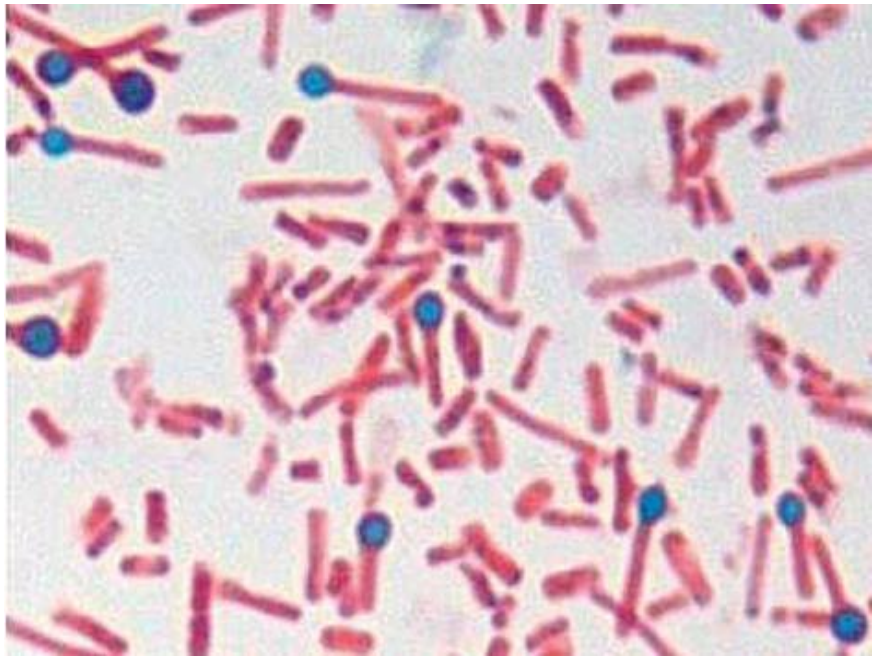
Clostridium tetani

- *C. tetani* is a large (0.5 to 2 × 2 to 18 µm), motile, spore-forming rod. The organism produces round, terminal spores that give it the appearance of a drumstick. *C. tetani* is **ubiquitous.** It is found in fertile soil and transiently colonizes the GI tracts of many animals, including humans.

- *C. tetani* produces two toxins, an oxygen-labile hemolysin **(tetanolysin)** and a plasmid-encoded, heat-labile neurotoxin **(tetanospasmin).**

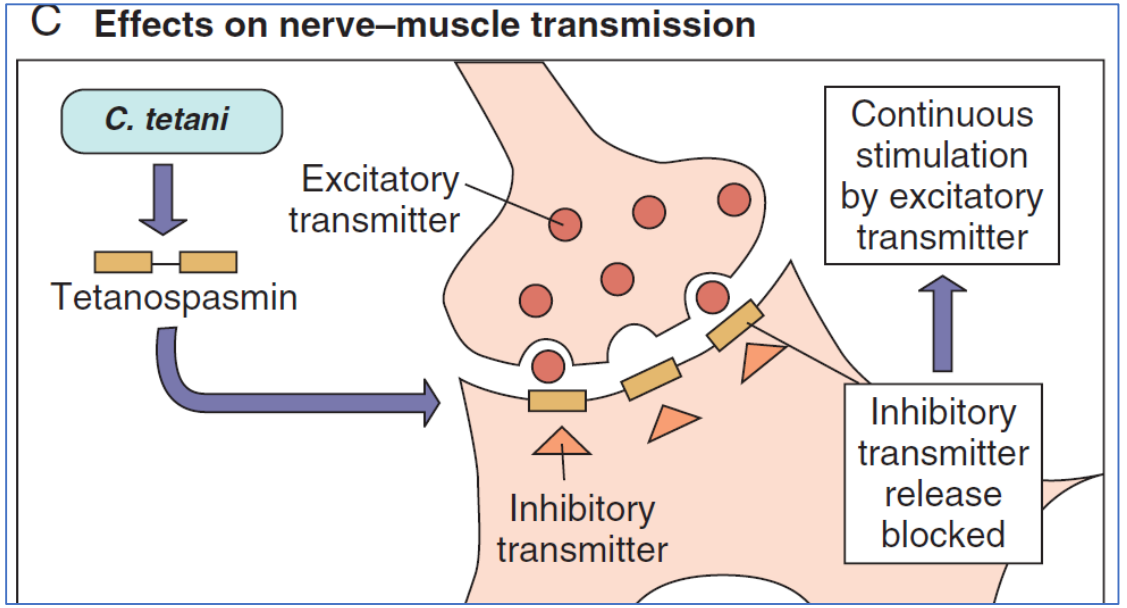
- Tetanospasmin **inactivates proteins that regulate release of the inhibitory neurotransmitters** glycine and gamma-aminobutyric acid (GABA). This leads to unregulated excitatory synaptic activity in the motor neurons, resulting in **spastic paralysis.**

- Disease is relatively rare because of the high incidence of **vaccine-induced immunity.**

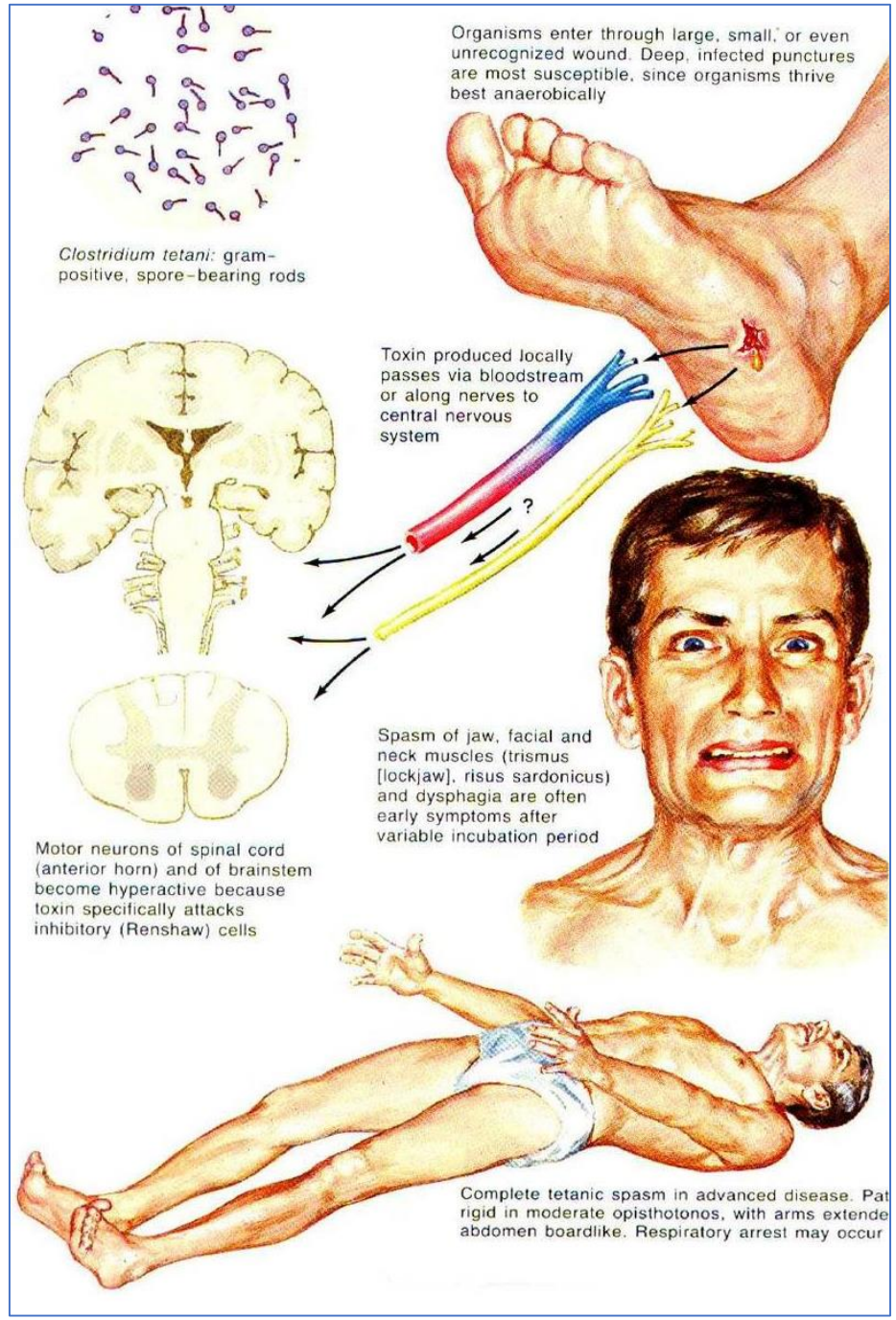


Clostridium tetani

Involvement of the masseter muscles (trismus or **lockjaw**) is the presenting sign in most patients. The characteristic **sardonic smile** that results from the sustained contraction of the facial muscles.



unregulated excitatory synaptic activity in the motor neurons, resulting in **spastic paralysis. Generalized tetanus** is the most common form.

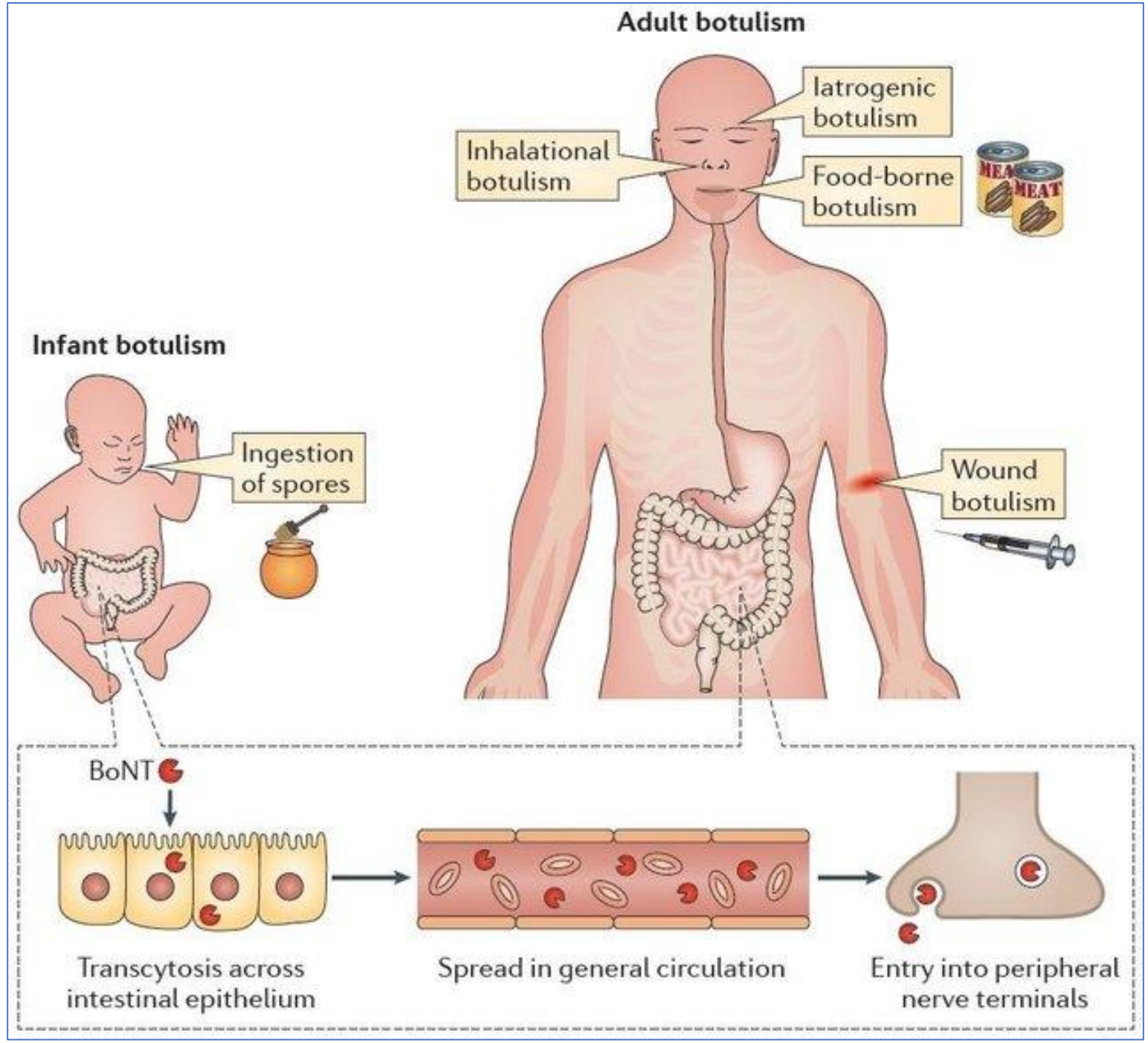

Organisms enter through large, small, or even unrecognized wound. Deep, infected punctures are most susceptible, since organisms thrive best anaerobically
Clostridium tetani: gram-positive, spore-bearing rods
Toxin produced locally passes via bloodstream or along nerves to central nervous system
?
Spasm of jaw, facial and neck muscles (trismus [lockjaw], risus sardonicus) and dysphagia are often early symptoms after variable incubation period
Motor neurons of spinal cord (anterior horn) and of brainstem become hyperactive because toxin specifically attacks inhibitory (Renshaw) cells
Complete tetanic spasm in advanced disease. Pat
rigid in moderate opisthotonos, with arms extende
abdomen boardlike. Respiratory arrest may occur

Clostridium botulinum

- The etiologic agents of botulism are a heterogeneous collection of large (0.6 to 1.4 × 3.0 to 20.2 µm), fastidious, spore-forming, anaerobic rods. *C. botulinum* is commonly isolated in soil and water samples throughout the world.

- Patients with **foodborne botulism (**most are associated with consumption of home-canned foods) typically become weak and dizzy 1 to 3 days after consuming the contaminated food. Bilateral descending **weakness of the peripheral muscles** develops in patients with progressive disease (flaccid paralysis), and death is most commonly attributed to **respiratory paralysis**.

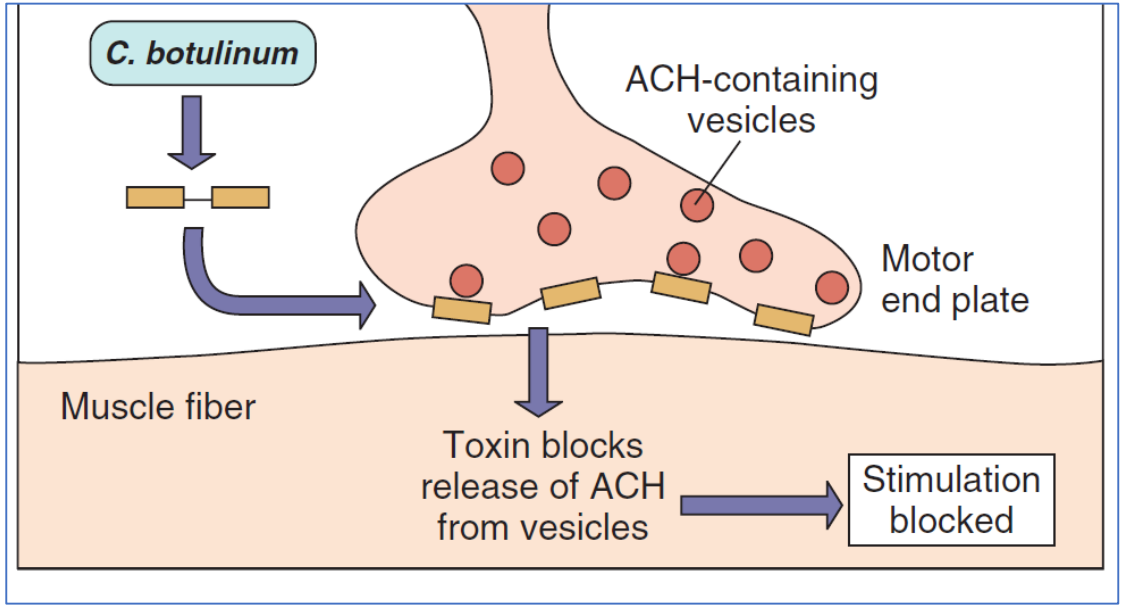
- **Infant botulism:** Associated with consumption of foods (e.g., honey, infant milk powder) contaminated with botulinum spores and ingestion of spore-contaminated soil and dust. In contrast with foodborne botulism, this disease is caused by neurotoxin produced in vivo by *C. botulinum* colonizing the GI tracts of infants.


Adult botulism
Iatrogenic botulism
Inhalational botulism
Food-borne botulism
MEAT
Infant botulism
Ingestion of spores
Wound botulism
BoNT
Transcytosis across intestinal epithelium
Spread in general circulation
Entry into peripheral nerve terminals

Clostridium botulinum

- Seven antigenically distinct botulinum toxins (A to G), human disease is associated with types A, B, E, and F.

- The botulinum neurotoxin remains at the neuromuscular junction, The botulinum endopeptidase then **inactivates the proteins that regulate release of acetylcholine,** blocking neurotransmission at peripheral cholinergic synapses. The resulting clinical presentation of botulism is a **flaccid paralysis.**



NON–SPORE-FORMING ANAEROBIC BACTERIA

- **Anaerobic Gram-Positive Cocci**: The anaerobic gram-positive cocci normally colonize the oral cavity, gastrointestinal (GI) tract, genitourinary tract, and skin. They produce infections when they spread from these sites to normally sterile sites.

- Although anaerobic cocci can be isolated from infections at all body sites, a predisposition for certain sites has been observed. In general, ***Peptostreptococcus*** species have been recovered more often **from subcutaneous and soft tissue abscesses and diabetes-related foot ulcers** than from intra-abdominal infections. Peptostreptococcus infections occur more often in chronic infections. Many infections caused by *peptostreptococcus* bacteria are **synergistic.**

Further reading:

- Murray - Medical Microbiology 8th Edition
  Section 4: Bacteriology
    Chapter 20: Bacillus
    Chapter 30: Clostridium