Clostridium

Clostridium

- anaerobic g+ bacilli, forming **endospores**
- ubiquitous
  - soil, water, sewage,GIT humans and animals
- more than 130 species, most are harmless
- Ability to survive in adverse environment
- Rapid growth in oxygen deprived conditions
- Production of **toxins**
  - „pore-forming“ toxins (gas gangrene, GIT diseases)
  - Neurotoxins (tetanus, botulism)

Histotoxic group

***C. perfringens* type A-E** - exogenously acquired more commonly than endogenously

C. *septicum, novyi, histolyticum, sordellii* - endogenously-acquired

- **tissue** infections, wounds, surgical infections
- crush type injuries, open fractures (soil contamination), knife/gunshot wounds (bowel contents leakage)
- Sudden onset, foul-smelling wounds, gas bubbles

  a. crepitant cellulitis (diabetic patients)

  b. myonecrosis (gas gangrene)

  c. Gynecologic infections (*C. sordellii* – abortion)

  d. Endophtalmitis (surgery)

Enterotoxigenic group

- **food poisoning - *C. perfringens* type A**

  8-30h after ingestion of large numbers of organisms on contaminated **meat products**, enterotoxin produced, cramps and diarrhea, no fever, no vomiting, self-limiting

- **enteritis necroticans** - ***C. perfringens* type C**
  - ischemic necrosis of the jejunum
  - alfa and beta toxin-producing strains – outbreak in developing countries

- **necrotizing enterocolitis – *C. perfringens***
  - **GIT of premature infants – bowel necrosis**

- ***C. difficile* –** endo or exogenously-acquired person-to-person in hospital, nosocomial inf.!
  - antibiotic-associated diarrhea (CDAD)
  - antibiotic-associated **pseudomembranous colitis – toxic megacolon**

Tetanus

- exogenously acquired — **C. *tetani***
- Puncture wound
- **Neurotoxin – tetanospasmin**
  - secreted as inactive polypeptide – cleaved by protease to active form – affect GABA neurotransmitters – block inhibitory impulses to CNS neurons – result in prolonged muscle spasms
- Preventable by tetanus toxoid
- 1 mil. cases per year
- Forms:
  - neonatal - contaminated umbilical stump
  - In drug users

Botulism

- exogenously acquired — ***C. botulinum***
- **neurotoxin – 8 serotypes (A - H)**
- A, B, and E – botulism in humans
- **Toxin - 3 domains** – 2 bind to nerve terminals (translocation of toxin to cytosol), 1 domain contain metalloprotease that inhibit release of neurotransmitter by peripheral nerves – flacid paralysis

a. foodborne - intoxication,1-2days incubation period

b. infant - ingestion of spores in honey

c. wound - symptoms similar to foodborne, but 4 or more days incubation

d. Other – adult intestinal toxemia, iatrogenic botulism, bioterrorism agent – inhalation form

Spores

- *Clostridium* form endospores under **adverse environmental conditions**
- Spores are a **survival** mechanism
- Spores are characterized on the basis of position, size and shape
- Most *Clostridium* spp., including ***C. perfringens*** **and** ***C. botulinum*****, have ovoid subterminal (OST) spores**
- ***C. tetani*** **have round terminal (RT) spores**

Clostridium Associated Human Disease

Species	Human Disease	Frequency
C. difficile	Antibiotic-associated diarrhea, pseudomembranous colitis	Common
C. perfringens	Soft tissue infections (i.e., cellulitis, suppurative myositis, myonecrosis or gas gangrene), food poisoning, enteritis necroticans, septicemia	Common
C. septicum	Gas gangrene, septicemia	Uncommon
C. tertium	Opportunistic infections	Uncommon
C. botulinum	Botulism	Uncommon
C. tetani	Tetanus	Uncommon
C. barati	Botulism	Rare
C. butyricum	Botulism	Rare
C. histolyticum	Gas gangrene	Rare
C. novyi	Gas gangrene	Rare
C. sordellii	Gas gangrene	Rare

Clostridium perfringens

Morphology and Physiology

- **gram+, large, rectangular bacilli** (rod)
- **spores - rarely seen** *in vitro* or in clinical specimens (ovoid, subterminal)
- **non-motile,** but **rapid spreading growth** on blood agar mimics growth of motile organisms
- aerotolerant

Lab Identification

- Broad spectrum of diseases
- Colonisation, mild or life-threatening disease
- Most commonly isolated in clinical specimens
- **gas** from glucose fermentation
- **double zone of hemolysis** on blood agar
- beta-hemolysis - theta toxin,
- alpha-hemolysis - alpha toxin
- **Nagler´s reaction-** precipitation on egg yolk media; alpha toxin (lecithinase)

Pathogenicity Determinants

four **major lethal toxins** (alpha (α), beta (β), epsilon (ε), and iota (ι) toxins) and an **enterotoxin**

- **six minor toxins** (delta(δ), theta(θ), kappa(κ), lambda(λ), mu(μ), nu(η)toxins) **& neuraminadase**
- subdivided into five types (A-E) on basis of production of major lethal toxins
- **C. *perfringens* Type A** only major lethal toxin is alpha toxin responsible for **histotoxic** and **enterotoxigenic** infections in humans;
- **C.perfringens Type C** - necrotizing enteritis, beta toxin

C. perfringens Virulence Factors

	Virulence Factors	Biologic Activity
Major	α toxin	Lethal toxin; phospholipase C (lecithinase); increases vascular permeability; hemolysin; produces necrotizing activity
Major	β toxin	Lethal toxin; necrotizing activity
Major	ϵ toxin	Lethal toxin; permease
Major	ι toxin	Lethal binary toxin; necrotizing activity; adenosine diphosphate (ADP) ribosylating
Minor	δ toxin	Hemolysin
Minor	θ toxin	Heat- and oxygen-labile hemolysin; cytolytic
Minor	κ toxin	Collagenase; gelatinase; necrotizing activity
Minor	λ toxin	Protease
Minor	μ toxin	Hyaluronidase
Minor	ν toxin	Deoxyribonuclease; hemolysin; necrotizing activity
	Enterotoxin	Alters membrane permeability (cytotoxic, enterotoxic)
	Neuraminidase	Alters cell surface ganglioside receptors; promotes capillary thrombosis

Exotoxins Associated with C. perfringens Types A-E

Type of Isolate	Major Lethal Toxins			
	α	β	ε	ι
A	+	−	−	−
B	+	+	+	−
C	+	+	−	−
D	+	−	+	−
E	+	−	−	+

Diagnosis/Treatment

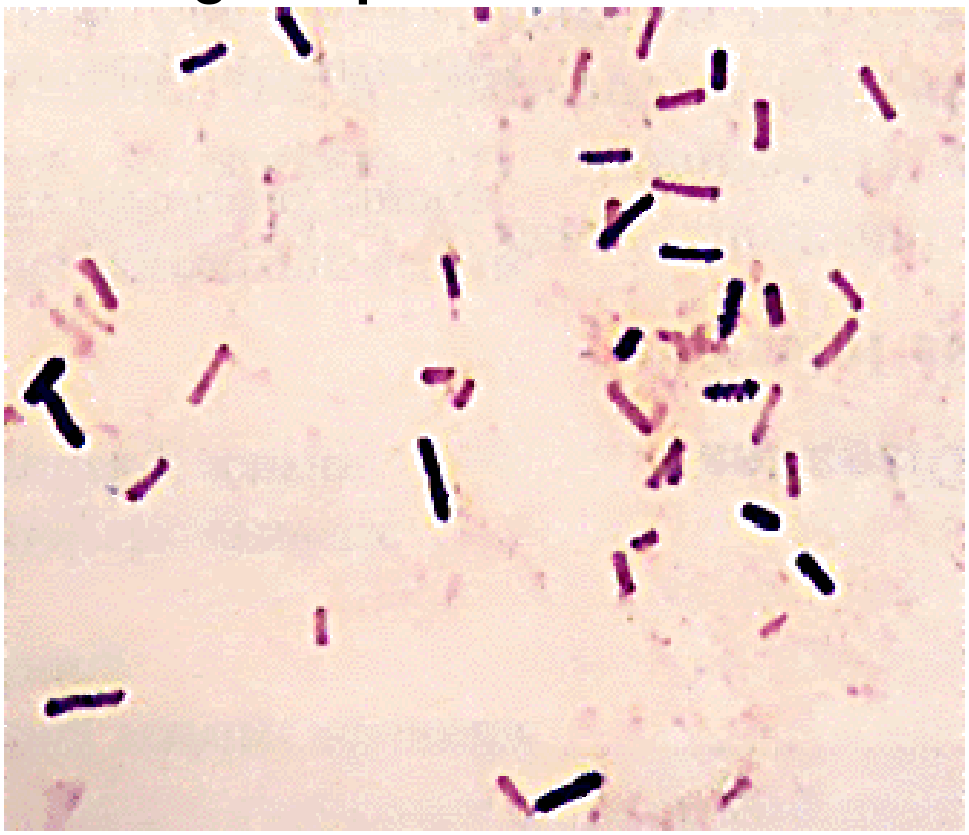
- Early diagnosis and aggressive treatment essential
- removal of necrotic tissue **(surgical debridement)**
- **Penicillin G in high doses** if more serious infection

Of poorly defined clinical value are:

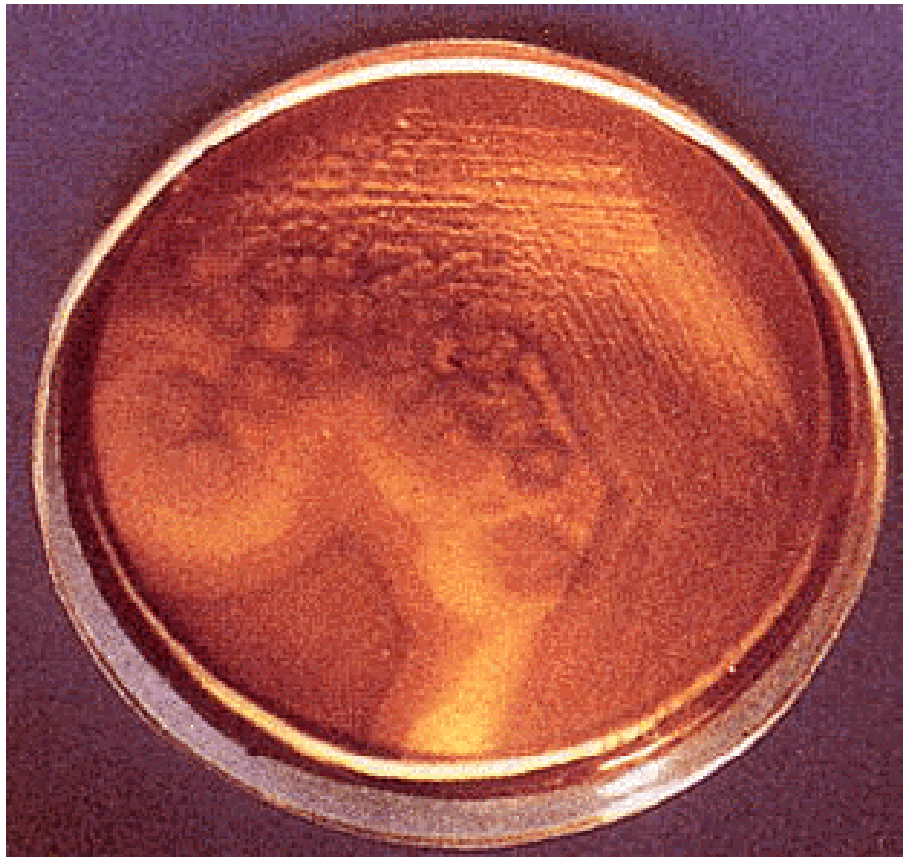
- administration of antitoxin
- hyperbaric oxygen (dive chamber) adjunct therapy (??inhibit growth of anaerobe??)

Micro & Macroscopic C. perfringens

NOTE: Large rectangular gram-positive bacilli

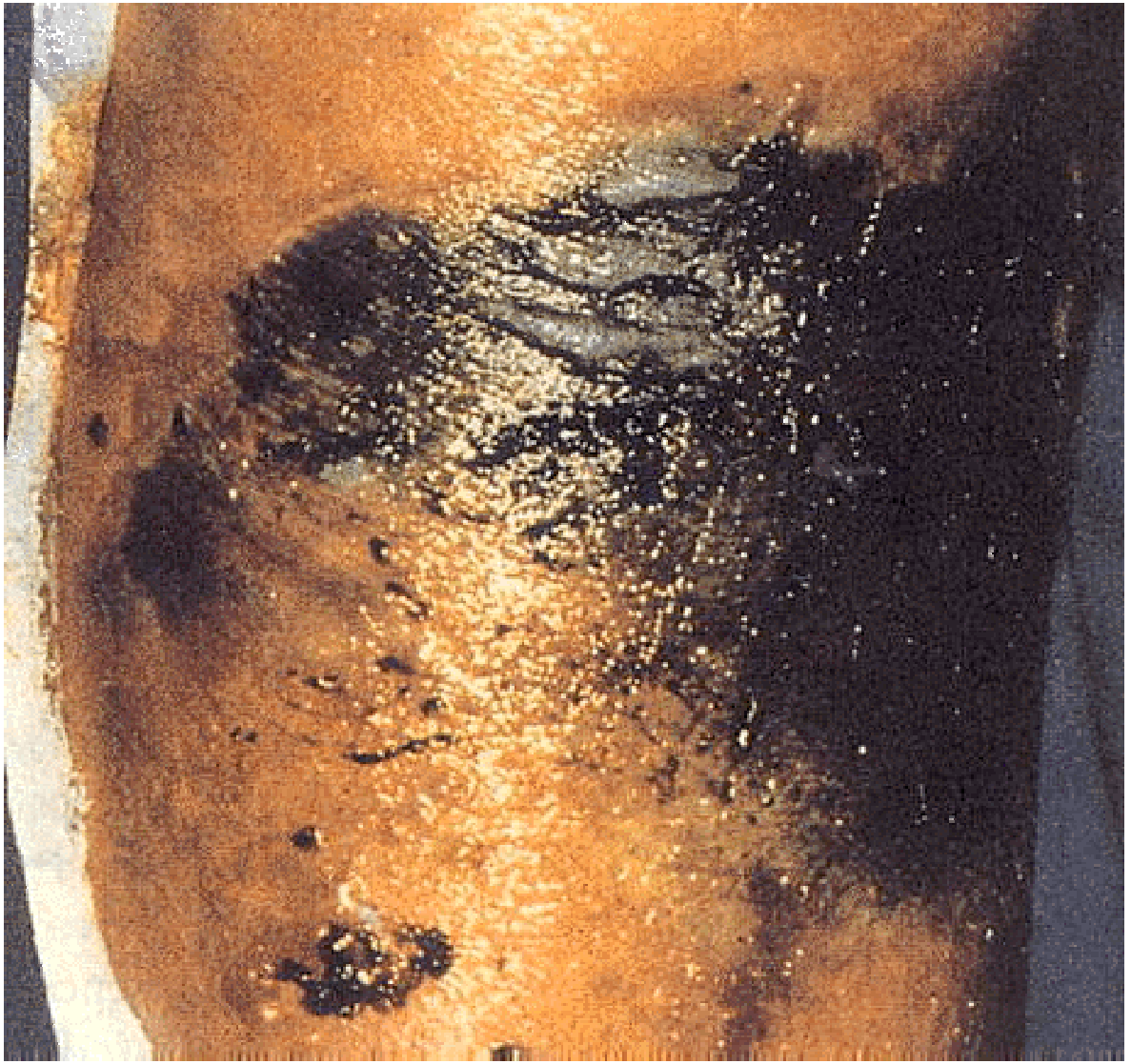


NOTE: Double zone of hemolysis

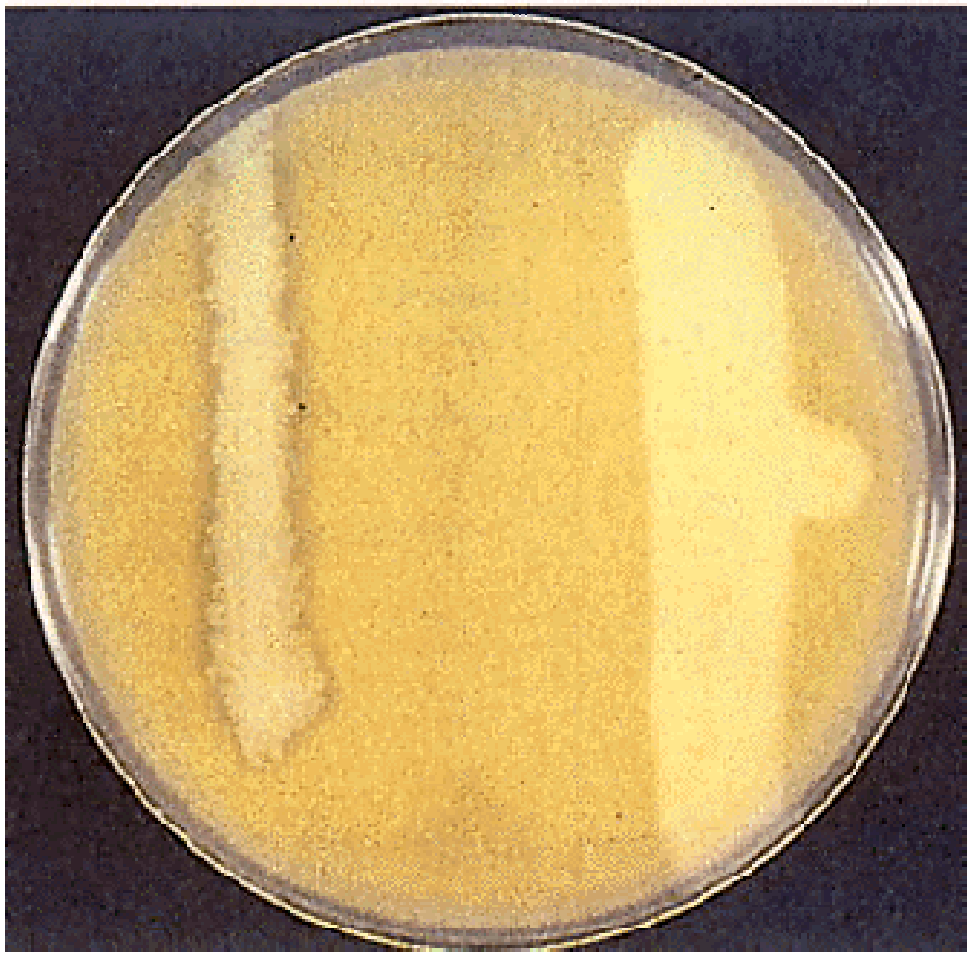


Inner beta-hemolysis = θ toxin
Outer alpha-hemolysis = α toxin

Clostridial Cellulitis



C. perfringens Nagler Reaction



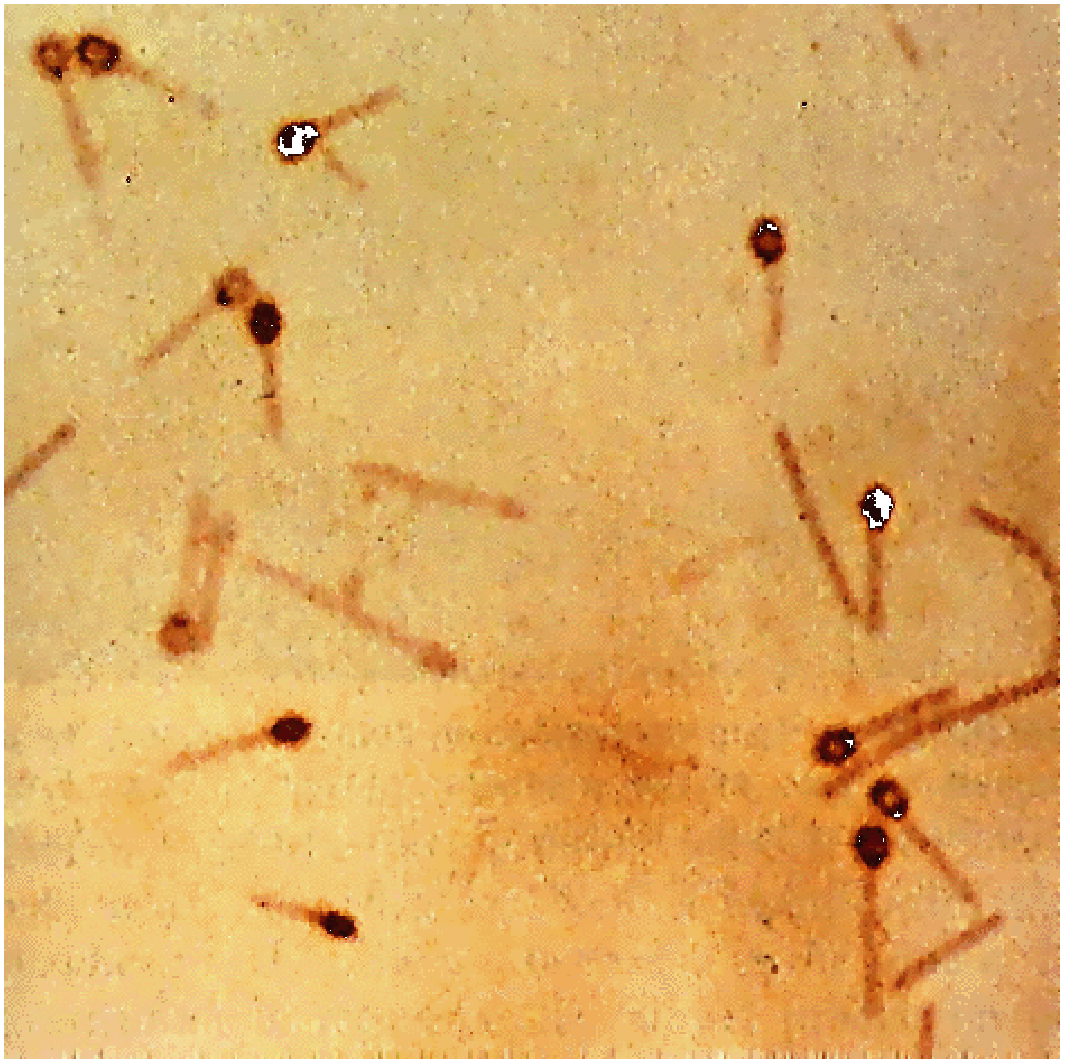
NOTE: **Lecithinase (α-toxin; phospholipase) hydrolyzes phospholipids in egg-yolk agar around streak on right. Antibody against α-toxin inhibits activity around left streak.**

Clostridium tetani

Morphology and Physiology

- long thin gram-positive organism that stains gram negative in old cultures
- **round terminal spore** gives drumstick appearance
- motile by peritrichous flagella
- grow on blood agar or cooked meat medium
- beta-hemolysis exhibited by isolated colonies
- spores resist boiling for 20 minutes

Clostridium tetani Gram Stain

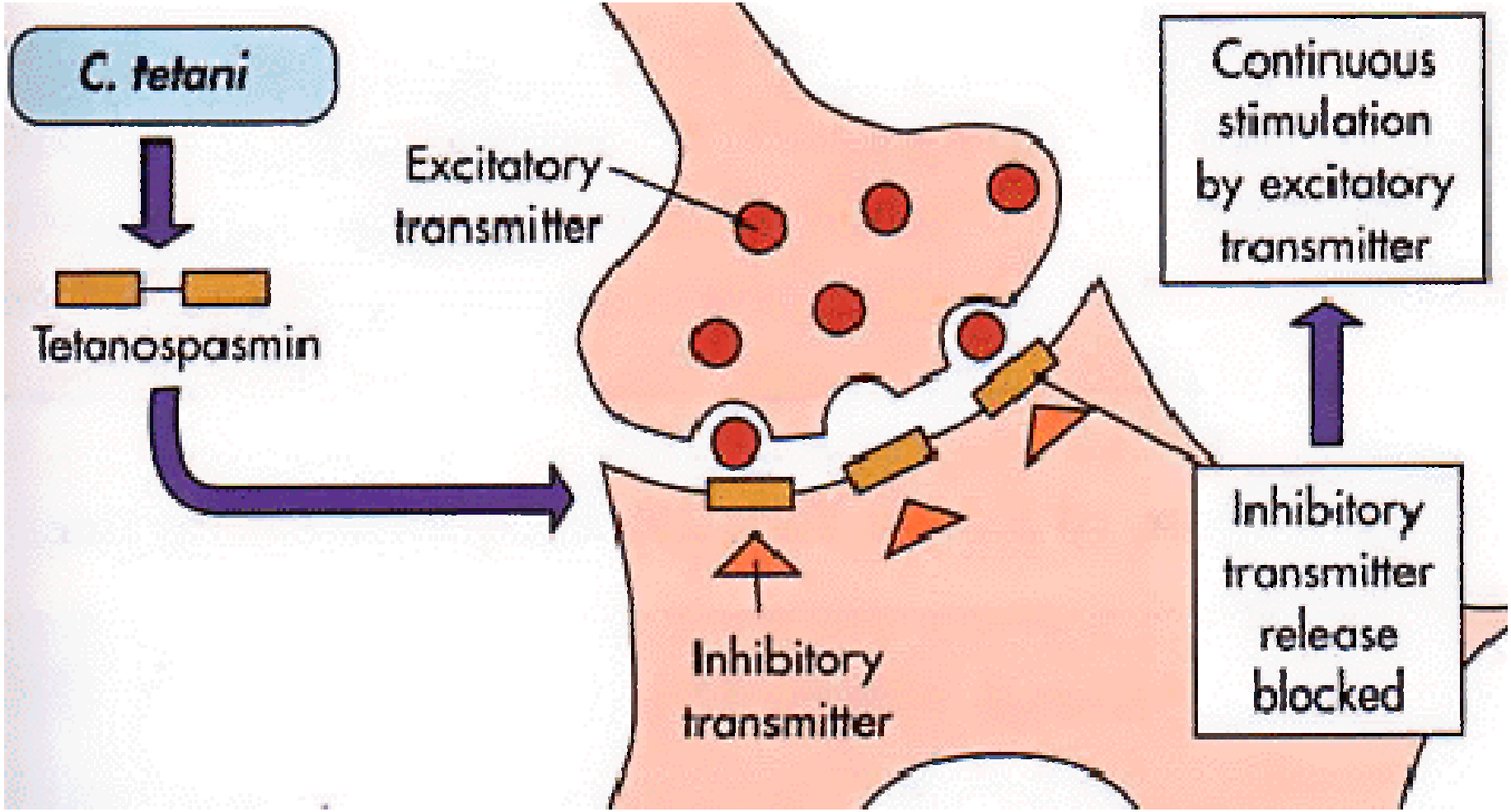


NOTE: **Round terminal spores give cells a "drumstick" or "tennis racket" appearance.**

Pathogenicity Determinants

- plasmid-mediated A-B neurotoxin **(tetanospasmin)** produced intracellularly
- acts by blocking the release transmiters for inhibitory synapses
- excitatory synaptic activity is unregulated
- muscle spasms (spastic paralysis) (trismus (lockjaw), risus sardonicus, opisthotonus),
- cardiac arrhythmias, fluctuations in blood pressure

Mechanism of Action of Tetanus Toxin

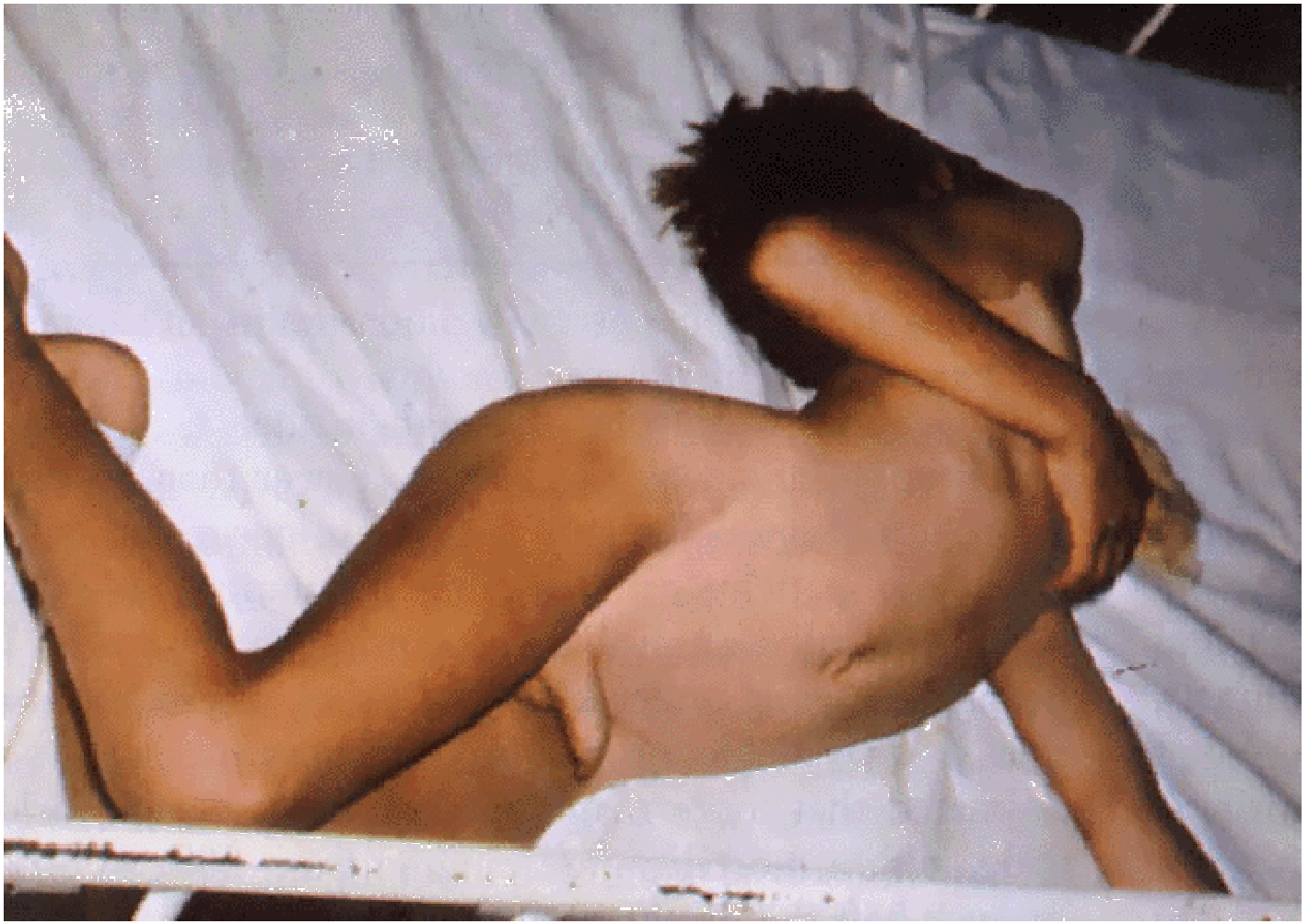


Diagnosis/Treatment/Prevention

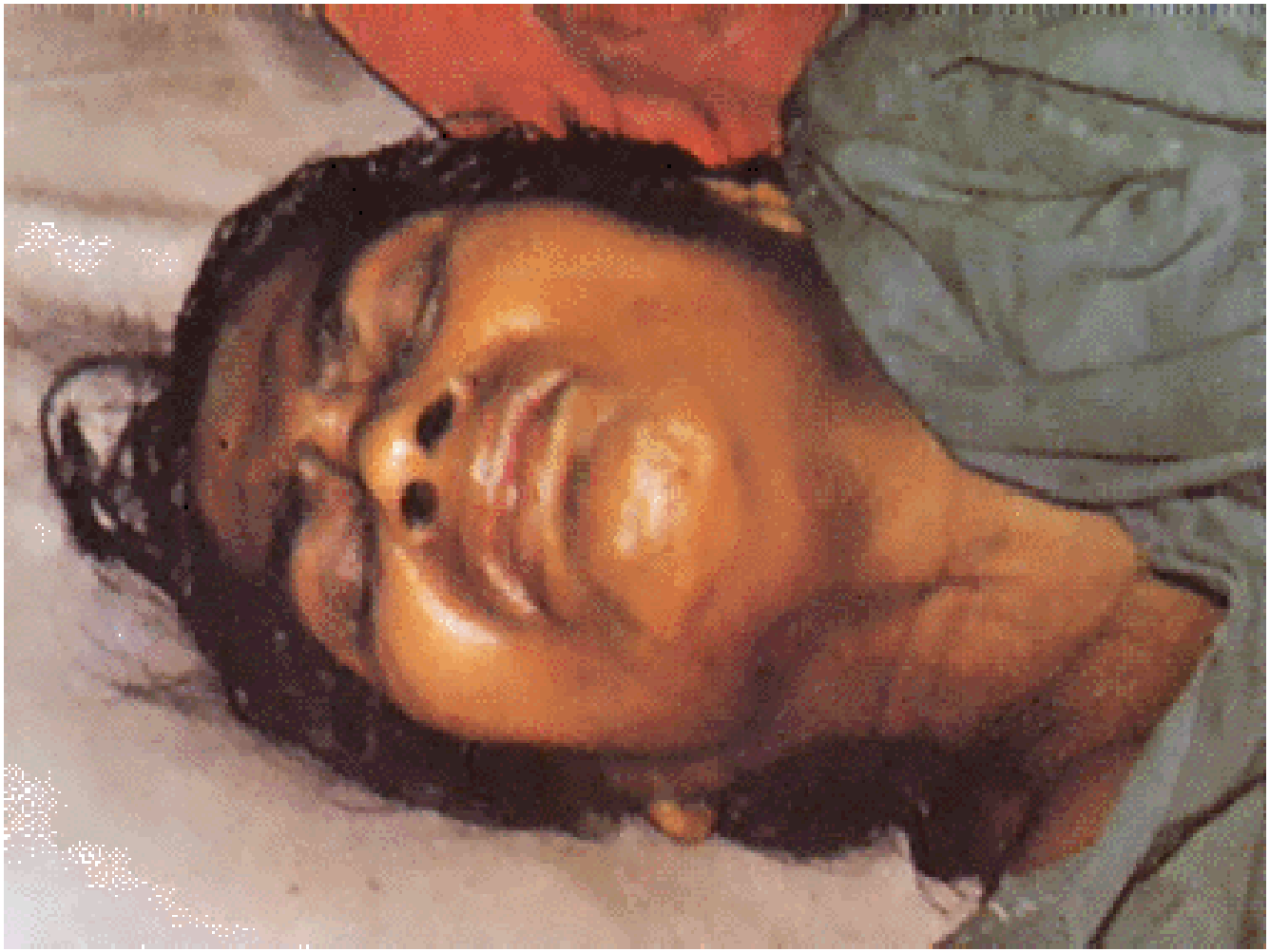
- DG: empirical on basis of clinical manifestations
- TH:
- **antitoxin** administered to bind free tetanospasmin
- **Surgical** debridement of the wound,
- **control spasms**
- **G-PNC** to eliminate vegetative bacteria that produce neurotoxin

- **passive immunity** (human tetanus immunoglobulin)
- **vaccination** (active) three doses of toxoid followed by boosters

Opisthotonos in Tetanus Patient



Risus Sardonicus in Tetanus Patient



Clostridium botulinum

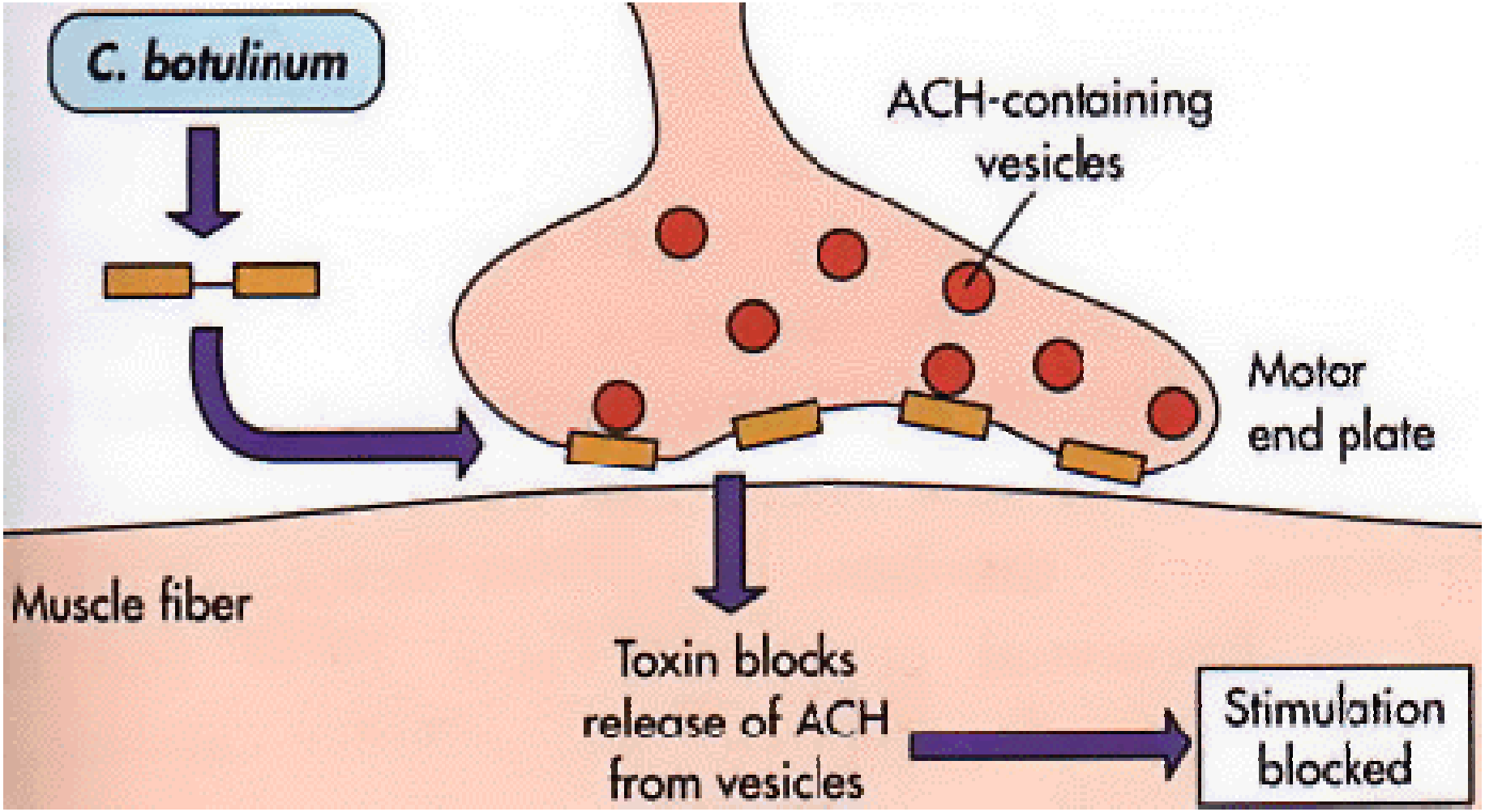
Morphology and Physiology

- heterogeneous group
- fastidious strictly anaerobic g+ bacilli
- motile by peritrichous flagella
- **heat-resistant spores (ovoid, subterminal)**
- 8 botulinum toxins A to H (A-B toxins)
- phage mediating systemic acting neurotoxin
- Spores can survive for more than 30 years

Pathogenicity Determinants

- lethal foodborne intoxication (not infection!)
- ID: 6 hrs – 8 days
- one of most extremely potent neurotoxins known
- „Good“ biological weapon
- Toxin enters bloodstream from mucosal surface
- blocks release of presynaptic **acetylcholine**
- blocking muscle stimulation
- Paralysis occurs beginnig with cranial nerves

Mechanism of Action of Botulinum Toxin



Categories of botulism

- Foodborne
  - eating food with botulotoxin
- Intestinal – neonates
  - ingesting spores which germinate and produce toxin in the intestine
- Wound
  - Spores germinate in the wound
- Inhalation
  - bioterrorism only, not natural

Clinical symptoms

- **Early:** nausea, vomiting,muscle weakness, dizziness, constipation
- **Later:** blurred/double vision, droping eylids, difficulty in swallowing and speaking, but patient is afebrile and alert
- **Final:** death due to respiratory paralysis

C.botulinum

- **Antigenic Structure**
- species divided into **four groups (I-IV)** based on type of toxin produced and proteolytic activity
- **eight antigenically distinct botulinum toxins (types A to H)**
- somatic antigens - heat stable and heat labile; spore antigens - more specific
- **Lab Identification**
- microscopic detection or culture are often unsuccessful (few organisms and slow growing)
- toxin detected and typed in lab via toxicity and antitoxin neutralization tests in mice or by ELISA

Diagnosis/Treatment/Prevention

- **crucial to rapidly diagnose** (symptoms often confusing); note the type of botulinum toxin involved
- treatment should be administered as quickly as possible on basis of clinical diagnosis
- **ventilatory support**
- **trivalent (A, B, E) antitoxin binds free toxin in bloodstream**
- administer **gastric lavage & penicillin eliminates organisms from GI tract**
- **care in home canning** and in heating of home-canned food; toxoid is available

Botulism diff. dg

- Guillain Barré sy
- Myastenia gravis
- CNS infection
- Poliomyelitis
- Tick paralysis
- Drug intoxication
- Psychiatric illness

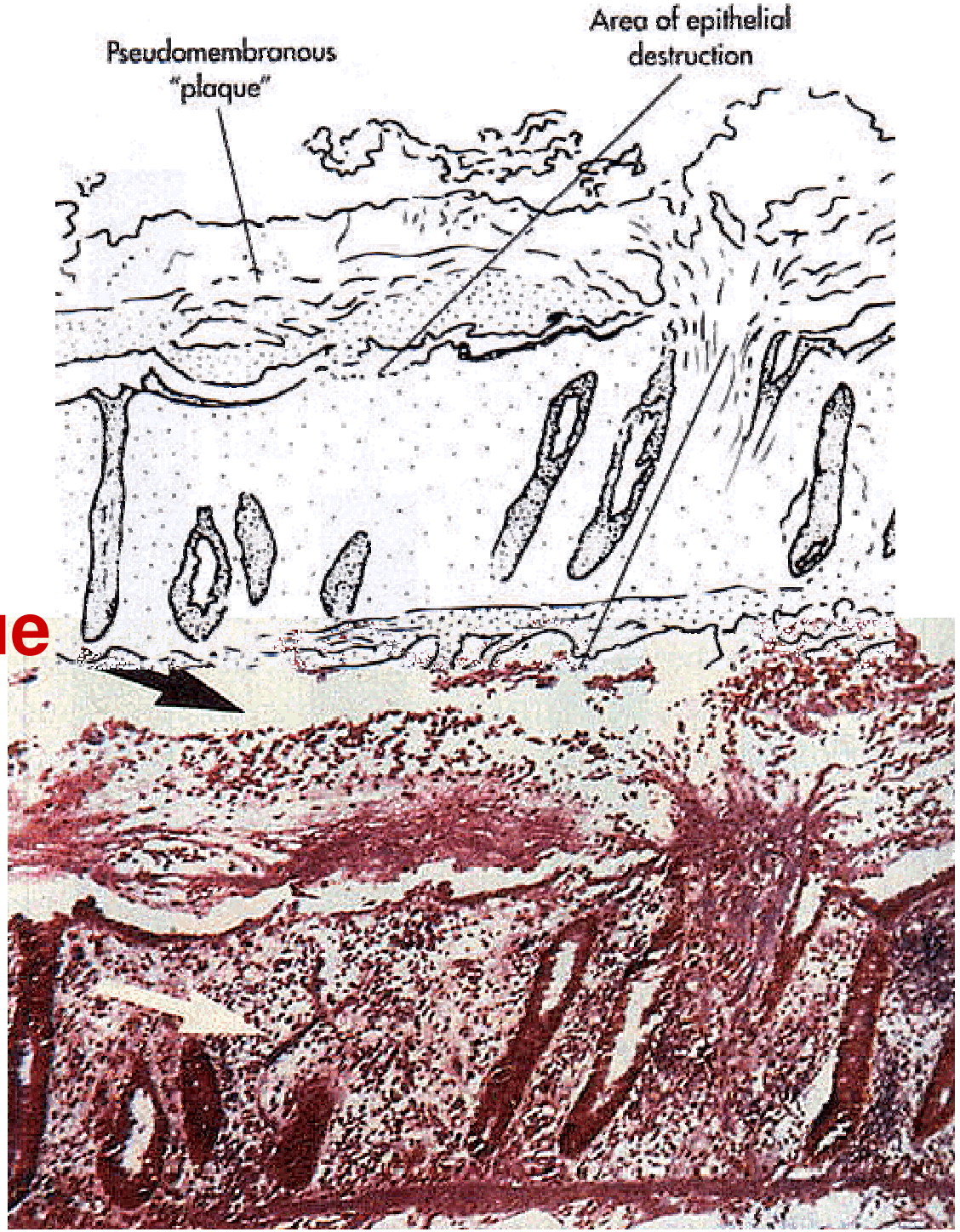
Therapeutic use

- Cosmetic use
- Sweating disorders
- Strabismus
- Smooth muscle hyperactive disorders
- Spasticity
- Involuntary muscle activity
- Localized muscle spasms and pain

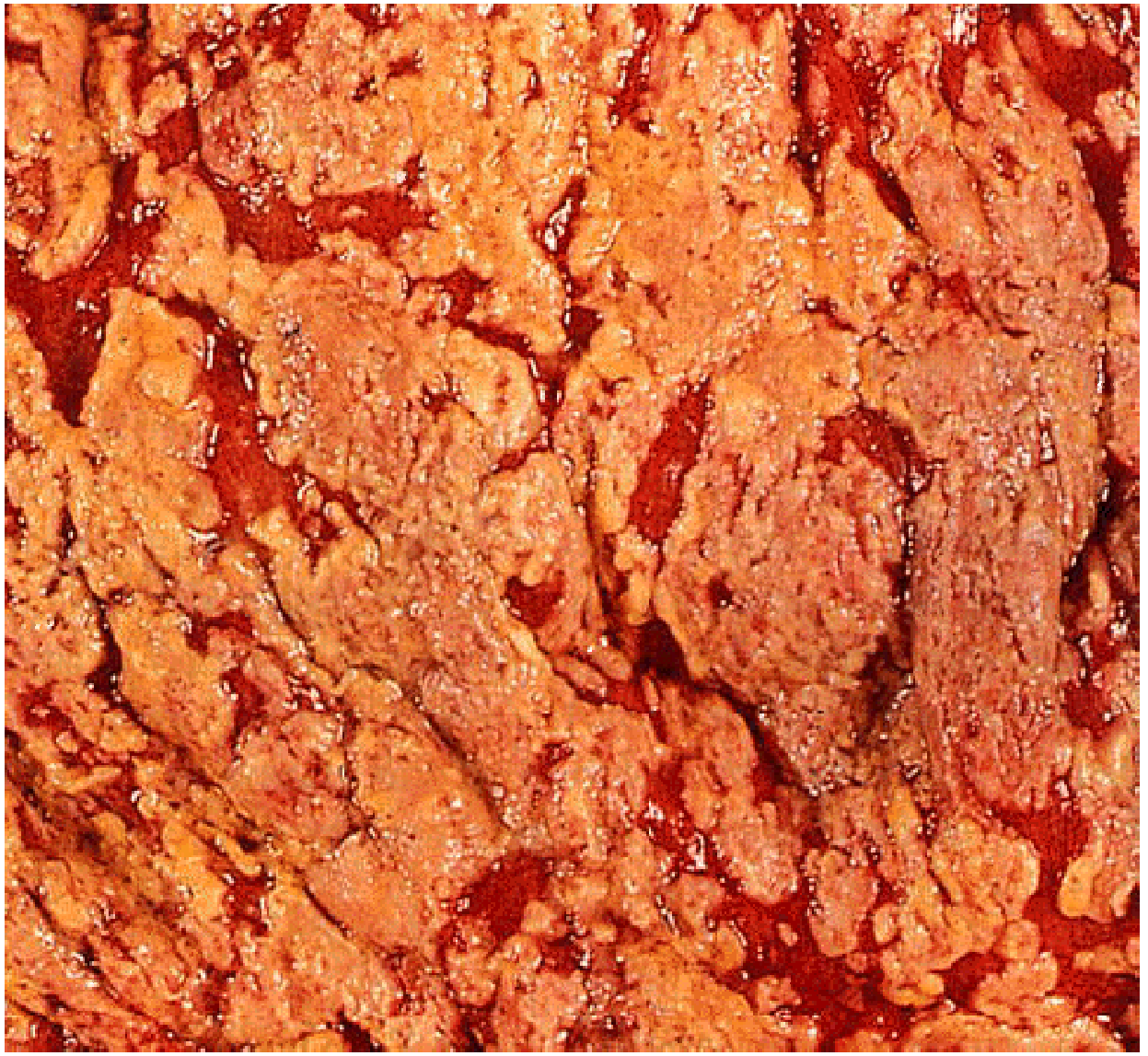
Clostridium dificile

Antibiotic-Associated Colitis

Plaque



Antibiotic-Associated Colitis



C. dificile Virulence Factors

Virulence Factor	Biologic Activity
Enterotoxin (toxin A)	Produces chemotaxis; induces cytokine production with hypersecretion of fluid; produces hemorrhagic necrosis
Cytotoxin (toxin B)	Induces depolymerization of actin with loss of cellular cytoskeleton
Adhesin factor	Mediates binding to human colonic cells
Hyaluronidase	Produces hydrolytic activity
Spore formation	Permits organism's survival for months in hospital environment