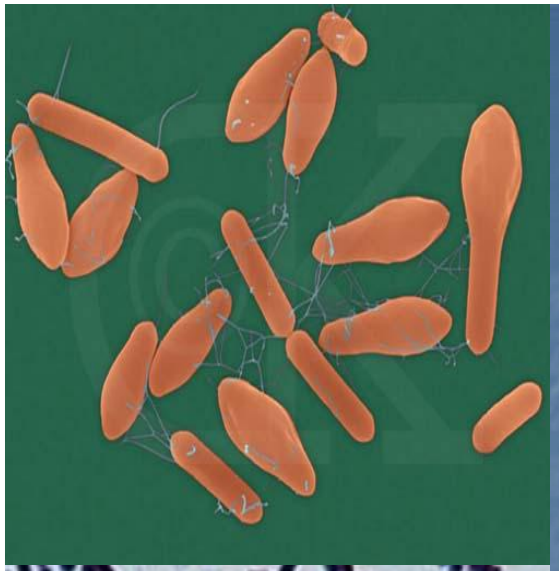
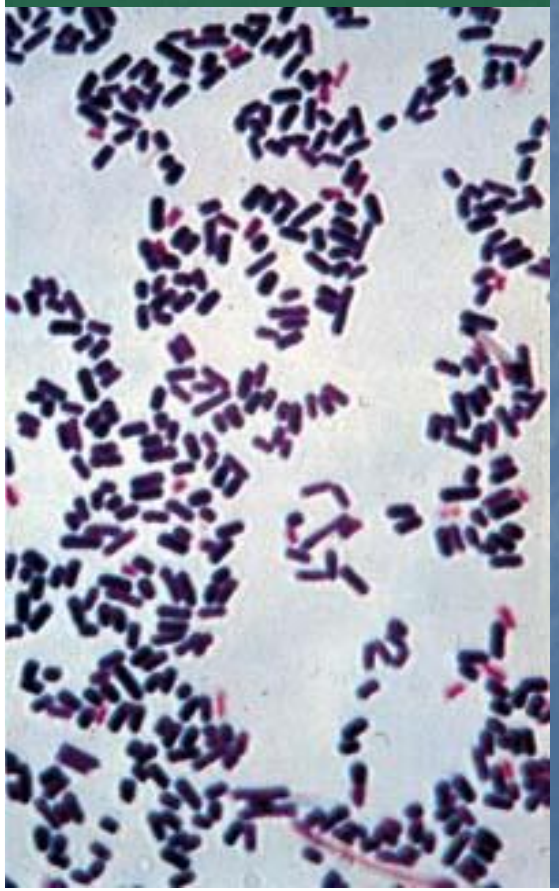
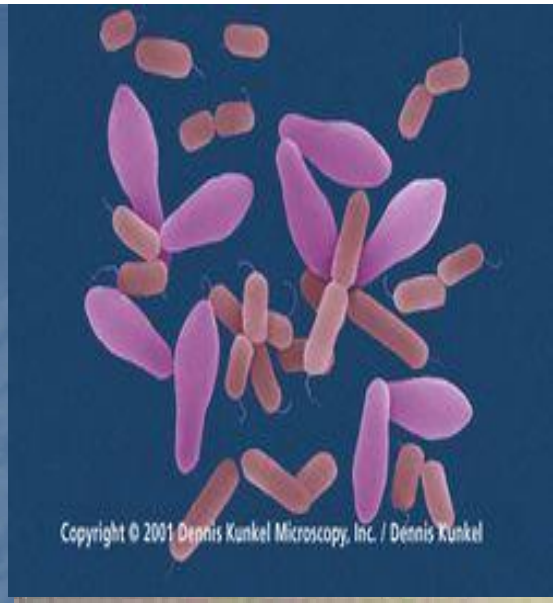
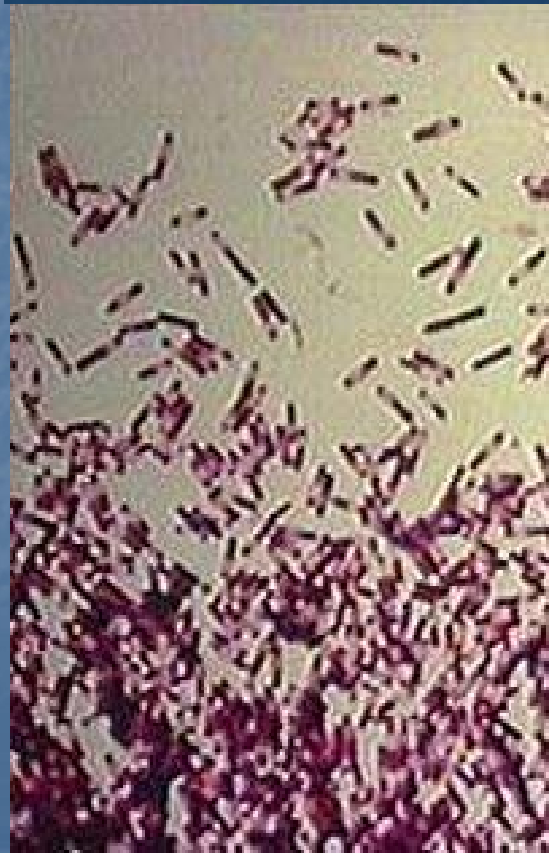




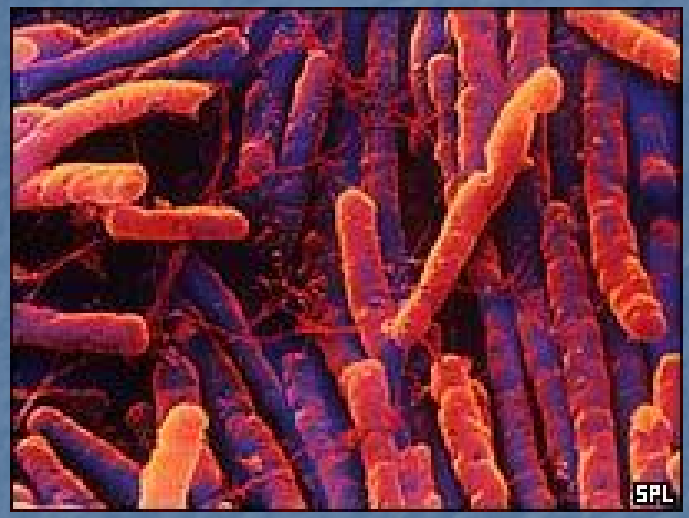
Clostridium





Introductory Characteristics

- Obligate anaerobes
- Gram positive
- Capable of producing endospores
- Rod-shaped, named after Greek word for spindle, *kloster*
- Club-shaped, as well: endospores form club end

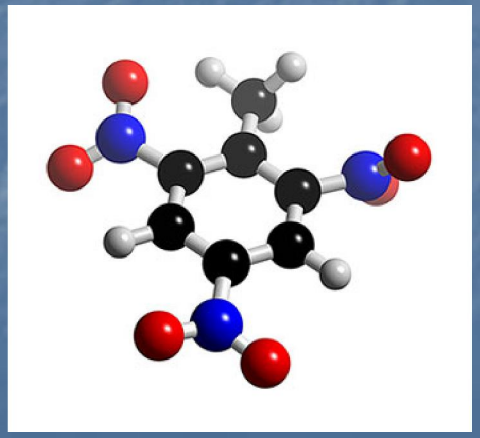


Connection to Aquatic Microbiology

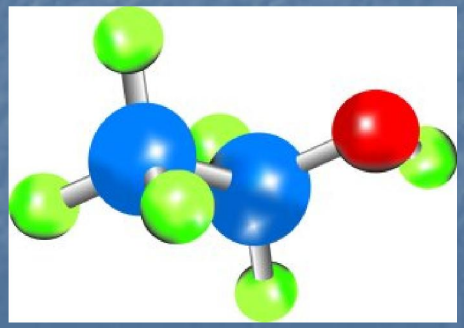
- Common habitat: anaerobic aquatic sediments
- Dormant spores restricted to fermentative metabolism: products include butyric acid, acetic acid, butanol, acetone, CO_2 and H_2 gas
- Play important role in biodegradation and carbon cycling

Free-living, non-pathogenic *Clostridium* bacteria

- *C. acetobutylicum*:
  - "Weizmann organism"
  - Used to generate acetone and biobutanol from starch since 1916 for use in gunpowder and TNT



- *C. thermocellum:*
  - Uses lignocellulosic waste to form ethanol
  - Requires no cooling system



Enumeration Method

- **TSC Agar**

  Tryptose Sulfite Cycloserine Agar

  1. Thin Pre-Poured surface and then overlay 1 m sample with 15 ml agar
  2. Pre-poured Plated – 0.1 ml sample and then thin overlay

- **Considerations**
  - Blend for only 1 min on low speed
    - Minimize Oxygen Incorporation
  - Gently Shake Dilution Bottles

TSC AGAR

- Selective and Differential
  - Cycloserine – Antibiotic (*C. perfingens* is resistant)
    - Add After Autoclaving
  - Sulfite
    - Reduced to Sulfide (Black)
  - Egg Yolk – Differential
    - Add After Autoclaving
    - NO Egg Yolk in Overlay
    - *C. perfringens* produces lecthinase

Typical Colonies

- Black
- Small
- Opaque zone surrounding colony
- Countable Plates
  - 20-200

Anaerobic Environments

- Reducing Compounds
  - Thioglycholate
  - Cystein
  - Anything with –SH
  - Must Use Indicator
- Gas Pack
  - Hydrogen + Oxygen = Water
  - Produces Hydrogen to Form Water in Jar

Anaerobic Incubation

- Anaerobic Jar
  - Impermeable to Oxygen
- Catalyst
  - Platinum or Palladium
  - In Lid or on Gas Pack
- Gas Pack
  - Uses Oxygen and Replaces with Carbon Dioxide

Anaerobic Incubation

- Plates should NOT be placed upside down
  - Water from Gas Pack Reaction Gets into Lids and then onto plates when inverted
- Redox Indicator
  - Methylene Blue
    - Low Redox – Blue
    - High Redox - White

Confirmation

- Select Typical Colonies
- Thioglycollate Broth
  - Thioglycollic Acid – Maintains Low Redox Potential with –SH
  - Resazurin – Redox Indicator
    - NO Oxygen – White
    - Oxygen – Pink
  - Should be less than 30% Pink After Autoclaving
  - MUST Make Fresh

Iron-Milk Media

- Confirmation

1. Lactose Fermentation
    1. Lactose to Glucose +Galactose
    2. Glucose to Lactic Acid, Carbon Dioxide, Hydrogen and Butyric Acid
        1. Acid – Clot Formation
        2. CO2 and H – Break Up Clot

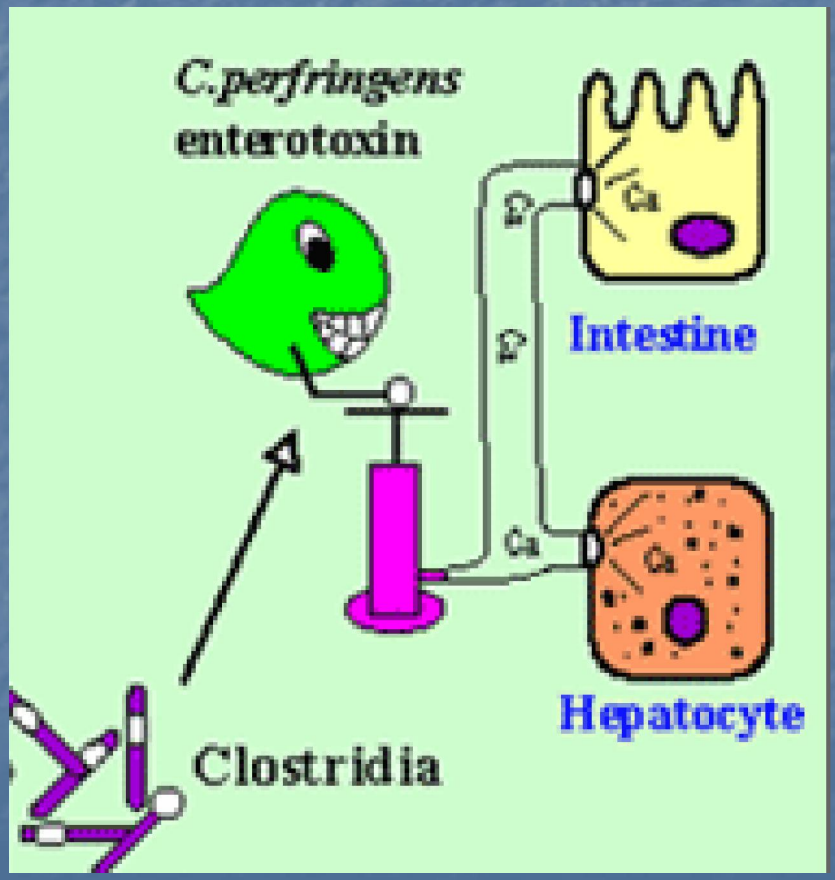
Iron Milk Media

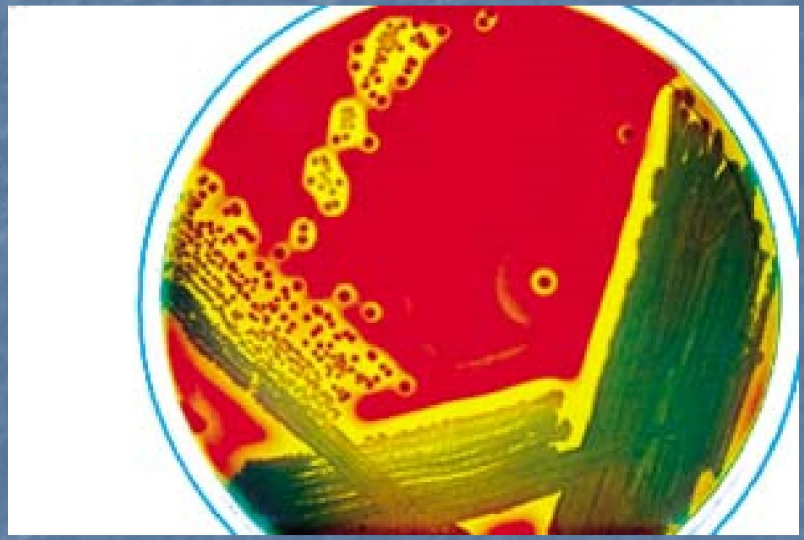
- Coagulation of Casein
  - Casein converted to Paracasein (curd)
  - *C. perfingenes* produces Rennin

Final Confirmation

- Motility Media
  - *C. perfringens* is NON-motile
    - Stab one STRAIGHT Line and then observe Growth
- Nitrate Test
  - *C. perfringens* Converts Nitrate to Nitrite
  - Commercial Kit
- Lactose Gelatin Test
  - *C. perfringens* will liquefy gelatin by producing acid from lactose
  - Must Refrigerate for 1 hour to make sure it stays liquid

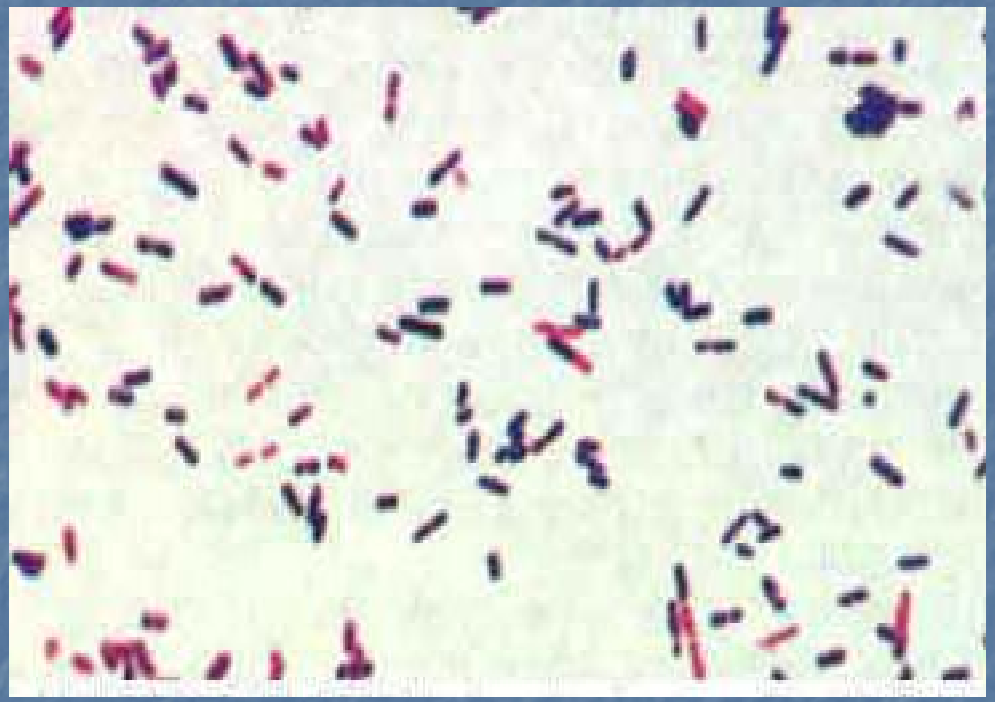
C. perfringens





The Basics

- Gram positive
- Rod-shaped
- Non-motile
- Anaerobic
- Five types of strains
  - A - E
- Four lethal toxins
  - Alpha, Beta, Epsilon and Iota



The Lethal Toxins

- Epsilon-toxin
  - Increases intestinal permeability causing vascular damage and oedema in major organs
  - Liver damage
  - Higher blood pressure
- Iota-toxin
  - Food-borne illness
- Alpha-toxin and Beta-toxins
  - Gas gangrene – necrotizing cell membranes
  - Food-borne illness

The Background Check

- Lives in soils and sediments
- Persists in human and animal intestinal tracts and fecal matter
- Optimal growth between 109-117°F
- One of the most common food-borne illnesses in the US
- Major threat to livestock

Polluted Regions

- Marine sediment at the base of sewage outfalls was found to have higher amounts of *C. perfringens*
  - Proportion of *C. perfringens* to total *Clostridium* populations
    - 56 - 71% near sewage outfalls
    - 0.4 – 4.1% for freshwater sediments and soils
- Fish guts collected from sewage outfalls in Puget Sound were found to store higher amounts of *C. perfringens*

Who is at Risk

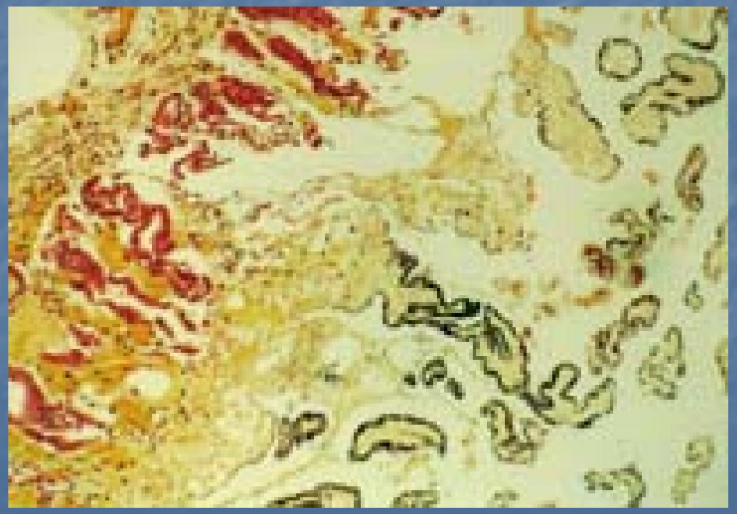
- People – especially elderly and children
- Animals
  - Domestic and wild
- ~10,000 cases reported in the US annually
  - 10-350x the reported case might be the actual numbers of people effected by disease

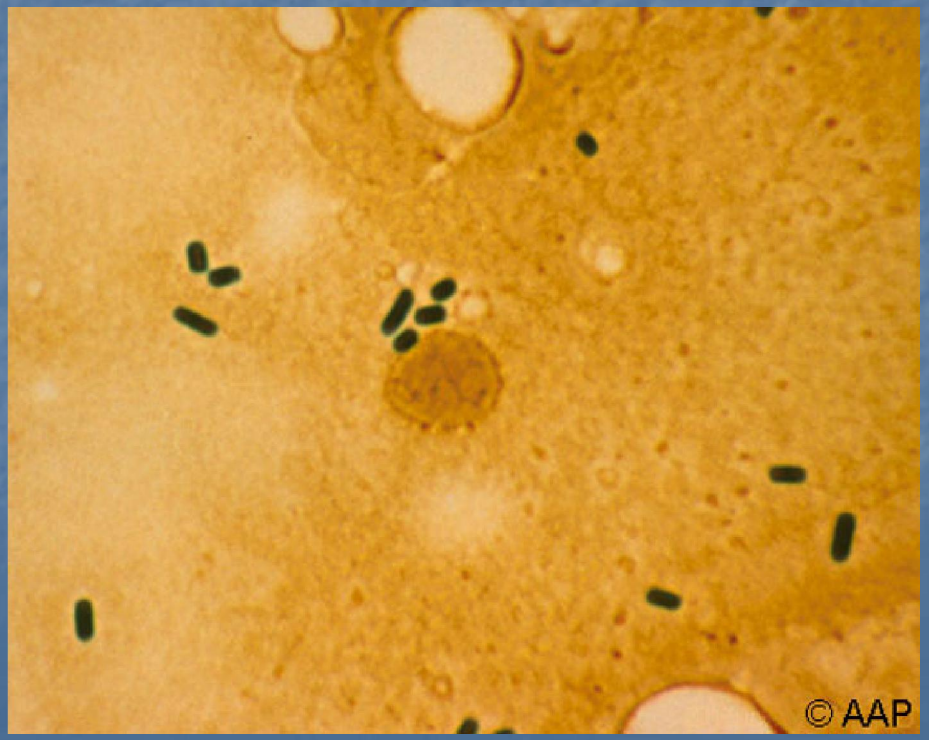
Enterotoxin

- Most common mediator for food-borne illnesses
- Can tolerate >100°F temperatures for more than 1 hour
- Can persist and multiply in animal intestinal tracts
- Temperature-abuse in cooked or raw food causes food contamination

Dangerous Amount

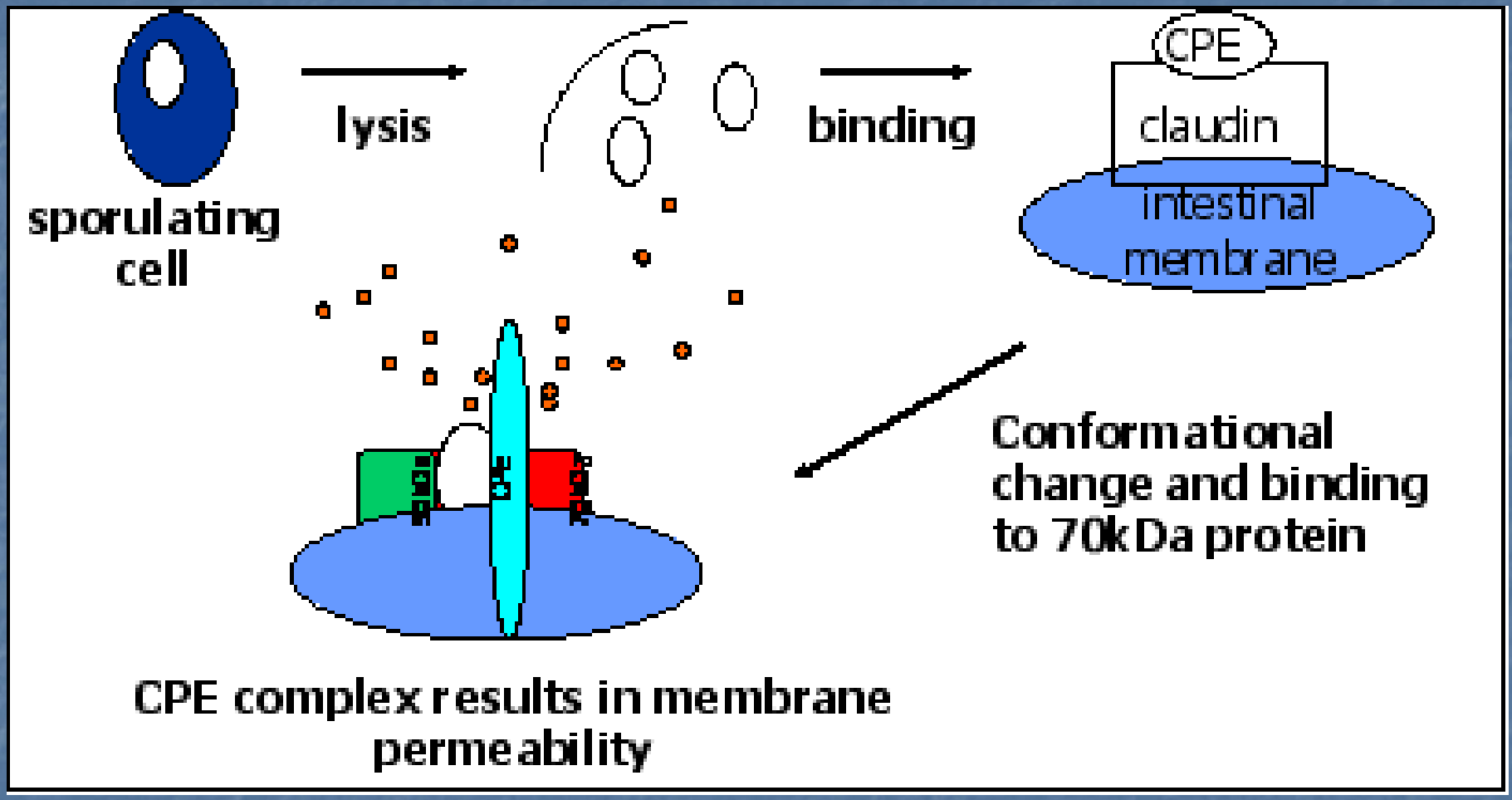
- ~10^5 spores/g can cause illness
- ~10^6 spores/g is considered food contaminated





Attack Method for Enterotoxin

- Stomach acids initiate spore germination
- When the cell lyses, it releases mature endospores
- Spores bind to intestinal epithelial cells and induce intestinal tissue damage
- Usually symptoms occur within 6-24 hours of ingestion and can last ~24 hours


sporulating cell
lysis
binding
CPE
claudin
intestinal membrane
Conformational change and binding to 70kDa protein
CPE complex results in membrane permeability

Results

- Can cause stomach aches, diarrhea and vomiting
- Rarely fatal in humans
- Very rapid death occurs in animals
- Mistaken for the “24 hour” flu

Areas of Outbreaks

- Usually in areas where large amounts of food are being served
  - Cafeterias, hospitals, nursing homes, catering
- In Nov. 1985, 44% of employees contracted food-borne illness from *C. perfringens* at an employee banquet
  - Gravy was culturing bacteria, improperly cooled and reheated

Treatment

- Depends on toxicity and type of strain ingested
- For Animals
  - Not much can be done once spores are ingested
- For Humans
  - Penicillin and other antibiotics are used for gas gangrene and wound infections
  - Surgery is used for cases in which severe tissue damages occur
  - Keep hydrated

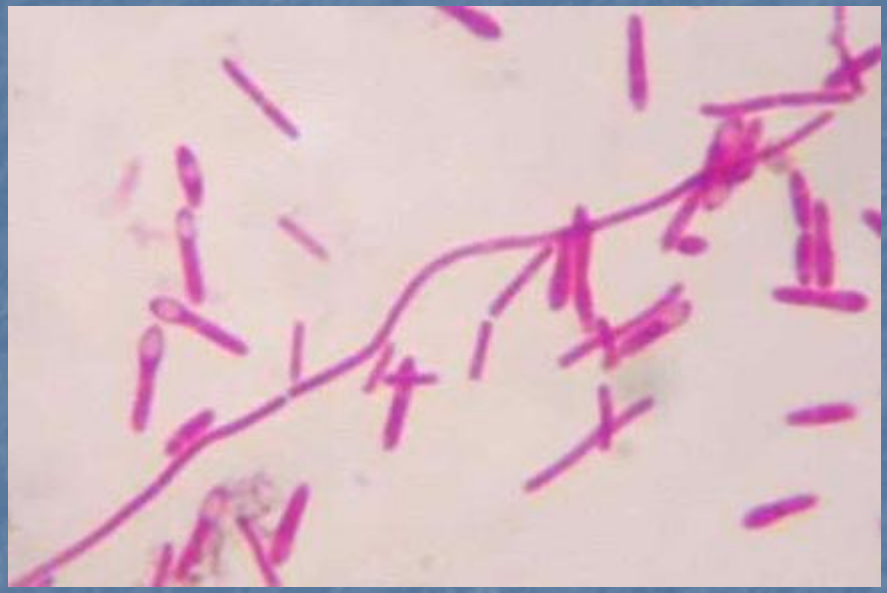
Prevention

- Handling foods properly, especially meats
- Use of correct temperatures when cooking and cooling food
  - 165° F kills bacteria
  - Must be cooled quickly and reheated to 165° F again
  - Maintaining raw meat at very low temperatures (<40° F)

C. perferingens

- *C. perfringens* is an anaerobic bacteria found in soils and sediments, especially in areas of pollution
- Persists in fecal matter and intestinal tracts of animals and humans
- Four lethal toxins are produced during spore germination
- Enterotoxin is most common cause for food-borne illnesses
- In humans, the illness only lasts ~24 hours
- Using the correct temperatures in cooking, cooling, or reheating food is crucial to inhibit bacteria growth in food

C. botulinum



Characteristics

- Anaerobic bacillus that forms sub-terminal endospores
- Heat resistant
- Found in soil, sediments of lakes, ponds, coastal waters, decaying vegetation
- Intestinal tracts of birds, mammals and fish
- Gills and viscera of crabs and shellfish
- Neutral or low acid environments
- Usually seen in canned foods
- Seven toxigenic subtypes of the organism:
  - A, B, C, D, E, F and G
  - Differ by pre-synaptic proteins bound at exocytosis stage

Clinical Syndromes

- Food-bourne, Wound, Infant and Unidentified
- Food-borne: ingested from foods that spores have germinated and grown in, considered an intoxication – most common form
- Wound: infects a wound and then produces toxins that spread through the bloodstream – very rare
- Infant: infection, establishes itself in the bowels of infants, colonizes and produces the toxin – common source is honey
- Unidentified: source is unknown, usually from intestinal colonization with *in vivo* production of toxin – usually from surgeries

Action of Toxin

- Structure: Synthesized as a polypeptide chain that cleaves into two chains, a light and heavy linked by disulfide bonds
- Binding occurs at the carboxy terminal
- Enters receptors via endocytosis
- Blocks release of Ach = failure to release neurotransmitter
  - Zinc-dependent endopeptidase that cleaves synaptobrevins
- Flaccid Paralysis
- Permanent damage

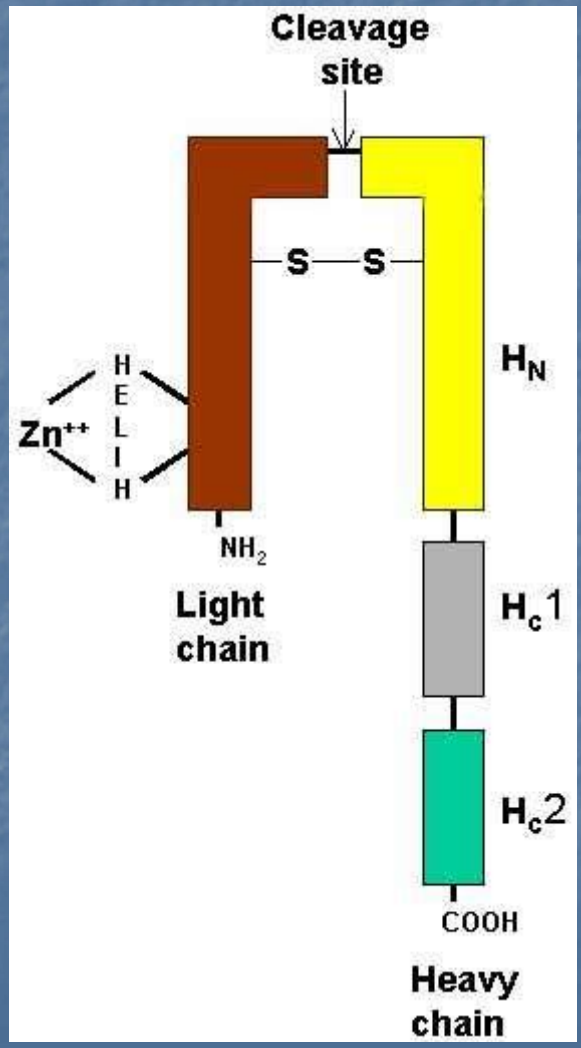
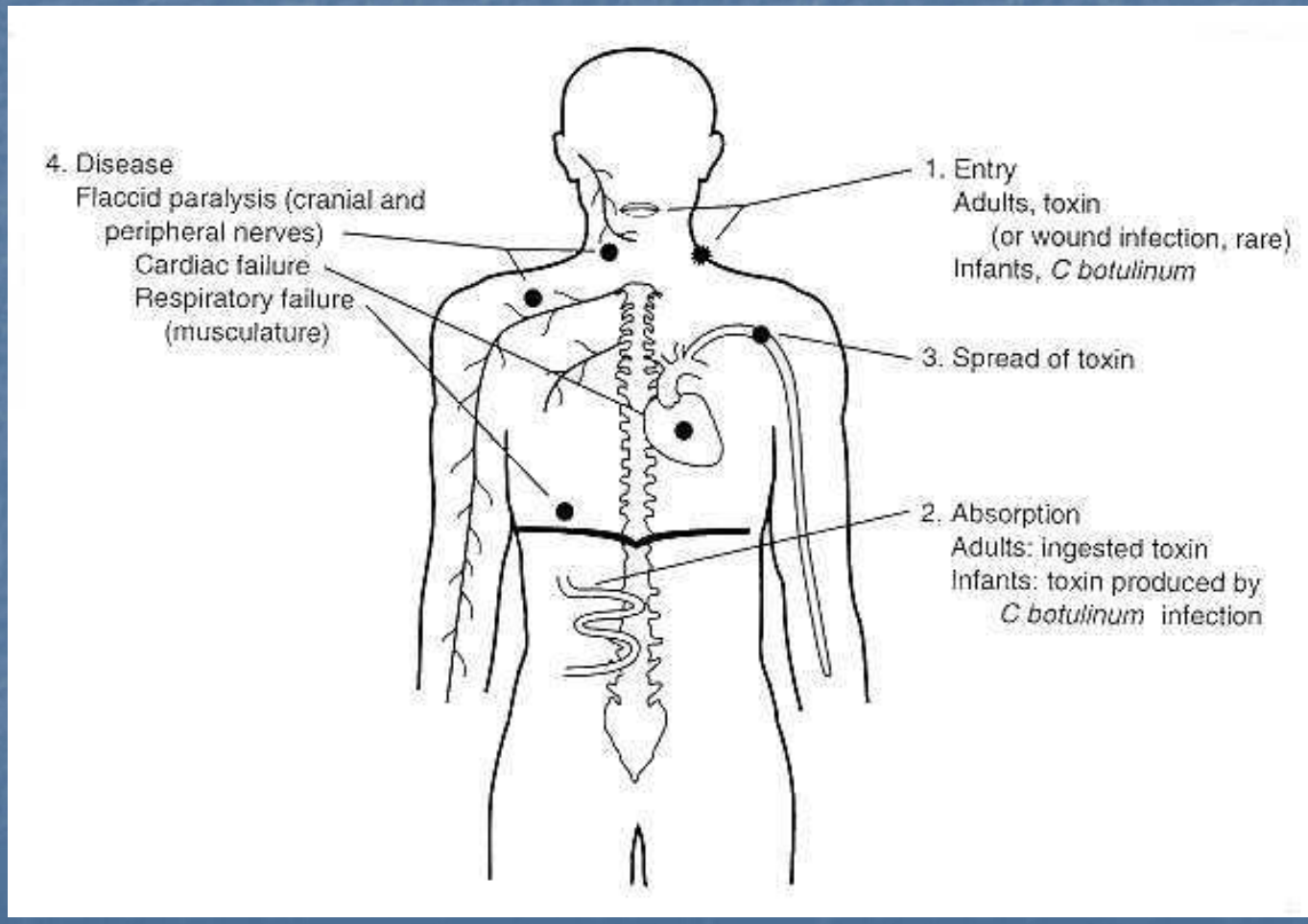


Diagram of Pathway



Symptoms

- Begin 8-36 hours after ingestion
- Length: 2 hours to 14 days after entering circulation
- Preliminary symptoms: weakness, dizziness, dryness mouth, nausea, vomiting
- After Neurological disturbance: blurred vision, inability to swallow, difficulty in speech, descending weakness of skeletal muscles and respiratory paralysis

Diagnosis and Treatment

- Electrodiagnostic testing = repetitive nerve stimulation
- Test serum or feces of the patient for the toxin
- Mouse neutralization test
  - 48 hours to complete
  - 5-7 days to culture specimens
- Neutralized by an antitoxin - only in circulation

Prevention

- Proper food handling and preparation
  - 80°C for 10minutes or longer
- Manufacturers use thermal processes designed to destroy spores
- Processors add salt or nitrites to reduce growth

Occurrences

- Found throughout the world
- 10-30 cases annually within the US
  - Over 2300 since discovered in late 1890s
  - About 1000 fatalities
- Common in commercially canned salmon

Outbreaks

- 1987: NYC and Israel, Kapchunka, contained E-type botulism
- 1995: Italy, eggplant in oil, B-type botulism
- April 17th, 2007: Italy, olives made by Charlie Brown di Rutigliano & Figli S.r.l
- May 20th, 2007: Lake Erie, Sheephead, Burbot and Perch had E-type botulism

Local botulism

- Pacific Coast, early 1960s
  - Dungeness Crab, intestinal tract, gills and shell
    - 60-70% of crabs sampled = B and E-type botulism
  - Salmon, Sturgeon and Steelhead, fish gills and viscera
    - 10-22% of fish sampled = E-type botulism
- Mid 1980s
  - Halibut, 27% sampled = A-type botulism
  - Rockfish, 30% sampled = A-type botulism
  - Prawns, Shrimp, Oysters = no botulism

Alternative botulism Uses

- Botox
  - A-Type botulism is an active ingredient
- Biological Warfare
  - Poisonous to Humans
  - World War II – Stanley Lovell
    - gelatin capsules with a lethal dose
    - slipped into food or drink
    - tested on donkeys
  - 1gram crystalline toxin dispersed evenly and inhaled = 1 million deaths
    - 70µg orally = lethal (70kg person)
    - 0.09µg – 0.15µg intravenously = lethal (70kg person)

Conclusion: *C. botulinum*

C.botulinum:

- Seven toxigenic subtypes
- Four clinical syndromes, all have similar symptoms but the mode of infection is different
- Action of toxin = blocks release of neurotransmitter
- Becoming more common (olives and wild fish)
- Always finding new ways to incorporate botulism into the medical field.