Clostridium spp, the Strict Anaerobes

Over 90 different species, all are anaerobic spore formers, but less than 20 are associated with human disease

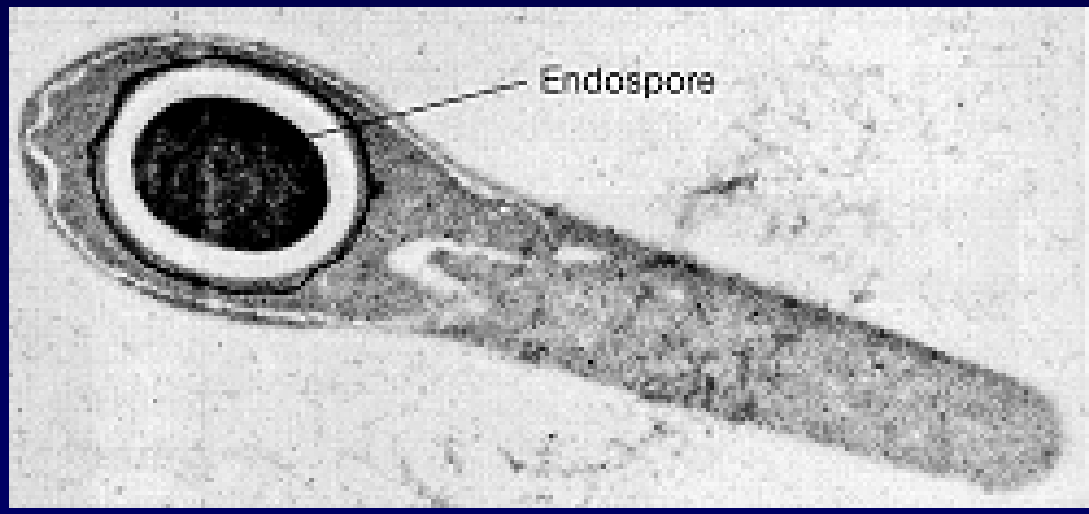
Biology of *Clostridium* spp

- Gram +, with ultra-cell wall structure,variable Gram stain
- Ubiquitous in environment
  - In soil, decaying vegetation, ocean sediment, GI tract of humans & animals
  - especially in fecal-contaminated soil
- Many human infections are endogenous, from normal flora
  - diseases produced by classical A/B toxins
    - also hemolysins, neuraminidase, enterotoxins, etc
    - tetanus, gas gangrene, food poising, and botulism, colitis, etc.

Clostridium tetani

- Gm+ obligate anaerobic bacillus
- Disease described by Egyptians & Greeks
  - associated with wounds followed by lethal spasms
- Looks like tennis racket or drumstick
  - spores highly resistant and long-lived

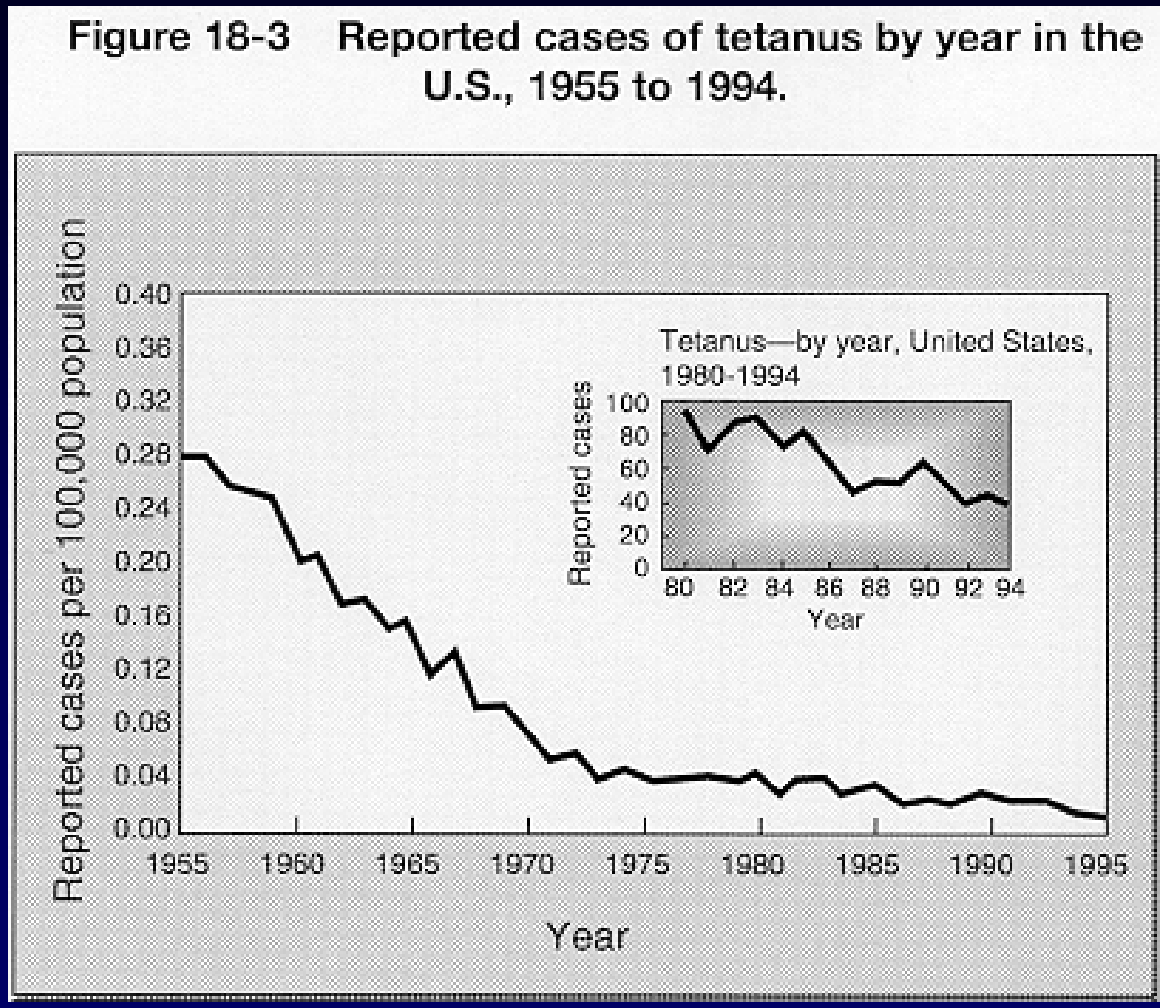
***C. tetani* with endospores**



Electron micrograph of endospore

Epidemiology of Tetanus

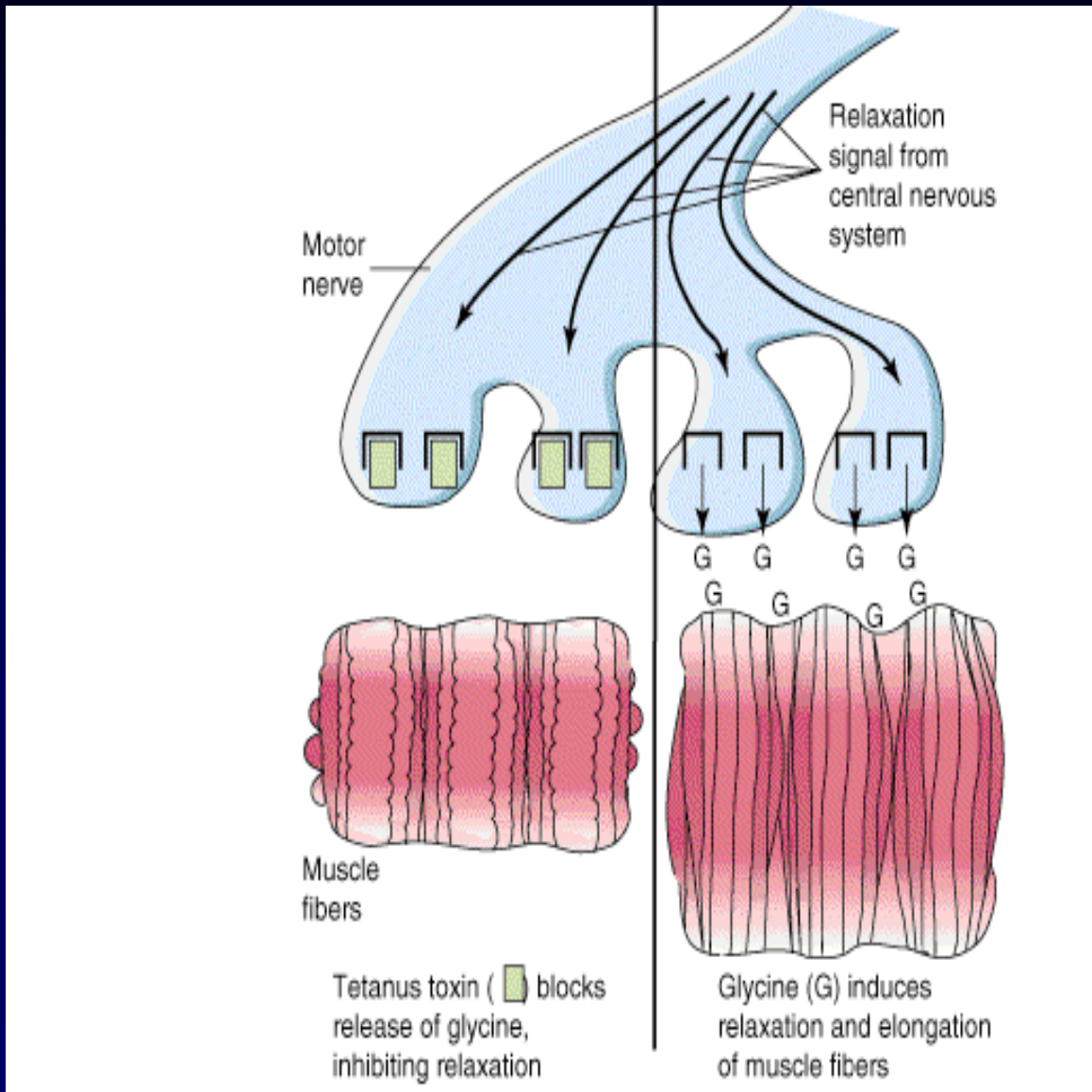
- Ubiquitous soil microbe
  - especially fecal-contaminated soils
- Low in USA, High in developing world
  - DPT vaccine
    - a toxoid vaccine
  - Neonatal tetanus
    - common in 3rd World
  - acute injuries
  - some cryptogenic infections



Pathogenesis of Tetanus

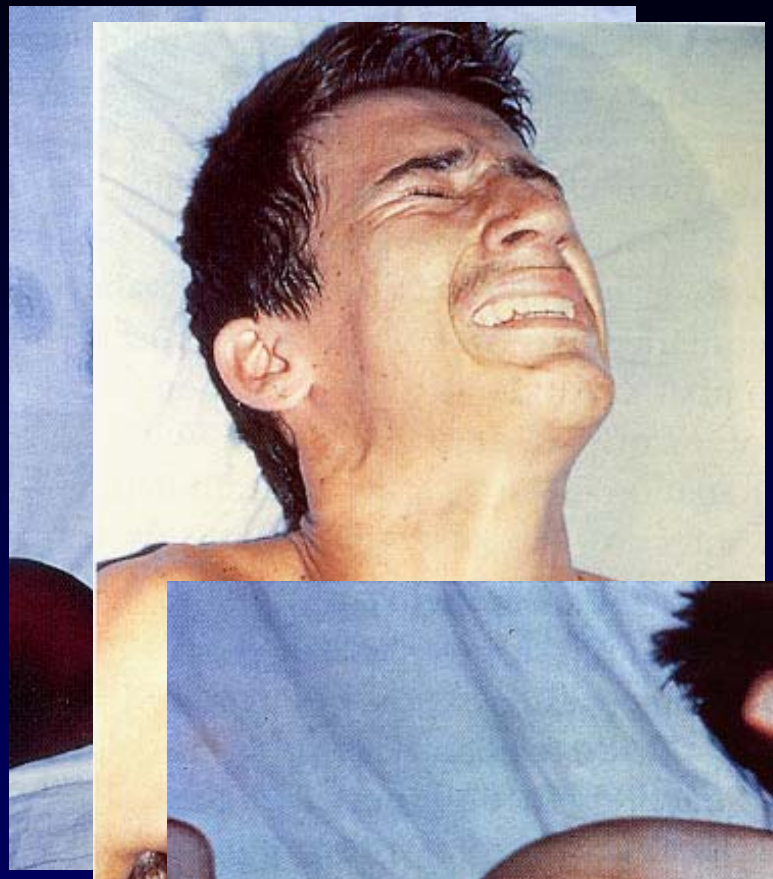
- **Tetanospasmin**, the Tetanus toxin
  - 4-10 days post exposure
  - plasmid encoded
  - classical A/B toxin
    - A=presynaptic inhibition of Glycine
    - B= spinal cord and brainstem neurons

Mode of Action of Tetanus toxin



Clinical features

- 4 distinct clinical types
  - generalized
    - most common
    - begins with masseter muscles (lock-jaw)
    - Opisthotonus posturing
    - takes two weeks
  - localized
    - near wound
    - mild to persistent
    - maybe due to partial immunity
  - cephalic
    - local paralysis of facial nerves & muscles
  - neonatal-90% mortality
    - rigidity, failure to nurse



Facies

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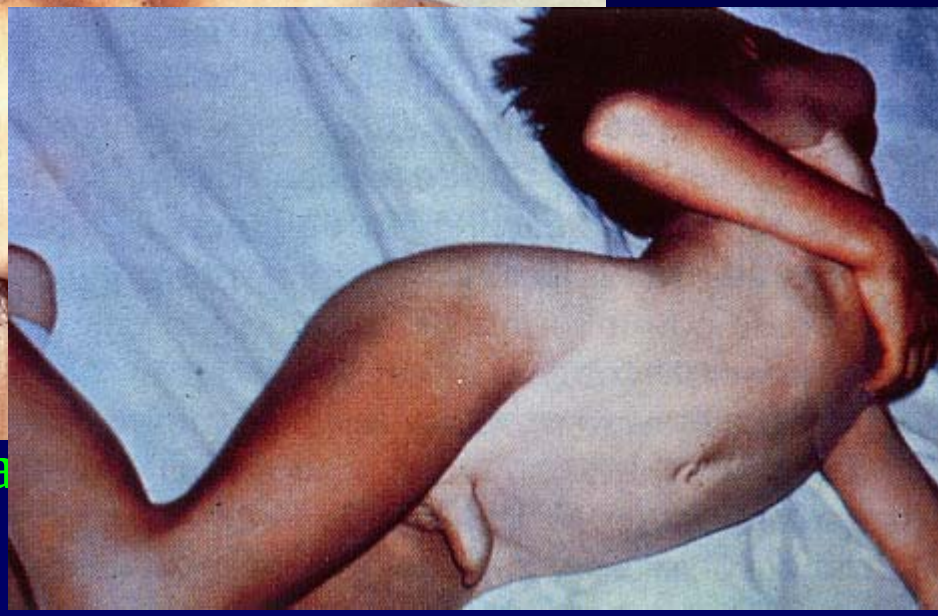


Opisthotonus posturing

Advanced Tetanus: a British Soldier

Neonatal Tetanus

- Rare in USA
- Common in 3rd World
  - lack of maternal immunity
  - poor hygienic conditions at delivery
  - rubbing dirt into umbilicus to stop bleeding (bad idea)
  - Weakness, failure to nurse, highly fatal

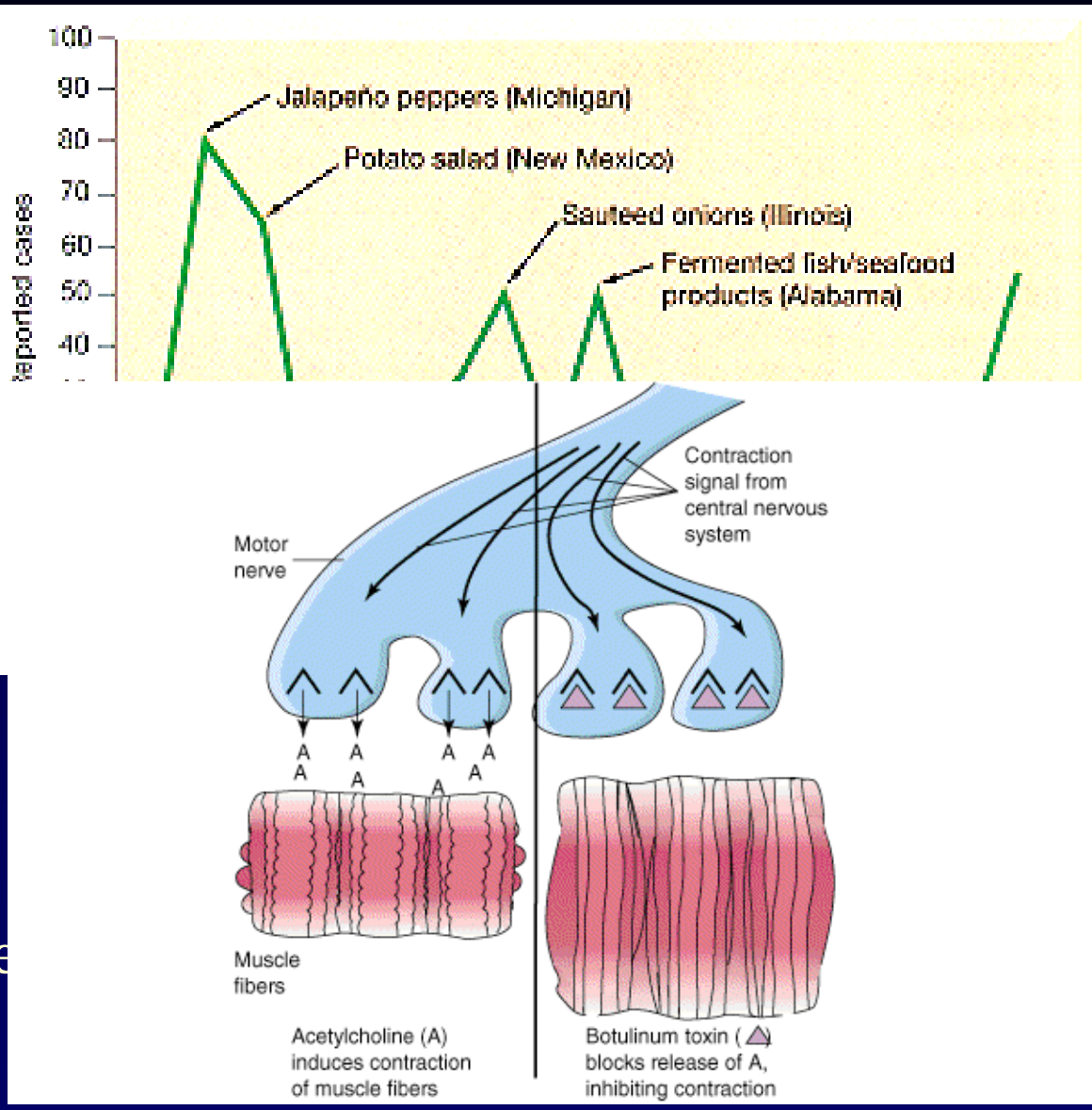
Neonatal Tetanus

Clostridium botulinum

- Botulism is not an infection, but the effects of the toxin can be catastrophic
- Botulinum toxin is the most potent known
  - 1 mg can kill 1 million guinea pigs!!!
  - Major biological warfare agent
- “botulinum” means sausage, because--
- Disease generally acquired from improperly processed foods-sausage, fish, etc.
  - generally non-acidic meats & vegetables
  - pressure cooking versus hot packing

Botulism

- More common in developed countries
  - USA, Canada, Japan, Germany, Poland, etc.
  - 124 cases in USA, 1976-84
- Spores very stable to boiling
- Toxin is heat labile
- Mode of action:
  - **A portion**: inhibits release of acetylcholine
  - **B portion**: peripheral motor-neural junctions



Mode of action of botulinum toxin

Clinical features

- Toxin causes “flaccid paralysis”
  - weakness of cranial nerves, limbs & trunk
  - blurred vision
  - dysphagia (can’t swallow)
  - respiratory embarrassment (1^{o} cause of death)
- Diagnosis--inject plasma into mice
- Treatment:
  - horse antiserum
  - respiratory support

Diagnosis of botulinal toxin

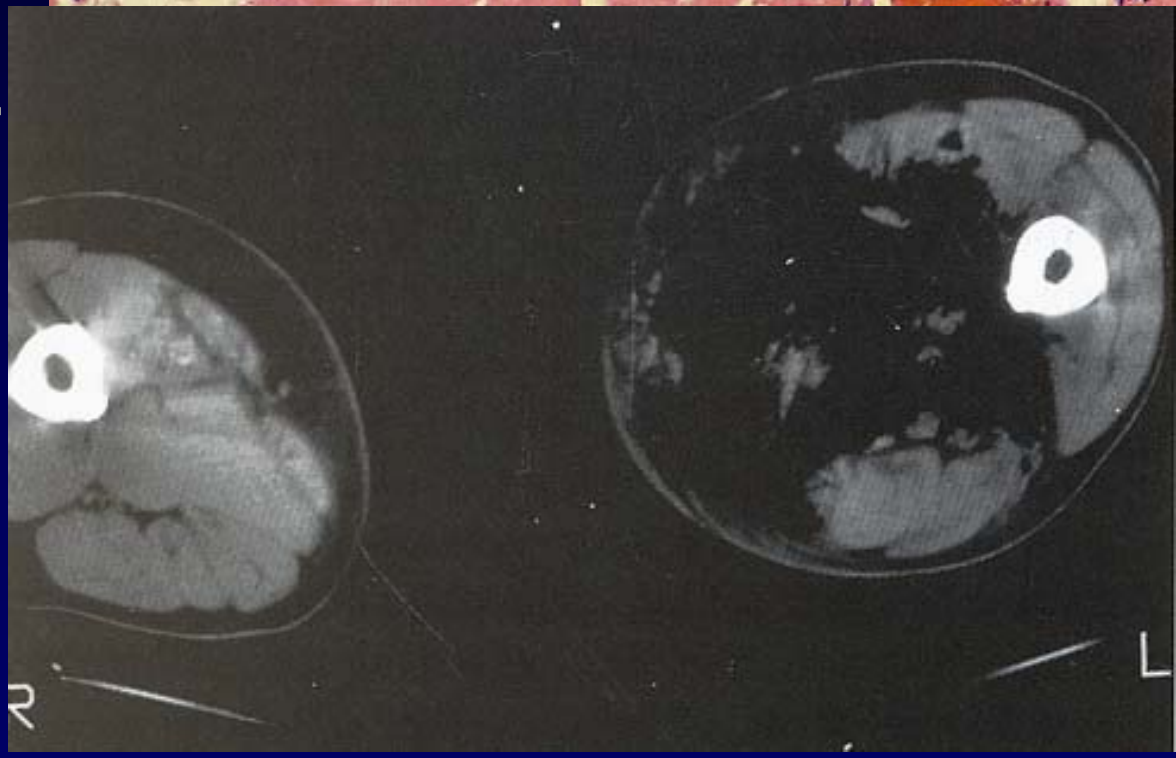
Clostridium perfringens

- Gas gangrene AKA **Clostridial myonecrosis**
- Also food poisoning
- Tissue damage filled with gas
  - due to potent enzymes
- *C. perfringens* from all soil except Sahara also in all feces & vagina**
  - 80% *C.p*, also *C. septicum, et al.***
  - tissue damage accelerated by damaged circulation
    - diabetes mellitus, etc.

- 12 toxic enzymes:
  - Alpha-toxin, a lecithinase, cytolysins, neuraminidase, etc. kill many cell types
- Gas production due to fermentation of muscle carbohydrates and AAs to CO_2 & H_2
  - Pressure-induced ischemia
- Common in:
  - traumatic injury--bleeding*
  - penetrating wounds--soil*
  - circulation insufficiency
  - colorectal, et al. cancers
  - self-induced abortions**

Clinical Features

- Short incubation 1-4 d.
- Sudden & severe pain
- pressure near wound
- edema of limb
  - pale then bronze color
  - discharge with sweet mousy odor
  - gas in tissues
  - tachycardia
  - fever & hypotension
  - renal failure
  - hemoglobinuria
  - comatose before death



CT scan of thighs, gas spaces on right leg

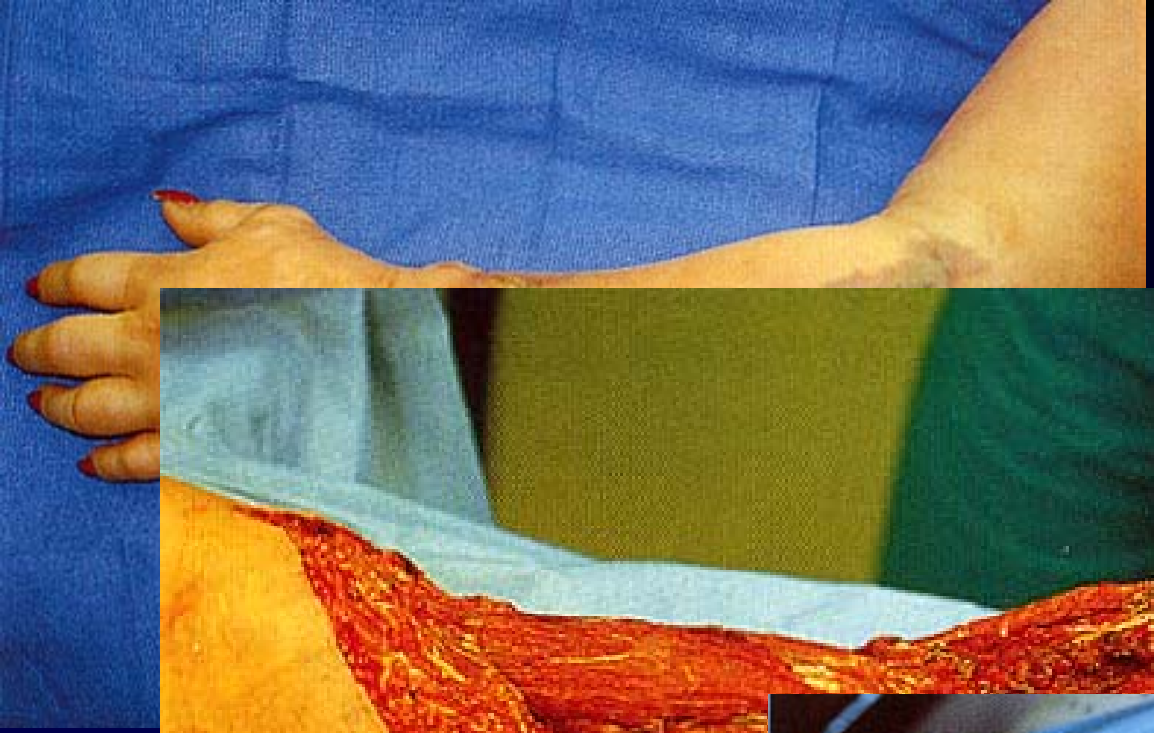
Clinical Features continued

- **Surgery**
  - Requires rapid and thorough debulking of affected tissues
    - systemic *a*-toxin**
  - Myonecrosis, no electrical stimulation of muscle
  - no bleeding of cut surface
- Further treatment:
  - Penicillin G may improve survival, but*
  - Hyperbaric oxygen therapy of **?**usefulness

Gar

Amputations required

Gangrene from drug use
(why drug use?)



Debulking remove

After skin graphing

Clostridium difficile

- **Pseudomembranous colitis**
  - serious, potentially fatal infection
  - intestinal overgrowth
    - endogenous infection
    - excess antibiotic use (AAPC)
    - ampicillin, clindamycin cephalosporins, etc.
    - debilitated persons, cancer, intensive-care patients, burn patients etc.

- Toxins
  - Toxin A: enterotoxin
    - induces alterations in liquid adsorption leading to severe diarrhea,
    - induces granulocyte tropism=inflammation
  - Toxin B: cytolysin
    - damages the lining of intestine leading to tissue necrosis and pseudomembrane formation

Clinical features

- Life-threatening diarrhea
  - develops during original treatment
- Patients remain severely ill after--
- Mucosae highly inflamed, ulcerated, necrotic
- Endoscopy--shaggy yellow & white exudate of dead tissue
- Difficult to treat requiring specific antibiotics
  - maintain electrolytes,
  - Vancomycin, bacitracin and metronidazole

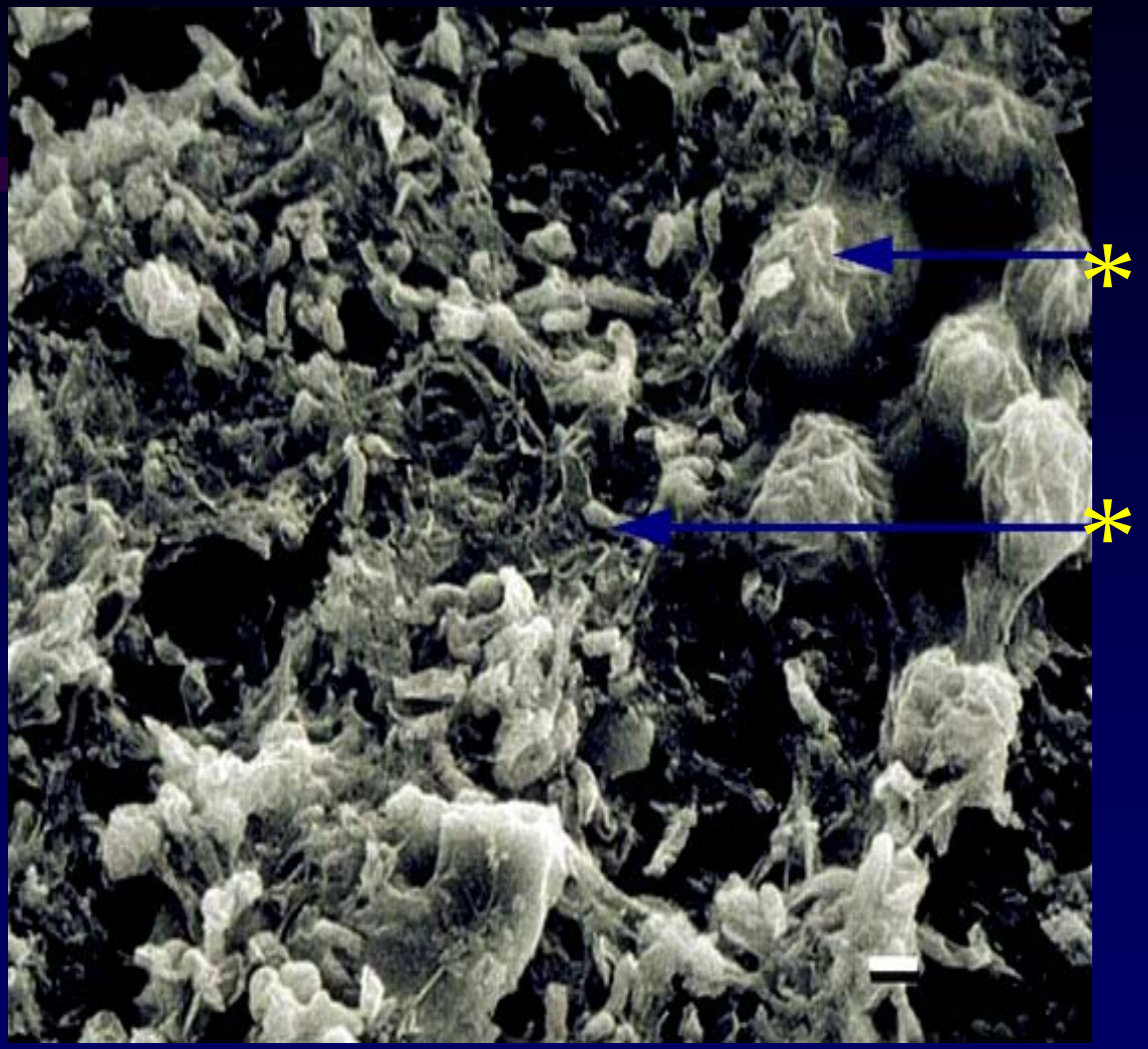
Discrete ulcers of Pseudm. Colitis

Other Anaerobes

- *C. perfringens* is also associated with food poisoning, especially sausages and other meats
- Many endogenous anaerobes also colonize other tissues and cause disease of the liver, kidney, heart, CNS, etc.
- Perforated colon (*Bacteroides* spp)
- Aspiration pneumonia
- However, one area of universal concern is the role they play in dental health--dental caries and periodontal disease---

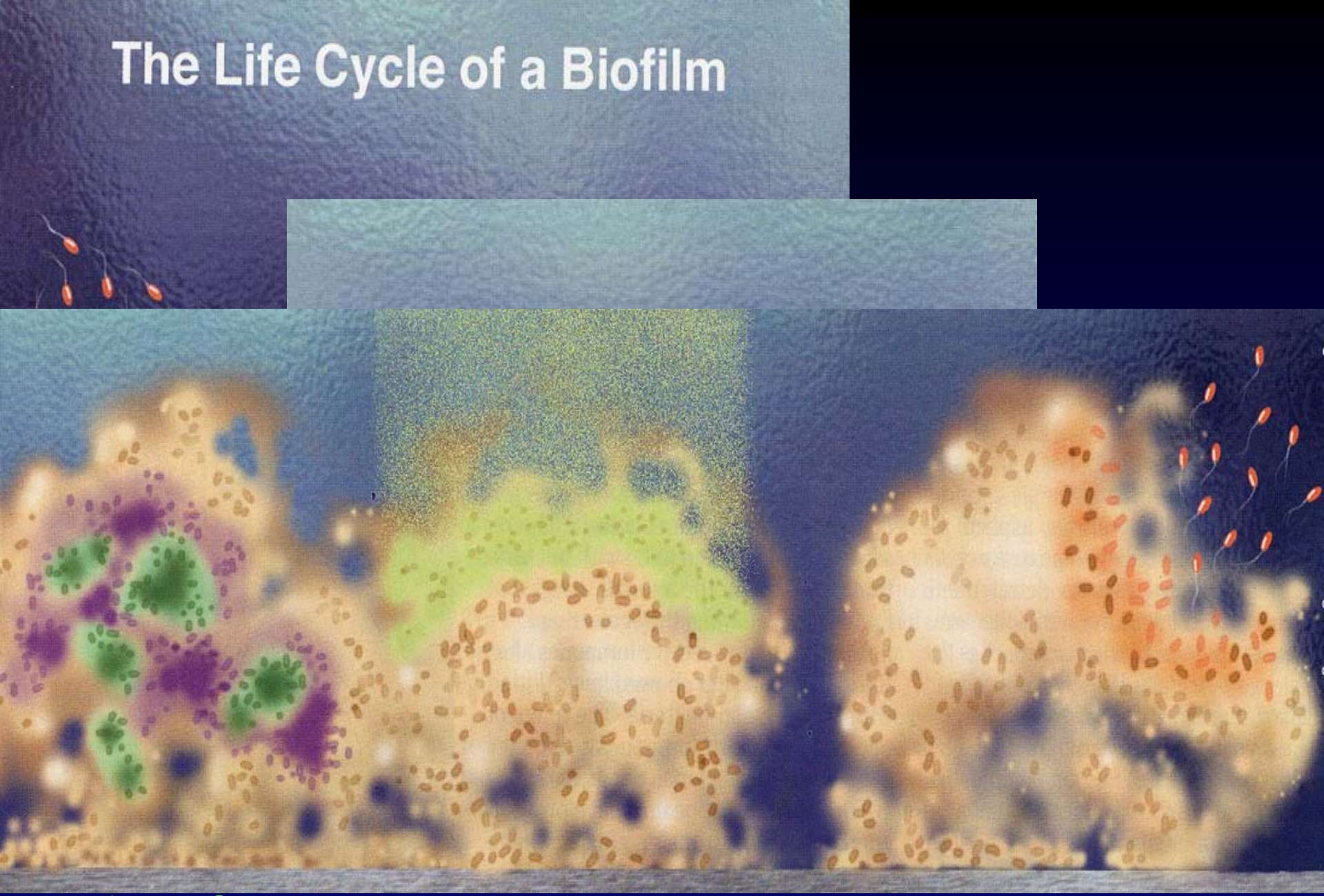
Biologic Aspects of Biofilms

- Slime or the Single cell?
  - Past views--single life
  - current thinking
- Development of biofilms
  - attachment
  - quorum sensing
  - cell-to-cell chatter
  - alginate matrix
  - complex structures
  - aerobic/anaerobic
  - shedding of cells



Biofilm from contact lens storage case

* **Acanthamoeba, and bacterial biofilm**



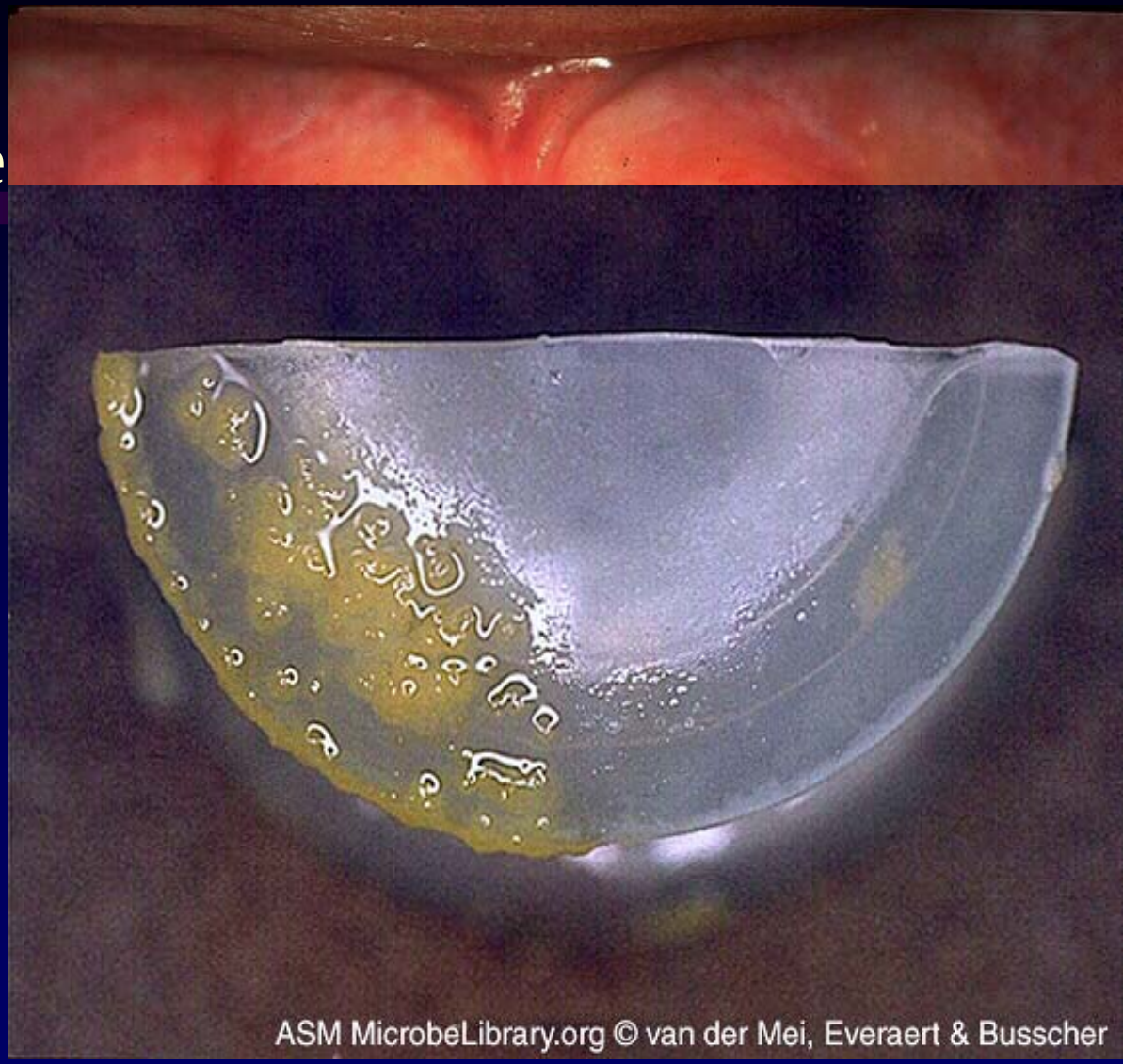
Variety of niches formed

Protection from antibiotics & toxins

Cells released from slime

Consequences of biofilms

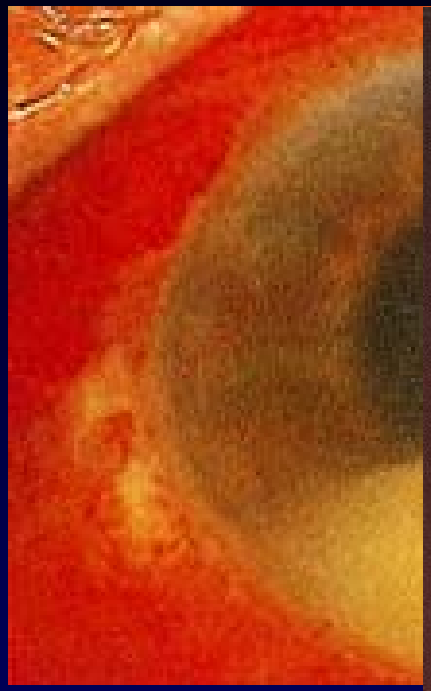
- ruminant nutrition
- antibiotic resistance
- antibody
- virulence factors
  - quorum sensing
- recurrent infections
- dental pathogens
- medical prostheses
  - heart valves, etc
  - contact lenses
- community water supplies



Biofilm on medical prosthesis

Diseases and biofilms

- To date:
  - Corneal necrosis from contact lenses
  - periodontal disease
  - prostrate infections
  - kidney stones
  - TB
  - Legionnaire's disease
  - middle ear infections
  - cystic fibrosis pneumonitis



Corneal necro
in cor

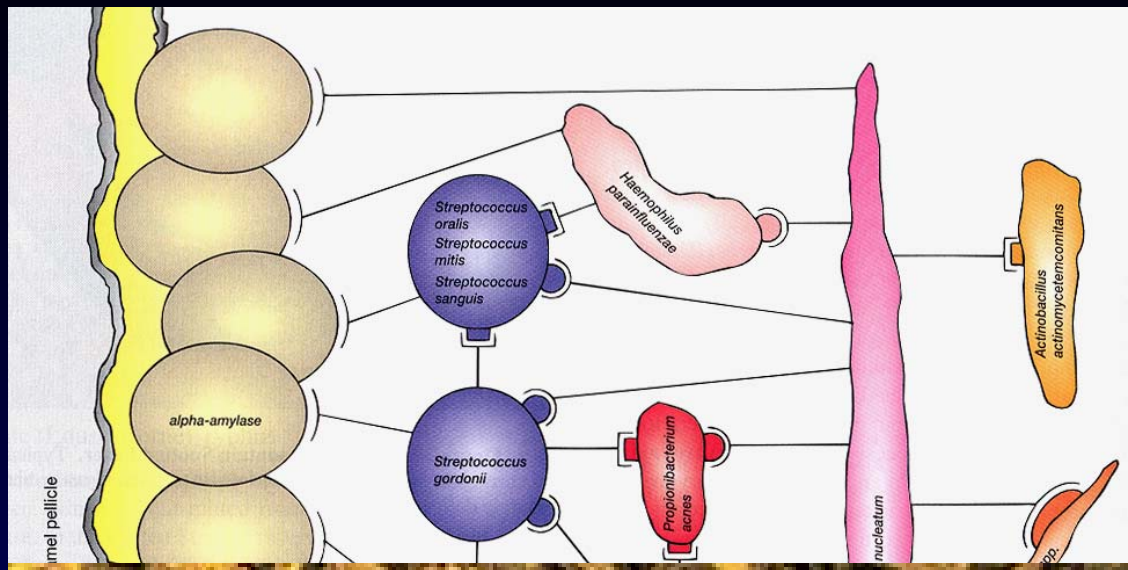
Biofilm of *V. cholerae*, 40X resistant to Cl^-

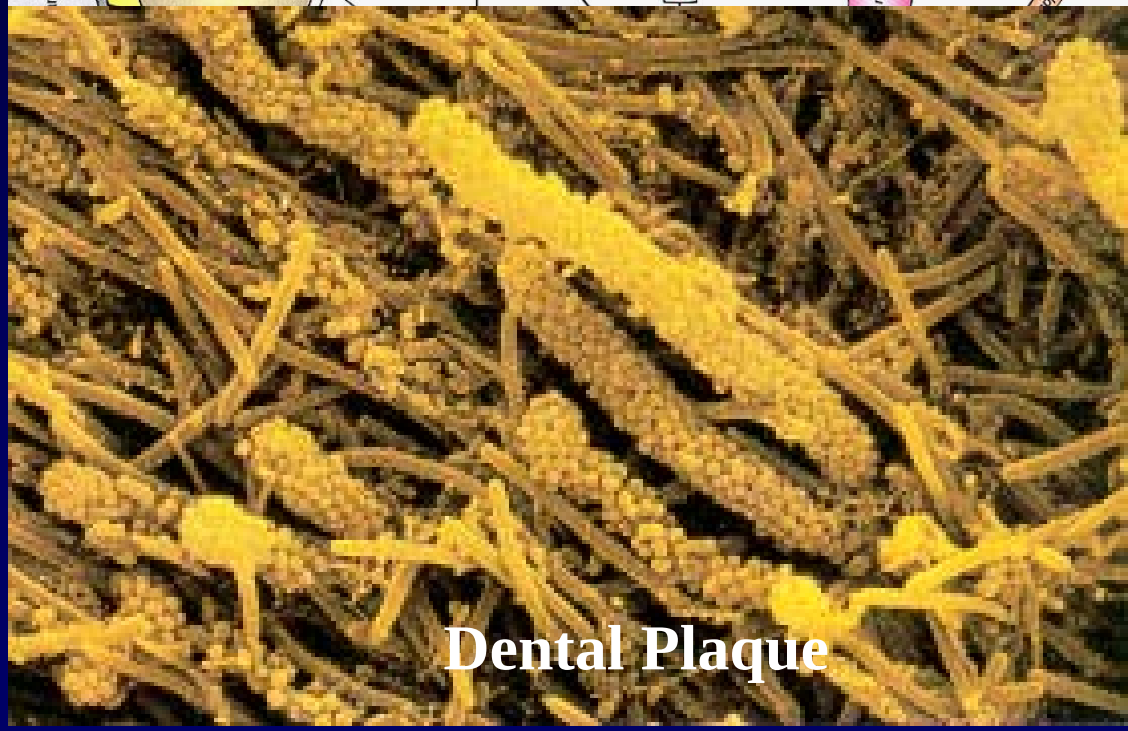
Dental Pathogens

- Odontopathogens induce dental disease, both caries and periodontal disease
- These form part of the normal endogenous flora and are not generally transmitted from person to person.
- Often the most important contributors to dental disease are anaerobes

Dental structure

- Teeth have natural defenses against caries
  - enamel adsorbs mucins
    - forming the negatively charged enamel pellicle
  - *Streptococcus* & *Actinomycetes* spp have tissue receptors, and adhere to teeth
  - layered symbiotic colonies form **plaque,** a typical biofilm
  - glucan from sugars cements the biofilm, breaking down the natural defenses



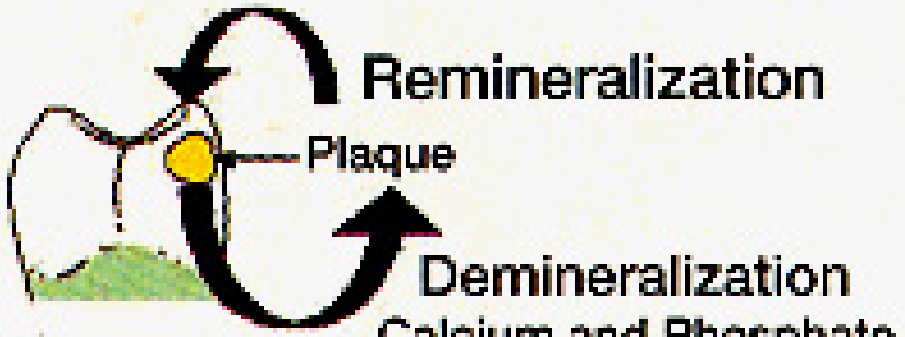


Dental Plaque & Caries

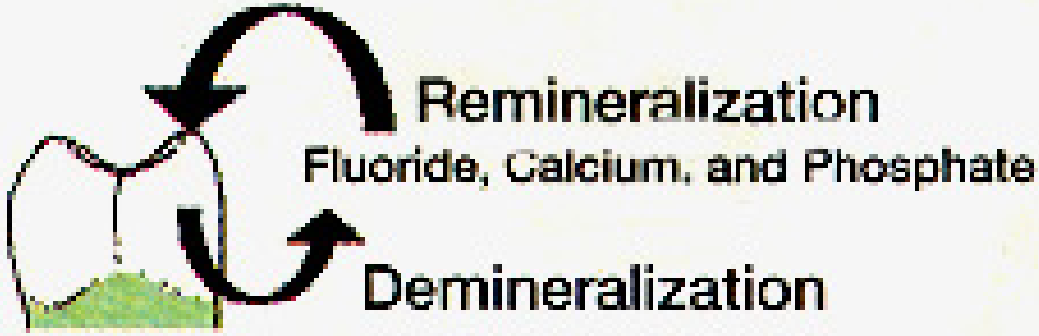
- Colonization requires bacteria to adhere to dental surfaces
- *Streptococci* & other spp. have specific fimbriae to attach to dental surfaces-- resisting the scrubbing& flushing action of food & saliva
- Plaque is a symbiotic biofilm requiring several spp of bacteria
- Plaque is the most concentrated col-lection of bacteria in the body (> 100 billion per gram)
  - *Streptococcus mutans* et al. produce acids (lactic, formic and acetic)from sugars
  - acids demineralize the enamel, forming caries
    - fluoride prevents this and helps with remineralization

Dental Caries and the role of Fluoride

Normal Conditions



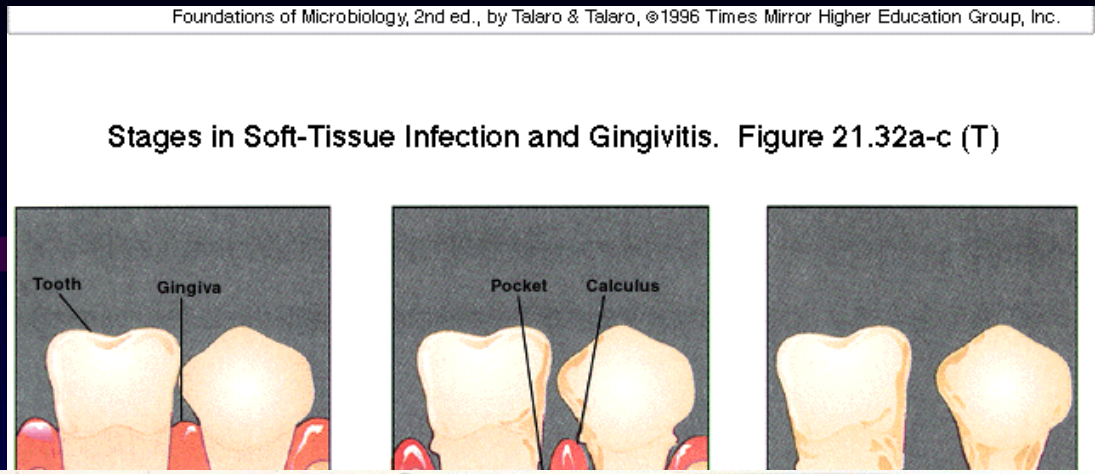
Cavity-Forming Conditions



Remineralizing Therapies

Periodontal disease

- Peridontium-- the supporting structure of teeth
  - gingivae, cementum, periodontal membrane, & bones of the jaw
  - **Subgingival plaque**
    - low O2 allows colonization of anaerobes: *Bacteroides*, and other anaerobs, causing inflammation & tissue necrosis leading to **Periodontal Disease**
      - The leading cause of bone damage & tooth loss in adults



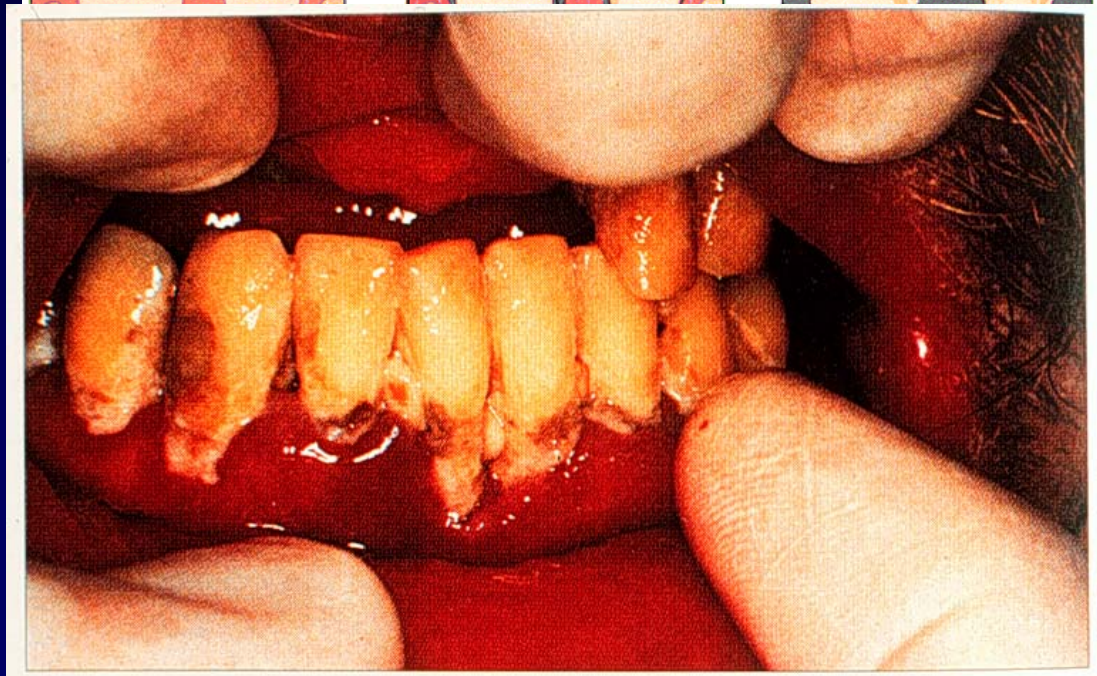


Fig. 15.11 Severe periodontal disease (adult periodontitis) in a 30-year-old male. Note the gingival swelling and detachment overlying probable extensive loss of alveolar bone.