Anaerobes

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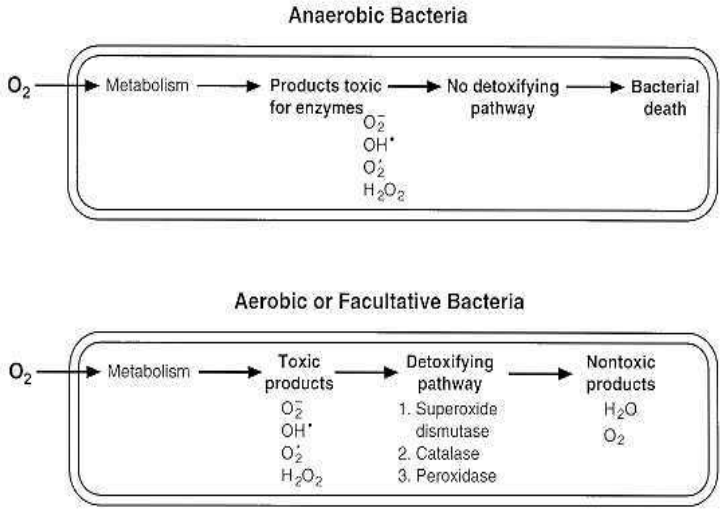
Definitions

- Anaerobes
  - Bacteria that require anaerobic conditions to initiate and sustain growth
- **Strict (obligate) anaerobe**
  - Unable to grow if > than 0.5% oxygen
- **Moderate anaerobes**
  - Capable of growing between 2-8% oxygen
- **Microaerophillic bacteria**
  - Grows poorly in air, but better in anaerobic conditions
- **Facultative bacteria (facultative anaerobes)**
  - Grows both in presence and absence of air



Classification of Medically Important Anaerobes

- Gram positive cocci
  - Peptostreptococcus
- Gram negative cocci
  - Veillonella
- Gram positive bacilli
  - *Clostridium perfringens, tetani, botulinum, difficile*
  - Propionibacterium
  - Actinomyces
  - Lactobacillus
  - Mobiluncus
- Gram negative bacilli
  - *Bacteroides fragilis, thetaiotaomicron*
  - Fusobacterium
  - Prevotella
  - Porphyromonas

Epidemiology

- Endogenous infections
  - Indigenous microflora
    - Skin: Propionibacterium, Peptostreptococcus
      - Prevalence in areas exposed to air explained by (1) oxygen consumption by aerobes (2) low oxidation-reduction potential microhabitats
    - Upper respiratory: Propionibacterium
    - Mouth: Fusobacterium, Actinomyces
    - Intestines: Clostridium, Bacteroides, Fusobacterium
    - Vagina: Lactobacillus
  - Flora can be profoundly modified to favor anaerobes
    - Medications: antibiotics, PPI, antacids, bowel motility agents
    - Surgery (blind loops)
    - Cancers
- Exogenous infections

Role of Anaerobes

- Role in normal host physiology
  - Prevent colonization & infection by pathogens
    - Bacterial interference through elaboration of toxic metabolites, low pH, depletion of nutrients
    - Interference with adhesion
  - Contributes to host physiology
    - *B. fragilis* synthesizes vitamin K and deconjugates bile acids

Clinical features of anaerobic infections

- The source of infecting micro-organism is the endogenous flora of host
- Alterations of host's tissues provide suitable conditions for development of opportunist anaerobic infections
- Anaerobic infections are generally polymicrobial
- Abscess formation
- Exotoxin formation

Sites of anaerobic infections

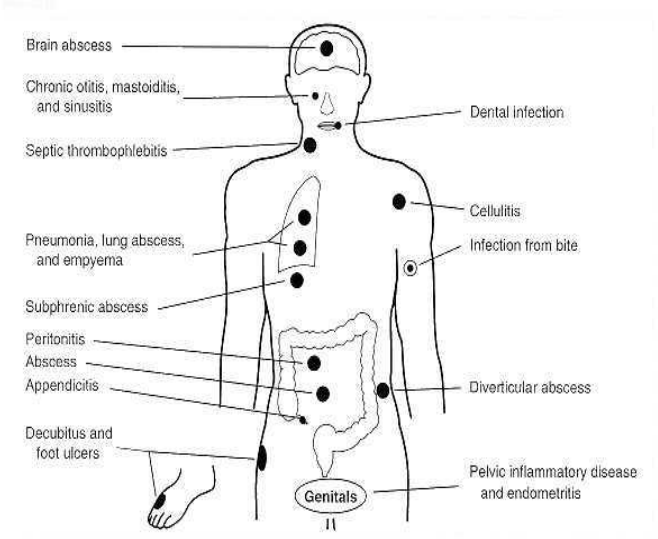


TABLE 20-2 Conditions Predisposing to Anaerobic Infection

- General
  - Diabetes
  - Corticosteroids
  - Leukopenia
  - Hypogammaglobulinemia
  - Immunosuppression
  - Cytotoxic drugs
  - Splenectomy
  - Collagen disease
- Decreased redox potential
  - Tissue anoxia
  - Tissue destruction
  - Aerobic infection
  - Foreign body
  - Calcium salts
  - Burns
  - Peripheral vascular insufficiency
- Specific clinical situations
  - Cancer
    - Colon, uterus, lung
    - Leukemia
  - Gastrointestinal and female pelvic surgery
  - Gastrointestinal trauma
  - Human and animal bites
  - Aminoglycoside therapy

Virulence factors

- Attachment and adhesion
  - Polysaccharide capsules and pili
- Invasion
  - Alteration in host tissue (trauma, disease)
  - Aerotolerance
- Establishment of infection
  - Polysaccharide capsule (*B. fragilis*)
  - Spore formation (Clostridium)
  - Maintenance of reduced environment
- Tissue damage
  - Elaboration of enzymes toxins

Anaerobic cocci

- Epidemiology
  - Normal flora of skin, mouth, intestinal and genitourinary tracts
- Pathogenesis
  - Opportunistic pathogens, often involved in polymicrobial infections
  - Virulence factors not as well characterized
  - Brain abscesses, periodontal disease, pneumonias, skin and soft tissue infections, intra-abdominal infections
- Peptostreptococcus
  - P. *magnus*: chronic bone and joint infections, especially prosthetic joints
  - P. *prevotti* and P. *anaerobius*: female genital tract and intra-abdominal infections
- Veillonella
  - Normal oral flora; isolated from infected human bites

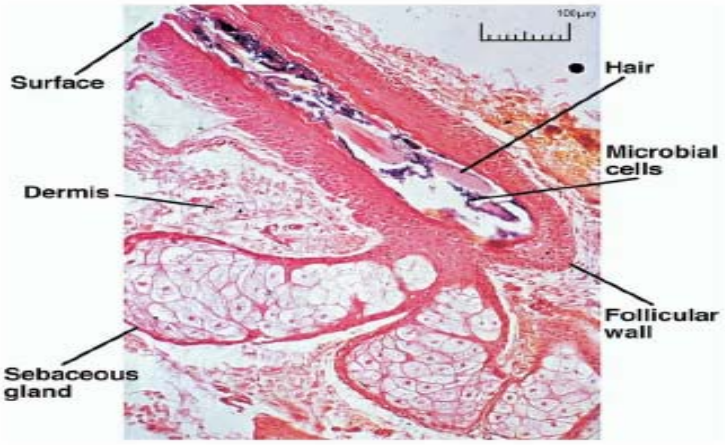
Anaerobic gram positive bacilli

- No Spore Formation
  - Propionibacterium
    - P. *acnes*
  - Actinomyces
    - A. *israelii*
  - Lactobacillus
  - Mobiluncus
- Spore Formation
  - Clostridium
    - C. *perfringens*
    - C. *difficile*
    - C. *tetani*
    - C. *botulinum*

Propionibacterium

- Anaerobic or aerotolerant, produces propionic acid as major byproduct of fermentation
- Colonize skin, conjunctiva, external ear, oropharynx, female GU tract
- *P. acnes*
  - Acne
    - Resides in sebaceous follicles, releases LMW peptide, stimulates an inflammatory response
  - Opportunistic infections
    - Prosthetic devices (heart valves, CSF shunts)

Pilosebaceous follicle



Actinomyces

- Facultative or strict anaerobe
- Colonize upper respiratory tract, GI, female GU tract
- Actinomycosis
  - Endogenous disease, no person-person spread
  - Low virulence; development of disease when normal mucosal barriers are disrupted
  - Diagnosis:
    - Macroscopic colonies of organisms resembling grains of sand (sulfur granules)
    - Culture

Actinomycosis

- Cervicofacial Actinomycosis
  - Poor oral hygiene, oral trauma, invasive dental procedure
  - Chronic granulomatous lesions that become suppurative and form sinus tracts
  - Slowly evolving, painless process
  - Treatment: surgical debridement and prolonged penicillin

Lactobacillus

- Facultative or strict anaerobes
- Colonize GI and GU tract
  - Vagina heavily colonized (10^5/ml) by Lactobacillus *crispatus* & *jensonii*
  - Certain strains produces H_2O_2 which is bactericidal to *Gardnerella vaginalis*
- Clinical disease
  - Transient bacteremia from GU source
  - Endocarditis
  - Bacteremia in immunocompromized host

Mobiluncus

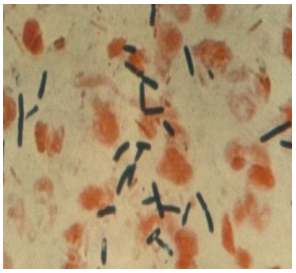
- Obligate anaerobes
- Gram variable
- Colonize GU tract in low numbers
- Associated with bacterial vaginosis
  - Detected in vagina of 6% of controls
  - As many as 97% of women with bacterial vaginosis

Case 1

- 12 year old boy with Acute Myelogenous Leukemia (AML) diagnosed 2 mo. ago
- Pancytopenia after cytarabine 2 wks ago
- Presents with painful ecchymotic areas on legs that rapidly progressed with marked swelling over several hours
  - Afebrile
  - Crepitus in both legs
  - Rapid progression to shock

Case 1

- Needle aspirate of ecchymotic area revealed gram-positive bacilli
- Blood cultures grew Clostridium perfringens

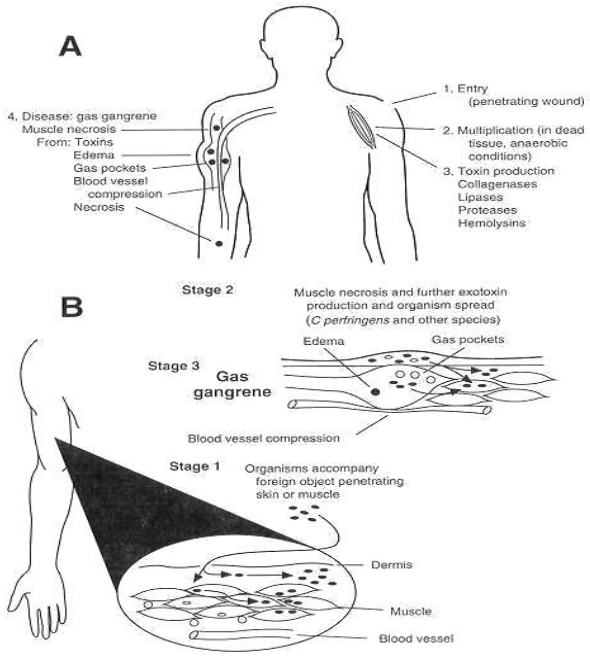


Clostridium

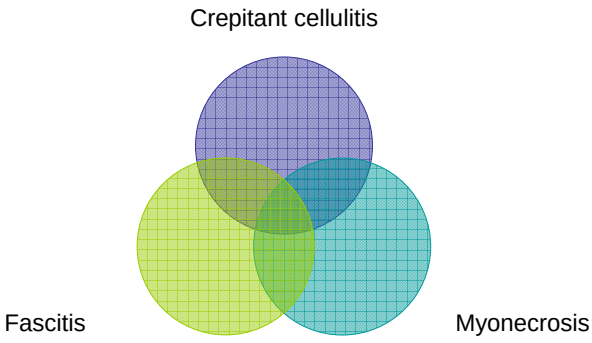
- Epidemiology
  - Ubiquitous
    - Present in soil, water, sewage
    - Normal flora in GI tracts of animals and humans
- Pathogenesis
  - Spore formation
    - resistant to heat, dessication, and disinfectants
    - can survive for years in adverse environments
  - Rapid growth in oxygen deprived, nutritionally enriched environment
  - Toxin elaboration (histolytic toxins, enterotoxins, neurotoxins)

Clostridium perfringens

- Epidemiology
  - GI tract of humans and animals
  - Type A responsible for most human infections, is widely distributed in soil and water contaminated with feces
  - Type B-E do not survive in soil but colonize the intestinal tracts of animals and occasionally humans
- Pathogenesis
  - **α-toxin**: lecithinase (phospholipase C) that lyses erythrocytes, platelets and endothelial cells resulting in increased vascular permeability and hemolysis
  - **ß-toxin:** necrotizing activity
  - **Enterotoxin:** binds to brush borders and disrupts small intestinal transport resulting in increased membrane permeability
- Clinical manifestations
  - Self-limited gastroenteritis
  - Soft tissue infections: cellulitis, fascitis or myonecrosis (gas gangrene)



Clostridial soft tissue infections



Myonecrosis

Clostridial myonecrosis

- Clinical course
  - Symptoms begin 1-4 days after inoculation and progresses rapidly to extensive muscle necrosis and shock
  - Local area with marked pain, swelling, serosanguinous discharge, bullae, slight crepitance
  - May be associated with increased CPK
- Treatment
  - Surgical debridement
  - Antibiotics
  - Hyperbaric oxygen

Case 2

- 80 year old woman who was treated for a pneumonia with a cephalosporin
  - Well upon discharge
  - 10 days later develops multiple, watery loose stools and abdominal cramps
  - Fever, bloody stools, worsened abdominal pain

Case 2

- Leukocytosis with 80% neutrophils
- Fecal leukocytes
- Stool culture neg. for salmonella, shigella campylobacter, Yersinia spp
- Colonoscopy
  - White plaques of fibrin, mucous and inflammatory cells

Clostridium difficile

- Epidemiology
  - Endogenous infection
    - Colonizes GI tract in 5% healthy individuals
    - Antibiotic exposure associated with overgrowth of *C. difficile*
      - Cephalosporins, clindamycin, ampicllin/amoxicillin
    - Other contributing factors: agents altering GI motility, surgery, age, underlying illness
  - Exogenous infection
    - Spores detected in hospital rooms of infected patients
- Pathogenesis
  - **Enterotoxin (toxin A)**
    - produces chemotaxis, induces cytokine production and hypersecretion of fluid, development of hemorrhagic necrosis
  - **Cytotoxin (toxin B)**
    - Induces polymerization of actin with loss of cellular cytoskeleton

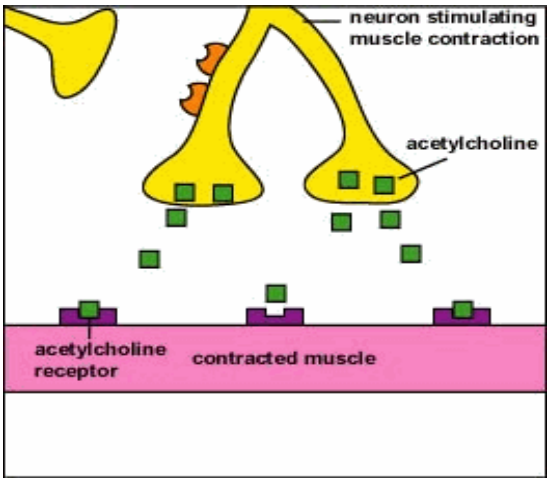
C. *difficile* colitis

- Clinical syndromes
  - Asymptomatic colonization
  - Antibiotic-associated diarrhea
  - Pseudomembranous colitis
- Diagnosis
  - Isolation of toxin
  - Culture
- Treatment
  - Discontinue antibiotics
  - Metronidazole or vancomycin
  - Relapse in 20-30% (spores are resistant)

Clostridium tetani

- Epidemiology
  - Spores found in most soils, GI tracts of animals
  - Disease in un-vaccinated or inadequately immunized
  - Disease does not induce immunity
- Pathogenesis
  - Spore inoculated into wound
  - **Tetanospasmin**
    - Heat-labile neurotoxin
    - Retrograde axonal transport to CNS
    - Blocks release of inhibitory neurotransmitters (eg. GABA) into synapses, allowing excitatory synapses to be unregulated. This results in muscle spasms
    - Binding is irreversible
  - Tetanolysin
    - Oxygen labile hemolysin, unclear clinical significance

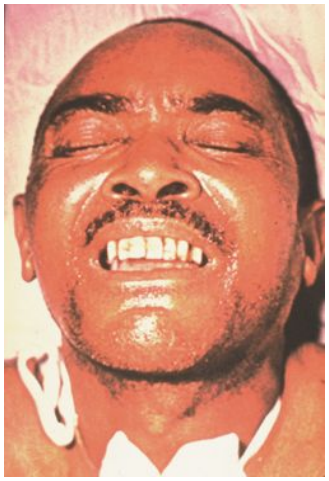
C. tetani exotoxin



Tetanus

- Clinical Manifestations
  - Generalized
    - Involvement of bulbar and paraspinal muscles
      - Trismus, risus sardonicus, opisthotonos
    - Autonomic involvement
      - Sweating, hyperthermia, cardiac arrythmias, labile BP
  - Cephalic
    - Involvement of cranial nerves only
  - Localized
    - Involvement of muscles in primary area of injury
  - Neonatal
    - Generalized in neonates; infected umbilical stump

Risus sardonicus and Opisthotonos of Tetanus



Tetanus

- Treatment
  - Debridement of wound
  - Metronidazole
  - Tetanus immunoglobulin
  - Vaccination with tetanus toxoid
- Prevention
  - Vaccination with a series of 3 tetanus toxoid
  - Booster dose every 10 years

Case 3

- 6 month old infant girl, full-term, previously healthy
- Progressive fussiness, poor oral intake, weak cry for 4 days.
- Uninterested in feeding or playing.
- Exam:
  - Listless
  - Afebrile, stable vital signs
  - Sluggish pupils, decreased tone, no reflexes bilaterally

Case 3

- No ill contacts or recent travel, lives with parents on Staten Island
  - Construction in neighborhood
- Diet: Breast milk & some rice cereal only
- No fever, vomiting, diarrhea, rash, seizures

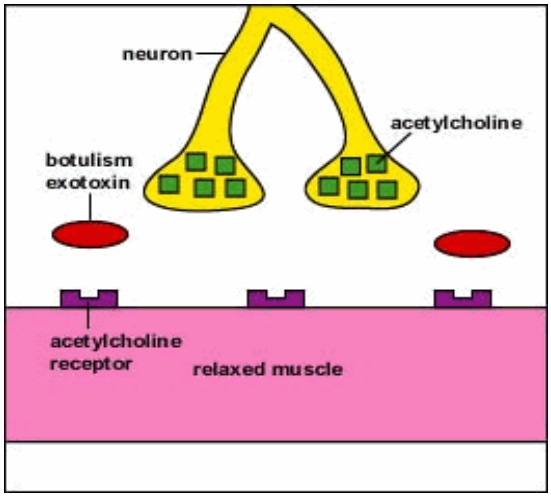
Case 3

- Serum, breast milk, stool sent to DOH for detection of Botulinum toxin
  - Stool POSTIVE for toxin type B
- Given Baby botulism immunoglobulin (Baby-BIG)
  - Regained movement of arm within a day
  - Began feeding in 4 days

Clostridium *botulinum*

- Epidemiology
  - Commonly isolated in soil and water
    - 20% soil samples
  - Human disease associated with botulinum toxin A, B, E, F
- Pathogenesis
  - Blocks neurotransmission at peripheral cholinergic synapses
  - Prevents release of acetylcholine, resulting in muscle relaxation
  - Recovery depends upon regeneration of nerve endings

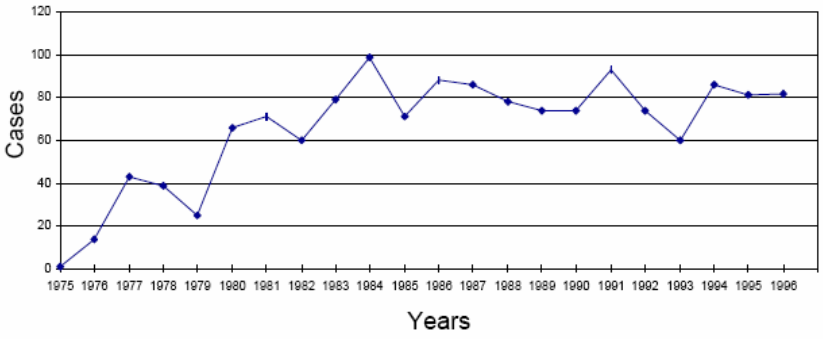
C. Botulinum Exotoxin



Botulism

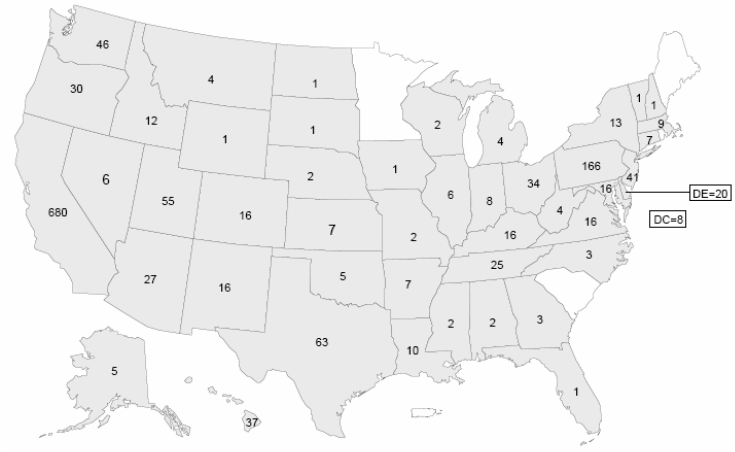
- Clinical Syndromes
  - Foodborne botulism
    - Associated with consumption of preformed toxin
      - Home-canned foods (toxin A, B)
      - Preserved fish (toxin E)
    - Onset of symptoms 1-2 days
      - Blurred vision, dilated pupils, dry mouth, constipation
      - Bilateral descending weakness of peripheral muscles; death related to respiratory failure
  - Infant botulism
    - Consumption of foods contaminated with botulinum spores
      - 6-10% of syrups or honeys
    - Disease associated with neurotoxin produced in vivo
    - Onset of symptoms in 3-10 days
  - Wound botulism
  - Asymptomatic adult carriage

Cases of Infant botulism 1976-1996



CDC, 1998

Outbreaks of infant botulism, by state, 1976-1996



CDC, 1998

Botulism: diagnosis

- Clinical features
- Identification of toxin or organism in stool or serum
  - Mouse bioassay most sensitive
- Electromygraphy
- Normal studies:
  - Blood, urine, CSF fluid analyses are usually normal
  - Edrophonium challenge negative
  - EEG/brain imaging normal

Botulism: Treatment

- Treatment
  - Supportive care
  - Elimination of organism from GI tract
    - Gastric lavage
    - Metronidazole or penicillin
  - Baby Botulinum Immunoglobulin (BIG): pooled plasma from adults immunized with pentavalent (ABCDE) botulinum toxoid
  - Trivalent equine Immunoglobulin (ABE)
- Prevention
  - Prevention of spore germination (Storage <4°C, high sugar content, acid PH)
  - Destruction of preformed toxin (20 min at 80°C)

Botox

Anaerobic gram negative bacilli

- Bacteroides
  - *B. fragilis*
  - *B. thetaiotaomicron*
- Fusobacterium
- Prevotella
- Porphyromonas

Anaerobic gram negative bacilli

- Epidemiology
  - Bacteroides and Prevotella are most prevalent organisms in human flora
  - **Oral cavity** (crypts of tonsils and tongue, dental plaques and gingival crevices)
    - Anaerobes become prominent after eruption of teeth
    - *Porphyromonas gingivalis* found in 37% of subjects, colonization concordance in families
    - Fusobacterium
  - **GI tract**
    - Anaerobes outnumber aerobes 1000:1
    - 10^{11}organisms per gram of fecal material
    - *Bacteroides* spp. (*vulgatus and thetaiotaomicron* most common)
  - **Vagina**

Anaerobic gram negative bacilli

- Clinical Diseases
  - Chronic sinus infections
  - Periodontal infections
  - Brain abscess
  - Intra-abdominal infection
  - Gynecological infection
  - Diabetic and decubitus ulcers

Case 4

- 37 year old woman with peri-umbilical pain, anorexia, and nausea
  - Given diagnosis of food poisoning in the ER
  - Develops sharp right lower abdominal pain and fever over next 4 days

Bacteroides

- Epidemiology
  - *B.* fragilis associated with 80% of intra-abd infx
    - Peritonitis, intraabdominal abcesses
  - Diabetic foot ulcers
- Pathogenesis
  - Polysaccharide capsule
    - Increases adhesion to peritoneal surfaces (along with fimbriae)
    - Protection against phagocytosis
    - Differs from LPS of aerobic GNR
      - Less fatty acids linked to Lipid A component
      - Less pyrogenic activity
    - Abscess Formation
  - Superoxide dismutase and catalase
  - Elaborate a variety of enzymes
  - Synergy

Abscess Formation

- Bacteroides Capsular Polysaccharide Complex (CPC)
  - 2 discreet polysaccharides (PS A & PS B) with oppositely charged structural groups
  - Injection of CPC into peritoneum of rat results in abscess formation
    - Chemical neutralization or removal of charged groups abrogated abscess induction
  - Vaccination with CPC results in protection against abscess formation
    - T cells important in abscess formation

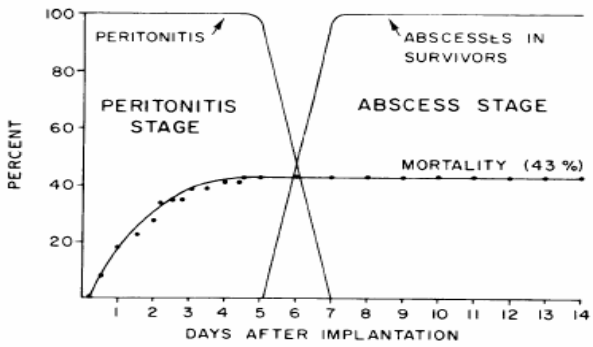


FIG. 1. *Mortality and abscess formation in 106 male Wistar rats receiving inoculum obtained from meat-fed animals. Mortality is expressed as cumulative percent.*

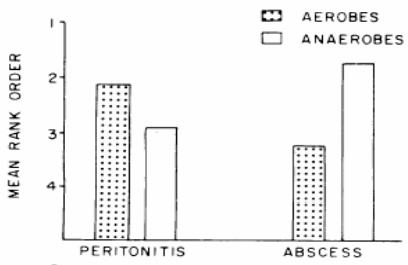


FIG. 2. *Comparison of the mean rank order for the two major aerobic isolates (E. coli and enterococcus) and the two major anaerobic isolates (B. fragilis and Fusobacterium) in peritoneal exudates and abscesses. In peritonitis exudates, the rank order of the two aerobes was greater than that of the two anaerobes; in abscesses, anaerobes outranked aerobes. Factorial analysis of variance showed that this difference in rank order was significant ($P < 0.001$).*

Abscess Formation

- Initial phase
  - Introduction of bacteria and inflammatory exudates (esp. fibrin)
- Microbial persistence (localization)
  - Impaired bacterial clearance: fibrin deposition, platelet clumping
  - Impaired neutrophil migration and killing: hypoxia, low PH
  - Impaired phagocytic function: fibrin, hemoglobin, by products
  - Complement depletion: necrotic debris
- Development of mature abscess
  - Central core of necrotic debris, dead cells, bacteria
  - Surrounded by neutrophils and macrophages and peripheral ring of fibroblasts and smooth muscle cells within collagen capsule

Conclusion

- Anaerobic infections
  - Endogenous or exogenous
  - Alteration of host tissue
    - Break in anatomic barrier
    - Devitalized tissue
  - Polymicrobial
    - Synergy between anaerobes and facultative bacteria
  - Abscess formation
  - Exotoxin elaboration