Anaerobic infections

PART 1: Infection with Gram-positive obligate anaerobes (Invasive *Clostridium* spp.)

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What is an obligate anaerobe?

- Obligate aerobes
  - acquire energy *ONLY* by respiration
  - cannot survive without oxygen
    - ex. *Mycobacterium tuberculosis, Pseudomonas aeruginosa*
- Obligate anaerobes
  - acquire energy *ONLY* by fermentation
  - most cannot survive in oxygen
    - ex. *Clostridium perfringens, Bacteroides fragilis*
- Facultative anaerobes (most bacteria)
  - acquire energy by either respiration OR fermentation
  - Can survive with or without oxygen
    - ex. *E. coli*, *Staphylococcus aureus*

Where do obligate anaerobes live?

- Endogenous (in the body)
  - GI tract of animals
  - Gingival crevices around teeth
  - Skin glands and hair follicles
- Exogenous (in the environment)
  - Soil

The Endogenous Microbiome

- Our bacteria > our own cells
- Roles of endogenous microbiota (microflora)
  - Digestion
  - Colonization resistance
  - Induction of antibodies (IgA, IgG, etc.)
  - Normal development of the immune response
- Should the microbiome be considered another organ of the body?

Sources of Anaerobic Infections

- Usually endogenous
  - Intestinal anaerobes
  - Oral anaerobes
- Usually exogenous
  - *Clostridium tetani* (tetanus)
  - *Clostridium botulinum* (botulism)
  - *Clostridium difficile* (antibiotic-associated colitis)
- Either endogenous or exogenous
  - Other Clostridial infections (e.g., gas gangrene)

What are these lectures about?

- *Part 1: Invasive Clostridium spp.*
  - gas gangrene/myonecrosis — *C. perfringens, C. septicum, C. histolyticum, C. novyi,* etc.
  - wound infection/abscess
  - food poisoning — *C. perfringens*
- *Part 2: Toxigenic Clostridium spp.*
  - tetanus — *C. tetani*
  - botulism — *C. botulinum*
  - antibiotic-associated colitis — *C. difficile*
- *Part 3: Gram-negative anaerobes*
  - abscesses — *B. fragilis, Bacteroides spp, Prevotella, Porphyromonas, Fusobacterium, anaerobic cocci*
  - other

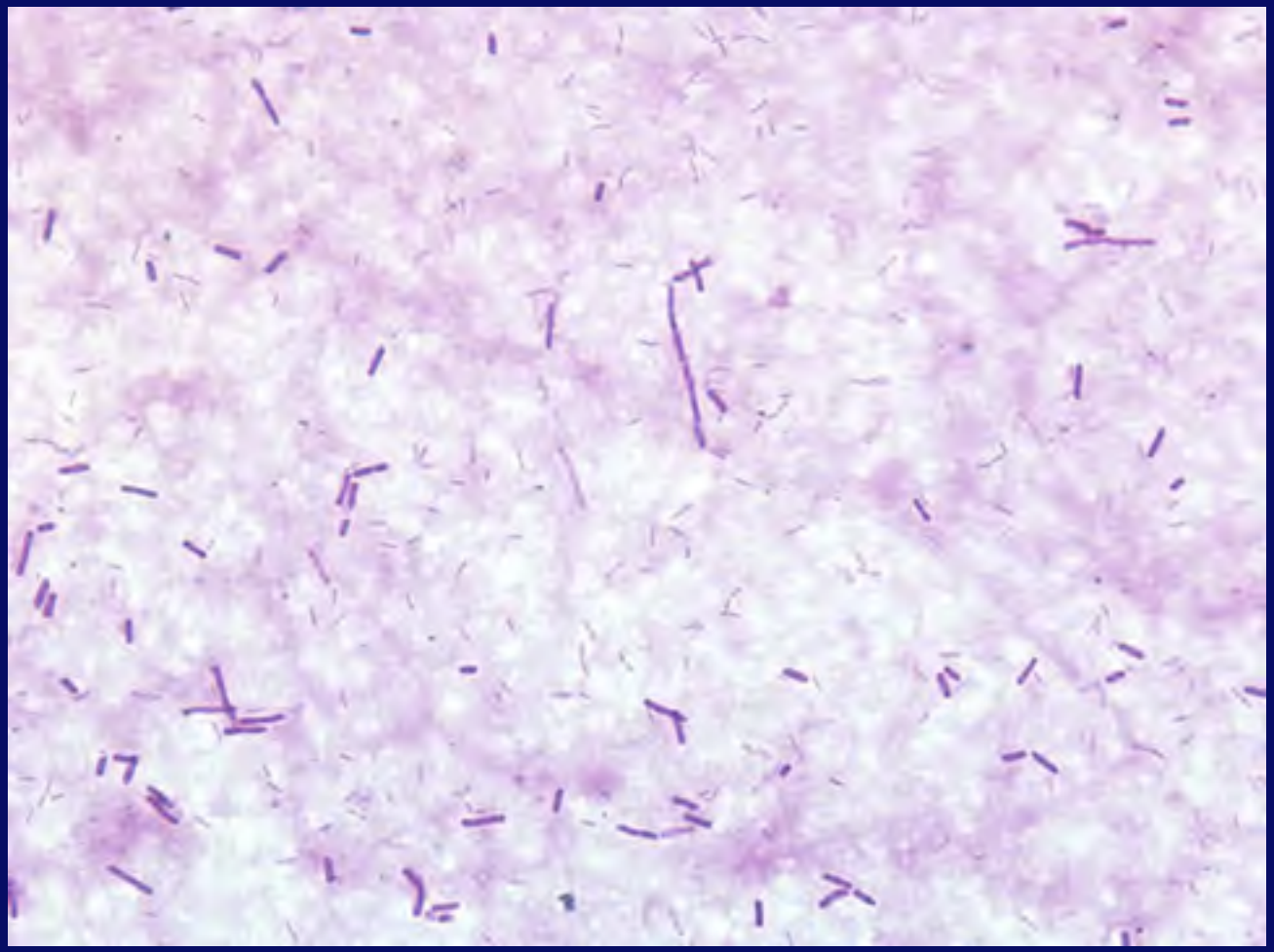
Case: infected wound

- 46 year old male construction supervisor fell ~4 meters onto an iron reinforcing rod at a construction site. The rod penetrated the left upper arm and tore the skin.
- The patient was told that he was diabetic many years earlier but was on no treatment.
- At the ED, the wound was sutured closed and bandaged. A pain killer and an oral antibiotic were prescribed. The patient took the pain killer only.

Case (continued)

- He returned 48 hours later with *extreme pain and tenderness* of the entire left arm
- Temperature=39.5C (*fever*).
- *Thin watery drainage* from the wound.
- "Crunchy" sensation under the affected skin (i.e., *crepitus*).
- An xray of the shoulder shows *gas in the tissues* under the affected skin.
- Routine bacterial culture: negative.

Gram-stain of watery discharge



PD-SELF Andreas Zautner, 2006

No intact PMNs were seen

Case (continued)

- The patient was hospitalized urgently and treated with high-dose penicillin
- He underwent several extensive debridements of devitalized muscle and skin, followed by muscle flap and skin graft closure.
- Anaerobic culture at his first surgery grew a Gram-positive rod with central spores.

Questions to consider?

- Where did these bacteria come from? Why were they at a construction site?
- Why are there no intact PMNs in the pus?
- Why is there gas in the tissue?
- Why was the routine culture negative?
- Why is this infection so aggressive in this patient?
- Why was it necessary to do repeated surgery if antibiotics are active?

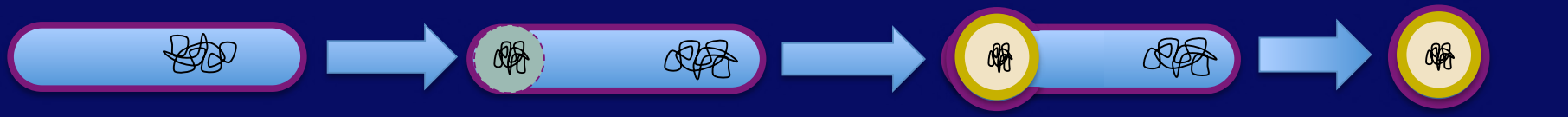
Clostridium species

- Gram-positive, spore-forming bacilli
- Sporulation occurs in nutrient-limiting conditions
- Spores are resistant to extremes of dryness, heat (boiling), and many chemical disinfectants
- They can persist in the environment for weeks to months and germinate deep in the soil where the conditions are anaerobic
- In favorable conditions (e.g., in human tissues), spores germinate and may produce toxins

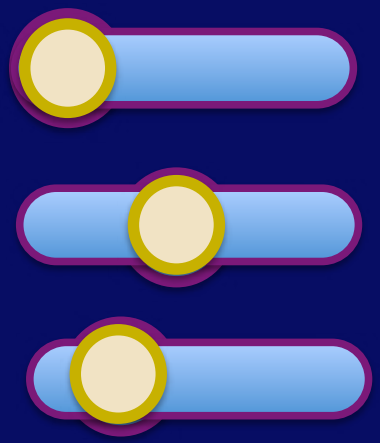
Sporulation

- Complex sequence of gene expression is triggered
- Asymmetric cell division, with partitioning of a chromosome into an internal spore (endospore)
- Elaboration of a thick, impermeable surface coat

Spores



Spore positioning and species



Terminal spore	*C. tetani*
Central spore	*C. perfringens*
Subterminal spore	*C. septicum, novyi, histolyticum difficile, botulinum*

Wounds and soft tissue infection

- *C. perfringens* type A is the most common invasive clostridial species (but other species can cause similar effects – *C. novyi, C. septicum*)
- The organism requires damaged tissue with anaerobic conditions, impaired blood supply, complex nutrients, and Ca^{++} ions
- Produces 12 toxins that attack membranes, including:
  - α-toxin (lecithinase, AKA "myotoxin")
  - zinc metallophospholipase (hydrolyzes phosphatidylcholine and sphingomyelin-kills cells)
- Toxins destroy PMNs, produce myonecrosis
- Organisms produce gas when they grow = crepitance in tissue

Management of gas gangrene

- Surgical debridement of all devitalized tissues (remove the anaerobic/necrotic focus)
- Penicillin and other beta-lactam antibiotics are effective but not sufficient
- Antitoxins are not effective
- Hyperbaric oxygen may be useful
- There is no vaccine

Clostridia may also be involved in milder non-necrotizing wound infections, often in combination with other pathogens, e.g., diabetic foot infections, leprous wounds

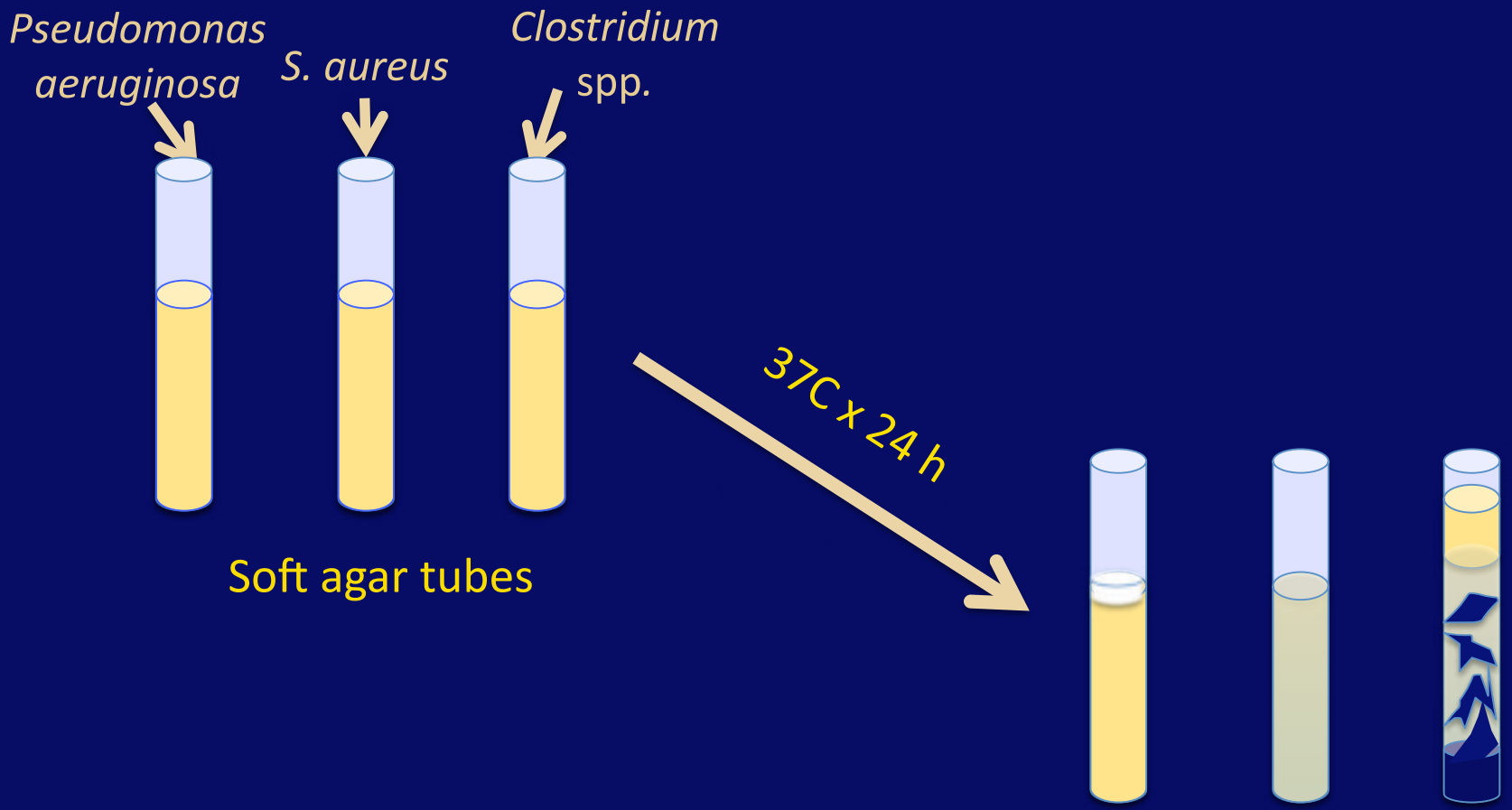
Laboratory diagnosis

- To cultivate anaerobes, the specimen should not be exposed to air

Anaerobic growth

- Anaerobes will grow at the bottom of tubes of static nutrient broth
- To separate facultative from obligate anaerobes you must plate out the growth from the bottom of the tubes

Thought experiment



Culturing anaerobes on plates

- Media (blood agar) should be pre-reduced
- Swabs should be pre-reduced when used
- Plating and culture is ideally done in an anaerobic environment

Tigerweet, Flickr. com

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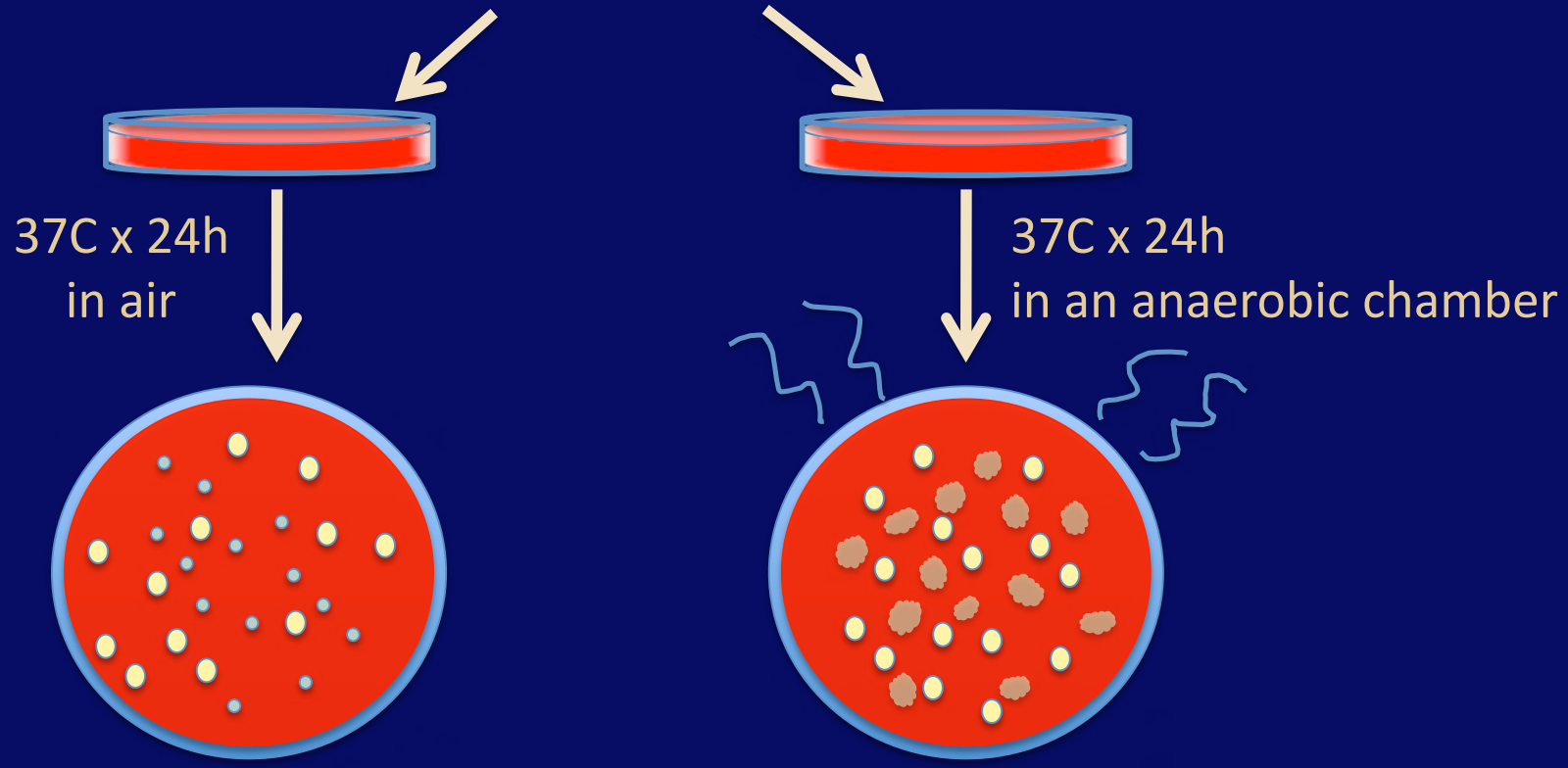
Another thought experiment

Mixture of: *Pseudomonas aeruginosa*
S. aureus
Clostridium sporogenes



Most obligate anaerobes produce a distinctly unpleasant odor!!

Questions to consider?

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Case: Spontaneous gas gangrene

- A 50 year old man developed gas gangrene of his right shoulder without any predisposing trauma.
- Culture of the debrided tissues grew *C. septicum*
- Two months later, colon cancer was diagnosed.

Spontaneous *C. septicum* gangrene

PD-INEL Mandell, G. Essential Atlas of Infectious Diseases

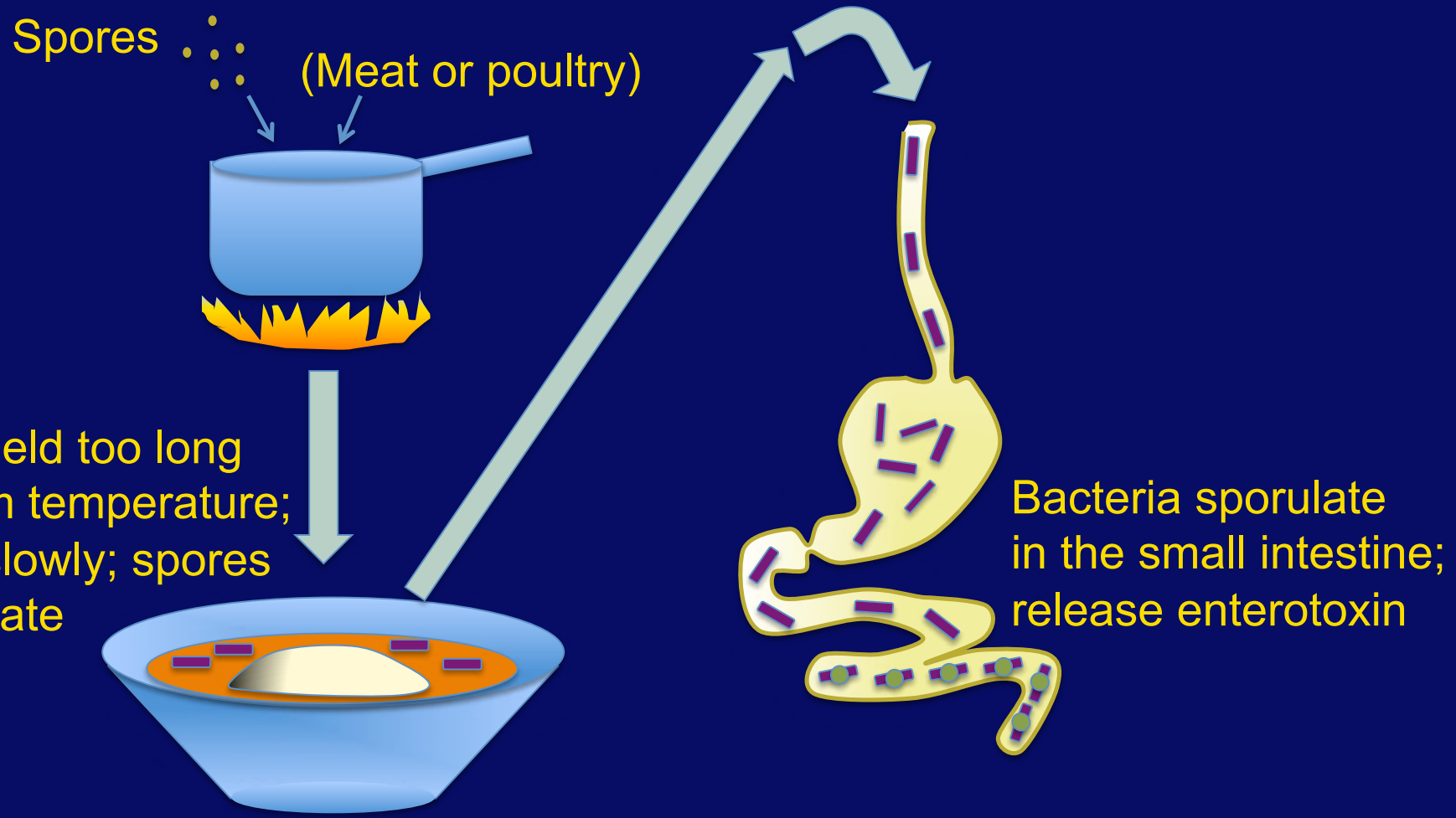
Clostridial diseases in which culture is not usually done

- Food poisoning
- Tetanus
- Botulism
- Antibiotic-associated colitis

C. perfringens food poisoning

- Improperly-handled food is contaminated with spores, which survive cooking temperature
- Spores germinate with heating (anaerobiasis) and if $>10^5$/g bacteria are ingested, illness may occur
- Sporulation in the small intestine releases enterotoxin
- Diarrhea (without fever) occurs 6-18 hours later, and resolves in 1-2 days

Clostridial food poisoning



Diagnosis Management of Clostridial Food Poisoning

- Usually recognized by multiple cases of diarrhea 6-18 hours after ingestion of food (usually in restaurants, not home)
- Culture of the patient is not helpful
- Treatment is supportive only (resolves spontaneously); no antibiotics
- Identify the food vehicle, (culture), and correct preparation problem

Generalizations about invasive Clostridium spp.

- Sporulation is critical for survival in the environment (soil)
- Exogenous infections = spores in wounds; Endogenous infections = vegetative bacteria released from colonized sites (e.g., colon)
- Disease is mediated by exotoxin-release from vegetative cells
- Simple antibiotics are effective, but not in non-viable tissues; surgery is often required
- Antibiotic resistance is not a problem

Summary of Key Points

- *Clostridium* spp. are Gram-positive spore-forming, obligate anaerobic bacteria that grow in devitalized tissues.
- Alpha-toxin is a lecithinase that destroy cell membranes, including PMNs and muscle cells.
- Gas gangrene and myonecrosis may result from wound infection with certain clostridia
- Antibiotics and surgery are critical if the patient is to survive.
- Clostridia may contribute to lesser, mixed bacterial wound infections
- *C. septicum* bacteremia may signal colon cancer
- Clostridial enterotoxin causes a self-limited diarrhea (food poisoning) but is not invasive most cases.

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