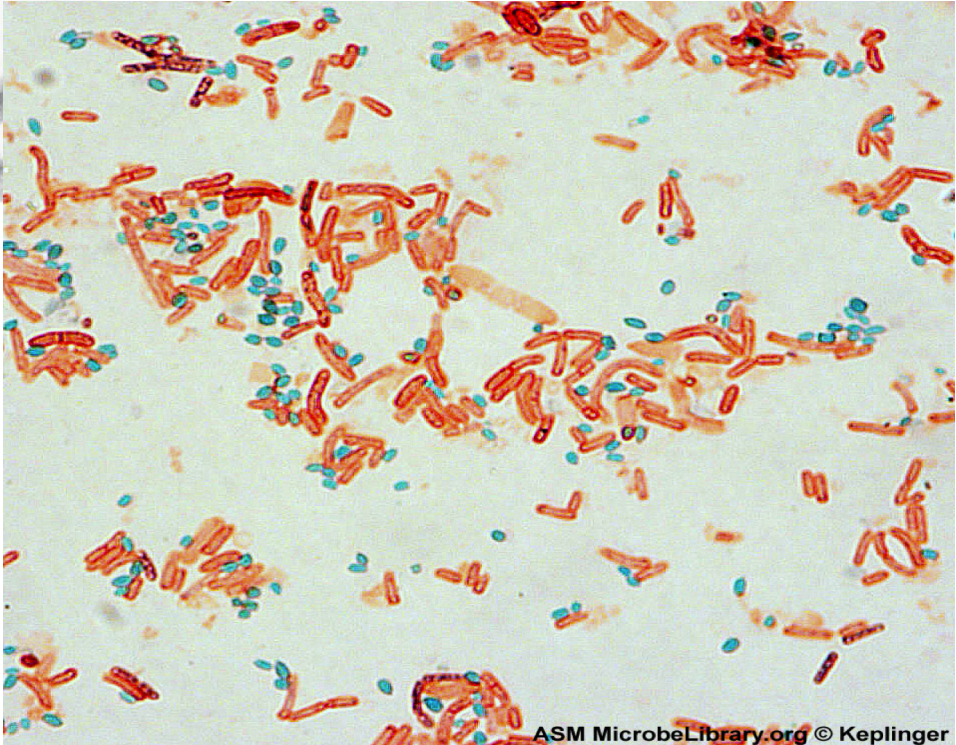
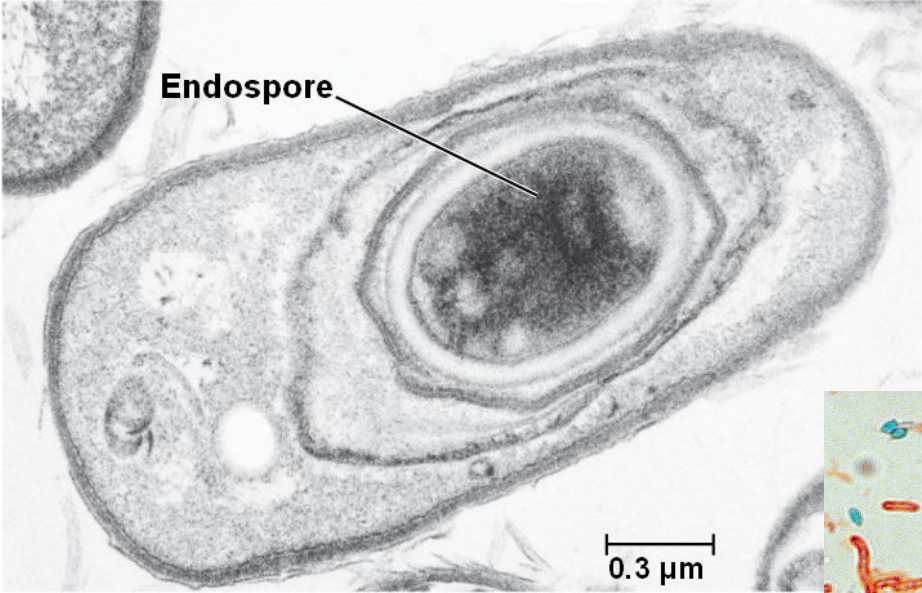


Exercise 3-9: Endospore Stain

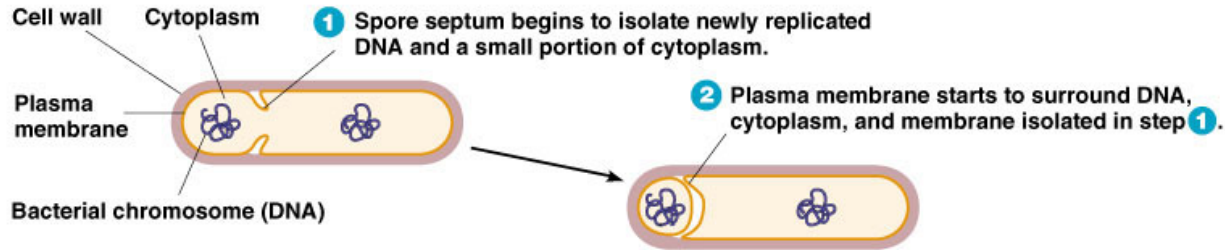


Differential Stain

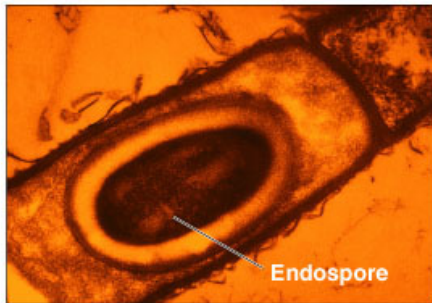
Endospores

- Genera: *Bacillus* (and new genera formerly classified as *Bacillus*) and *Clostridium*
 - Dormant structure
 - Extremely dehydrated structure → heat resistance
 - Not metabolically active → total absence of ATP
 - Allow bacteria to survive unfavorable environmental conditions
 - Heat
 - UV Radiation
 - Chemicals
 - Starvation
- * Endospores define conditions for sterility:
121°C, 15psi

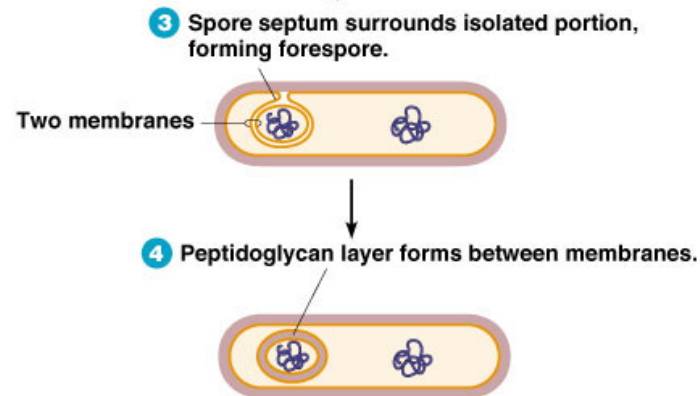
Sporulation



(a) Sporulation, the process of endospore formation



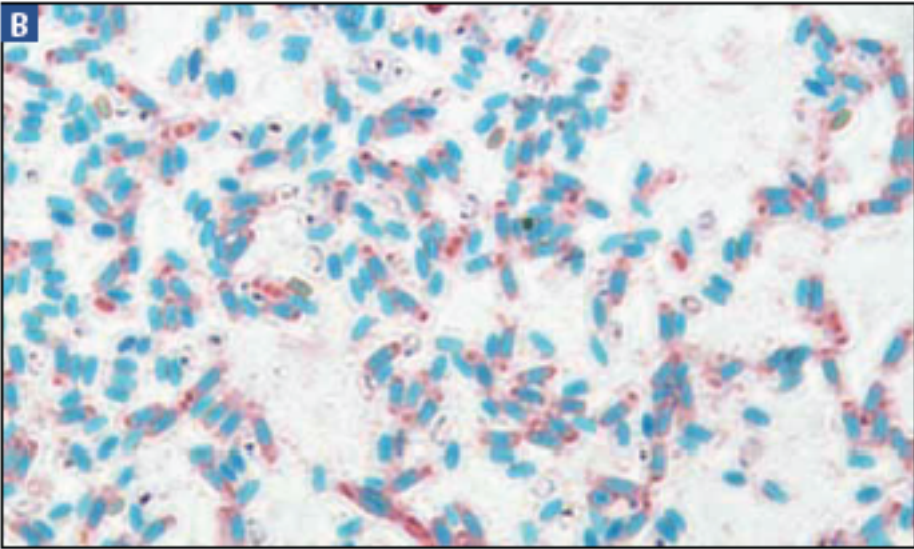
(b) An endospore in *Bacillus anthracis*



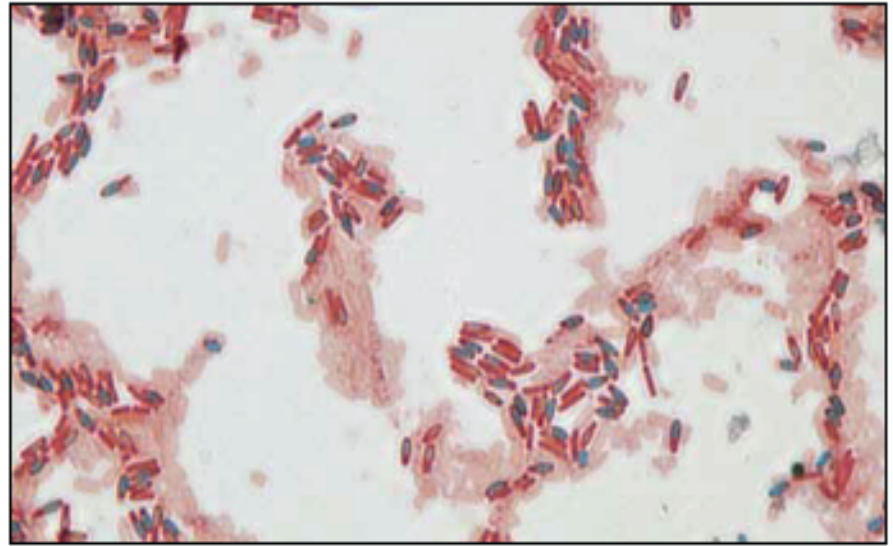
Germination

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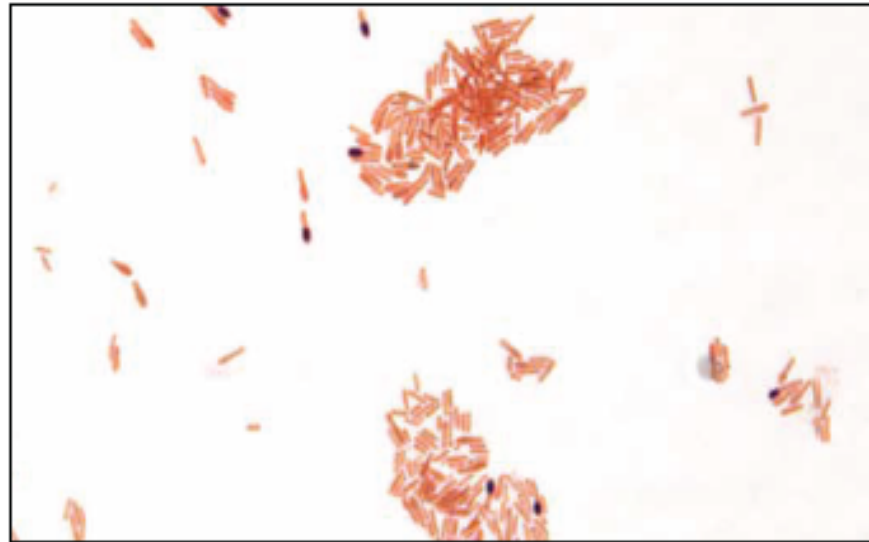
- Location of spore varies: **terminal**, **sub-terminal** or **central**
- Shape of spore varies: **spherical** or **oval** (elliptical)
- Distended (swollen) cells or not



Mostly Released Endospores



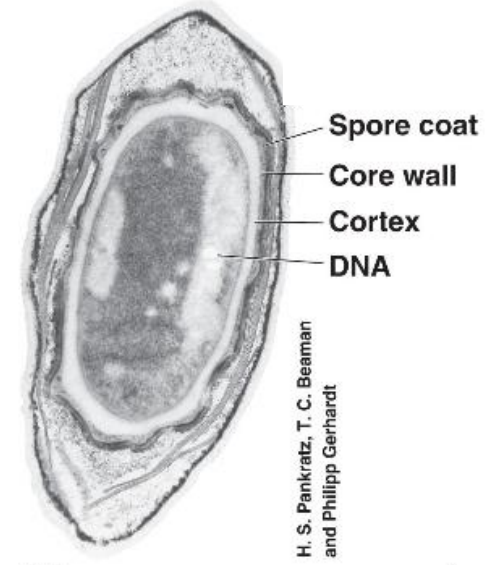
**Central Elliptical Endospores
No Distended Cells**



**Sub-Terminal/Terminal Elliptical Endospores,
Distended Cells**

Endospores

- **Spore coat:** tough outer barrier made of **keratin** and additional spore proteins
 - Provides protective barrier
 - Also resists normal staining techniques
 - Heat is applied to facilitate uptake of the dye
 - Dye penetrates the spore because of heat and is trapped within spore
 - Primary dye is left for longer:
 - Water soluble → will not penetrate cell
 - Vegetative cells can be decolorized with water, but not the spore



**Spore
producer**

**Spore
nonproducer**

Cells and spores prior to staining are transparent.



After staining with malachite green, cells and spores are green. Heat is used to force the stain into spores, if present.



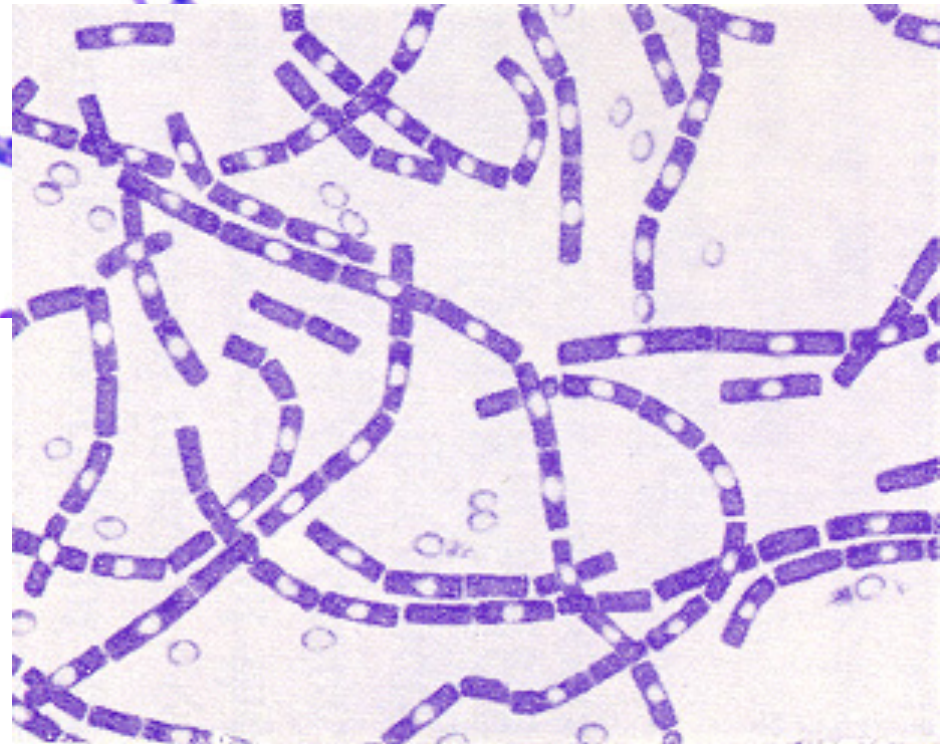
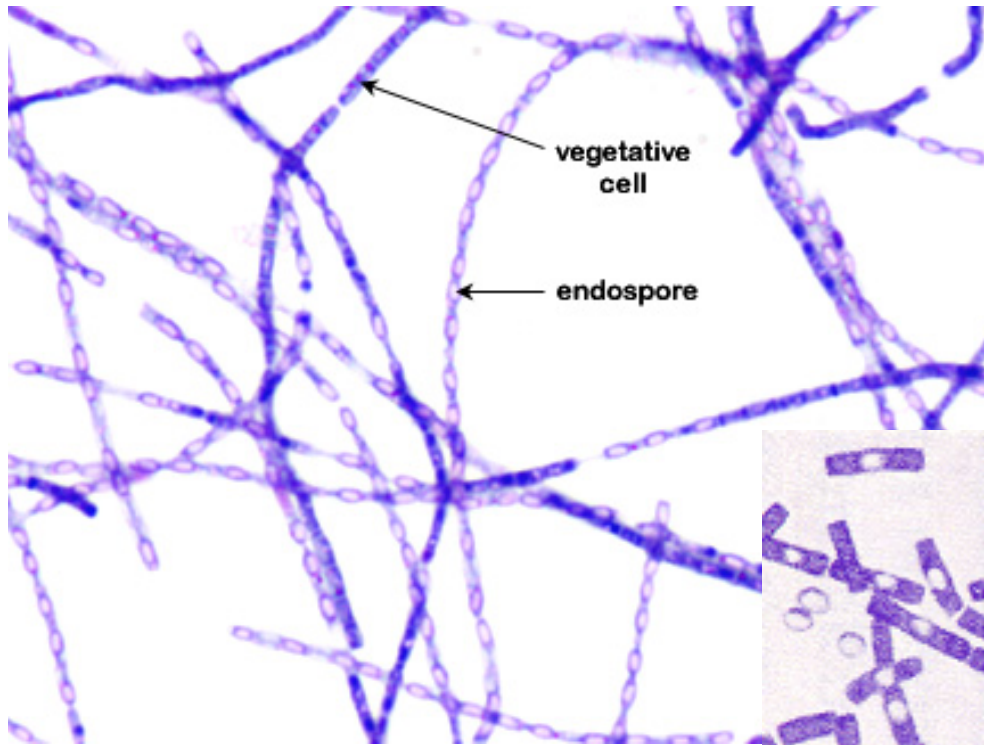
Decolorization with water removes stain from cells, but not spores.



Safranin is used to counterstain cells.



Simple or Gram stain of endospores



Schaeffer-Fulton Method



Modification: **Quick-Spore Stain**

Heat is the mordant in this staining technique - **NO steam used**

1. Smear Prep: **Heat-fix 20 times** instead (dry heat)
2. Malachite green for 15 minutes
3. Wash → Water functions as the decolorizer
4. Safranin for 1 minute
5. Wash
6. Dry

Results

Bacillus megaterium
Identify structures/cells

Magnification
Stain

