# Fate and transport of pathogens in water

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# Topics

- Source of waterborne pathogens
- Removal of waterborne pathogens
  - Wastewater treatment processes
  - Natural processes
- Persistence of waterborne pathogens in the environment
  - Agent factors
  - Environmental factors

# The source of waterborne pathogens

- Environment
  - *Mycobacterium avium*: drinking water distribution systems
  - *Legionella pnemophilia*: hot water systems
- Infected hosts

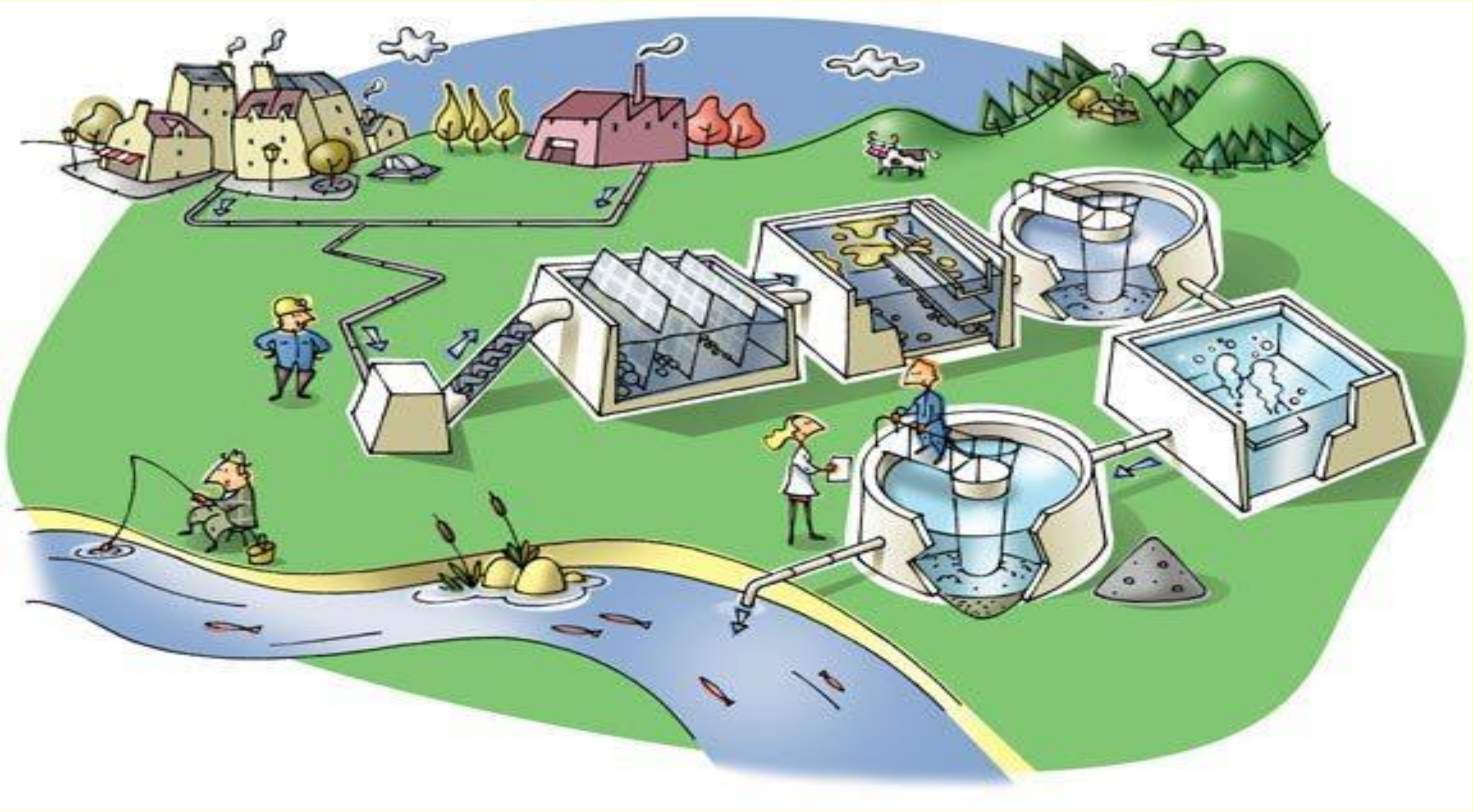
# Incidence and concentration of enteric pathogens in feces (USA)

| Pathogen | Incidence (%) | Concentration(/gram) |
| --- | --- | --- |
| Enteric virus | 10-40 | $10^{3}$-$10^{8}$ |
| Hepatitis A | 0.1 | $10^{8}$ |
| Rotavirus | 10-29 | $10^{10}$-$10^{12}$ |
| *Salmonella* | 0.5 | $10^{4}$-$10^{10}$ |
| *Giardia* | 3.8 | $10^{6}$ |
| | 18-54 | $10^{6}$ |
| *Cryptosporidium* | 0.6-20 | $10^{6}$-$10^{7}$ |
| | 27-50 | $10^{6}$-$10^{7}$ |

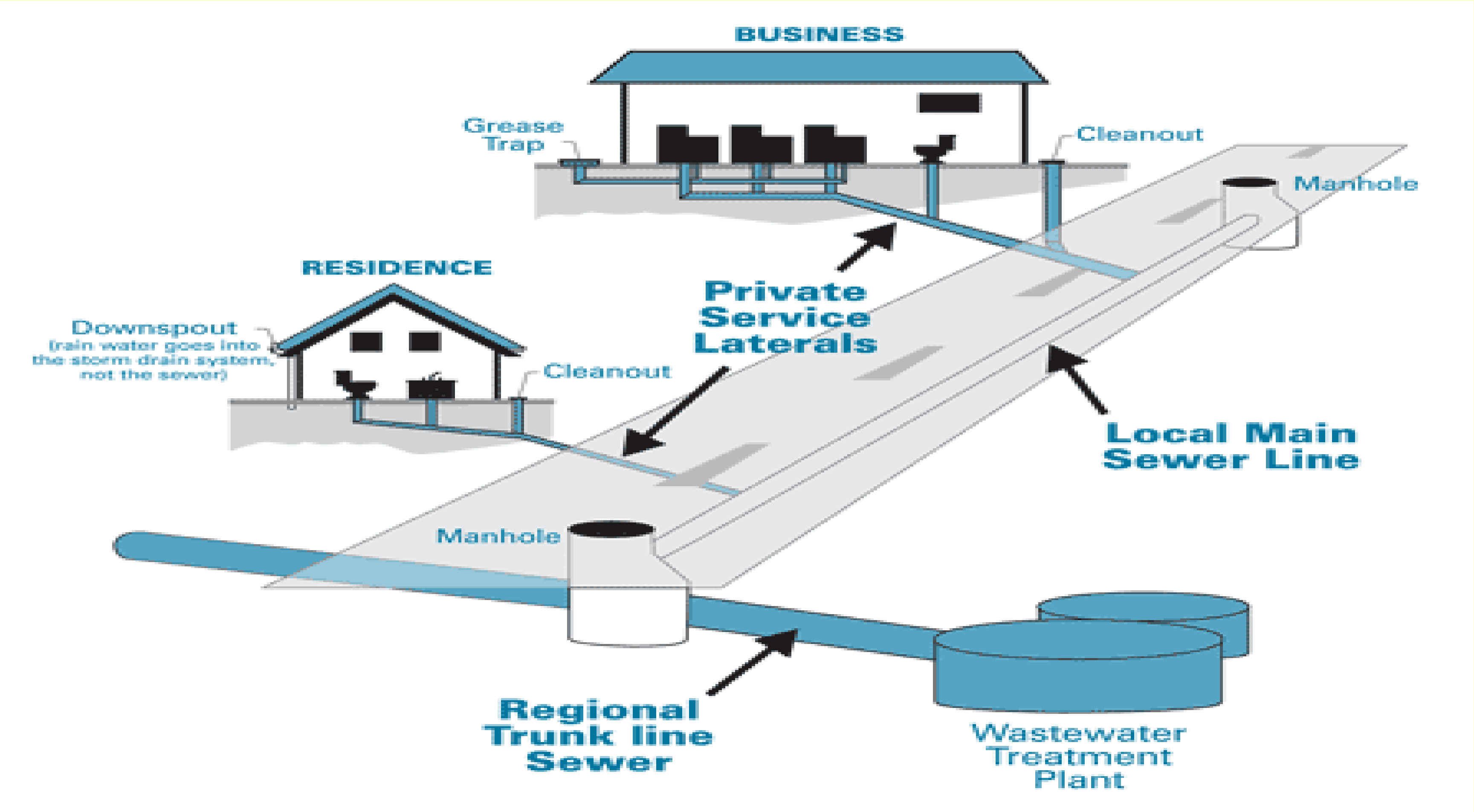
# A simple calculation

- Incidence = 10%, Concentration = $10^6$/gram of feces
- US population (250 million ($10^6$)) X incidence (10 %) = 25 million ($10^6$) cases/year
- Cases (25 million ($10^6$)) X concentration (1 million ($10^6$)/gram) X average weight of feces (500 gram) = 12.5 X $10^{15}$/year
- (12.5 X $10^{15)}$ X frequency of defecation (5) = 62.5 X $10^{15}$ /year
- 62.5 X $10^{15}$ / 365 days = 171 X $10^{12}$/day

# Typical municipal wastewater treatment system

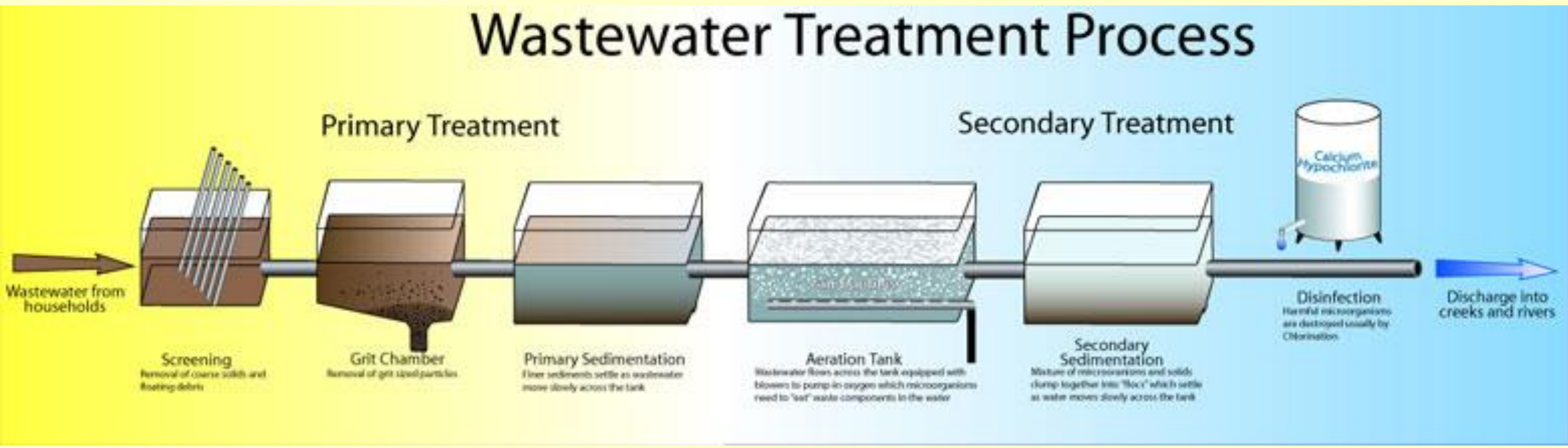


# Sewer systems



# Concentration of enteric pathogens in raw sewage (USA)

| Organism | Concentration (/liter) |
|---|---|
| Enteric virus | $10^4$-$10^5$ |
| *Salmonella* | $10^3$-$10^5$ |
| *Clostridium perfringens* | $10^4$-$10^7$ |
| *Cryptosporidium* oocysts | $10^2$-$10^4$ |
| *Giardia* cysts | $10^2$-$10^5$ |


Wastewater Treatment Process
Primary Treatment
Secondary Treatment
Calcium Hypochlorite
Wastewater from households
Screening
Removal of coarse solids and floating debris
Grit Chamber
Removal of grit sized particles
Primary Sedimentation
Finer sediments settle as wastewater move slowly across the tank
Aeration Tank
Wastewater flows across the tank equipped with blowers to pump-in oxygen which microorganisms need to "eat" waste components in the water
Secondary Sedimentation
Mixture of microorganisms and solids clump together into "flocs" which settle as water moves slowly across the tank
Disinfection
Harmful microorganisms are destroyed usually by Chlorination
Discharge into creeks and rivers

# Removal of pathogens by wastewater treatment processes

**TABLE 21.5 Pathogen Removal during Sewage Treatment**

| | Enteric viruses | *Salmonella* | *Giardia* | *Cryptosporidium* |
|---|---|---|---|---|
| Concentration in raw sewage (number per liter) | $10^5$–$10^6$ | 5,000–80,000 | 9,000–200,000 | 1–3,960 |
| Removal during | | | | |
| Primary treatment [a] | | | | |
| % removal | 50–98.3 | 95.8–99.8 | 27–64 | 0.7 |
| No. remaining/l | 1,700–500,000 | 160–3,360 | 72,000–146,000 | |
| Secondary treatment [b] | | | | |
| % removal | 53–99.92 | 98.65–99.996 | 45–96.7 | |
| No. remaining/l | 80–470,000 | 3–1075 | 6,480–109,500 | |
| Secondary treatment [c] | | | | |
| % removal | 99.983–99.9999998 | 99.99–99.999999995 | 98.5–99.99995 | 2.7[d] |
| No. remaining/l | 0.007–170 | 0.000004–7 | 0.099–2,951 | |

Data from Yates (1994); Robertson *et al.* (1995); Enriquez *et al.* (1995); Modore *et al.* (1987).

[a] Primary sedimentation and disinfection.

[b] Primary sedimentation, trickling filter or activated sludge, and disinfection.

[c] Primary sedimentation, trickling filter or activated sludge, disinfection, coagulation, filtration, and disinfection.

[d] Filtration only.

# Transmission of pathogens in water

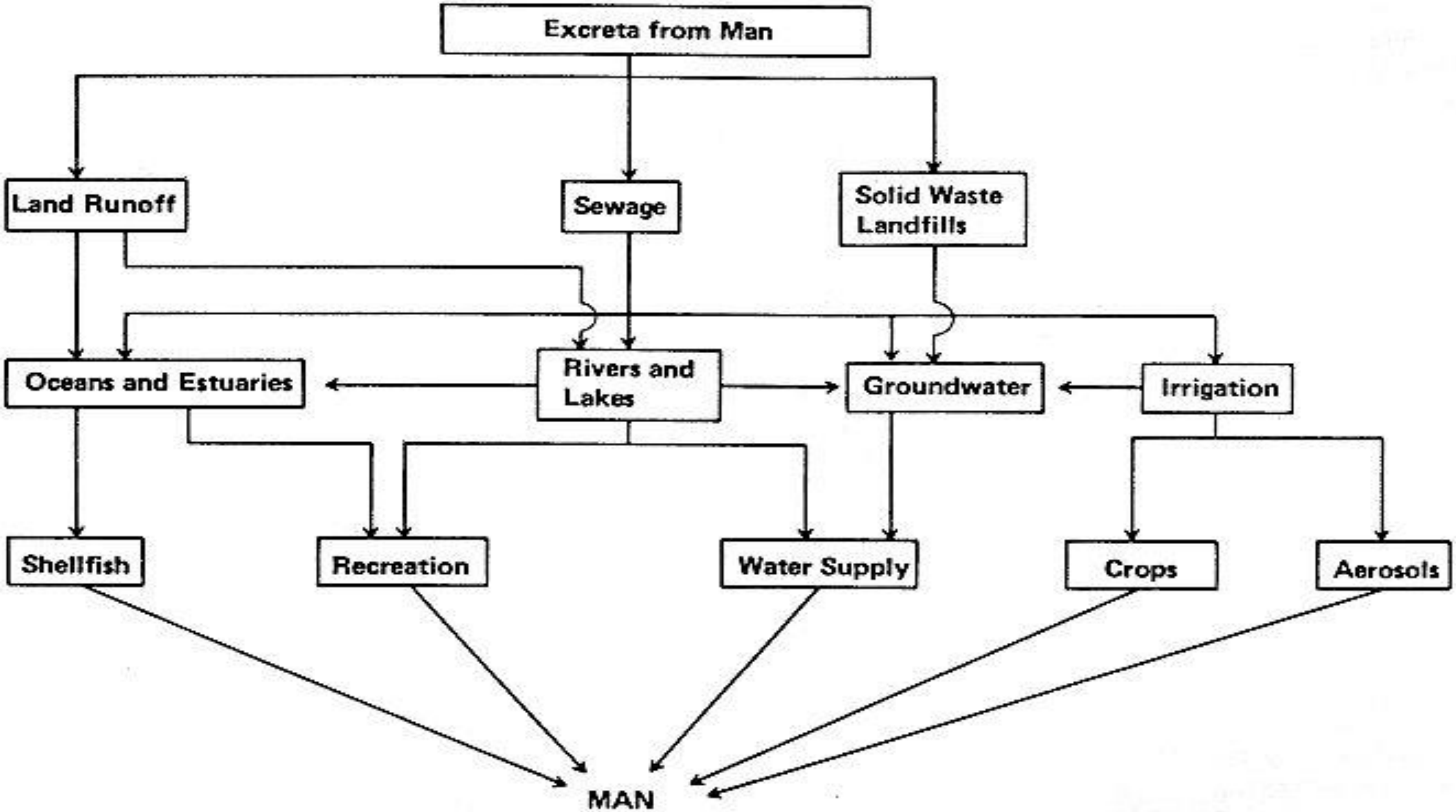



**FIG. 7.** Routes of potential enteric virus transmission in the environment (203).

# Persistence of waterborne pathogens

# Persistence of microorganisms in the environment

- Agent factor
- Environmental factors

# Agent factor

# Microbial Persistence in the Environment

- Viruses
    - non-enveloped viruses > enveloped viruses
        - Envelopes are relatively fragile compared to outer capsids (protein coats)
- Bacteria
    - Gram-positive bacteria (e.g., enterococci) > Gram-negative bacteria ( e.g., *E. coli)*
        - Gram positives have thicker peptidoglycan layer
- Protozoa
    - thick-wall (oo)cysts > thin-wall (oo)cysts >>active living stages (trophozoites, sporozoites)

# Structure of viruses

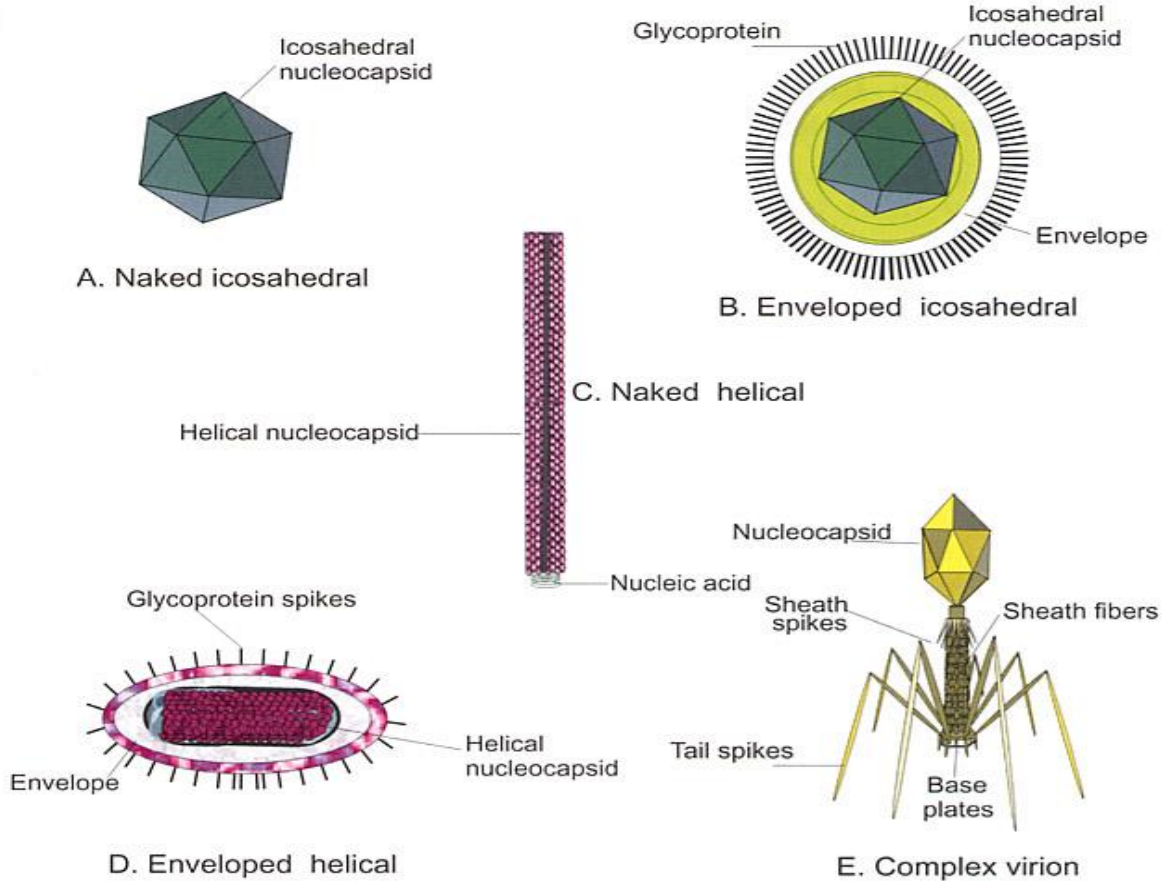



**FIGURE 2.4** Simple forms of viruses and their components. The naked icosahedral viruses (A) resemble small crystals: the enveloped icosahedral viruses (B) are made up of icosahedral nucleocapsids surrounded by the envelope: naked helical viruses (C) resemble rods with a fine regular helical pattern in their surface: enveloped helical viruses (D) are helical nucleocapsids surrounded by the envelope: and complex viruses (E) are mixtures of helical and icosahedral and other structural shapes.

# Structure of bacteria

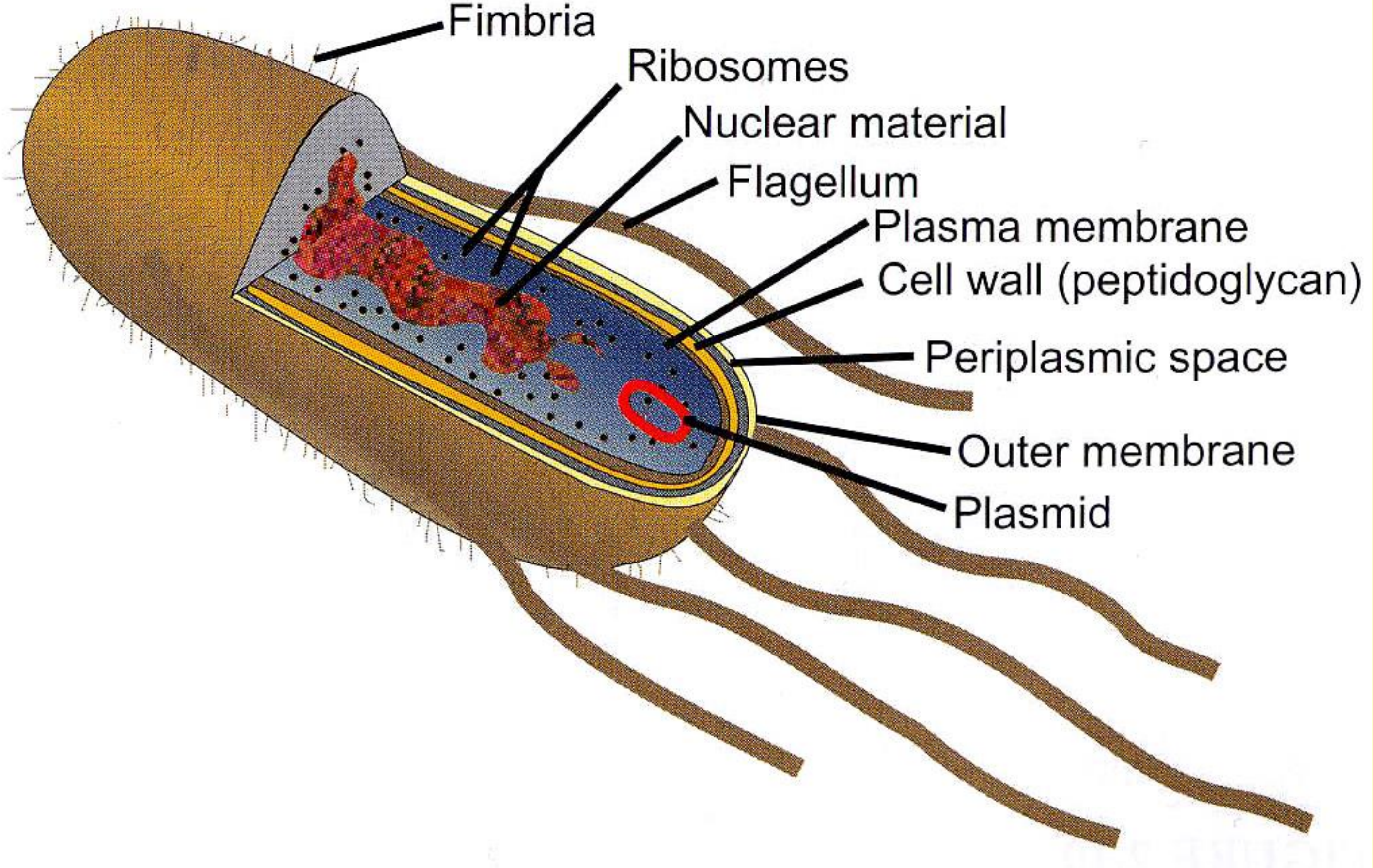



**FIGURE 2.9** Schematic representation of a typical bacterial cell.

# Structure of bacterial cell walls

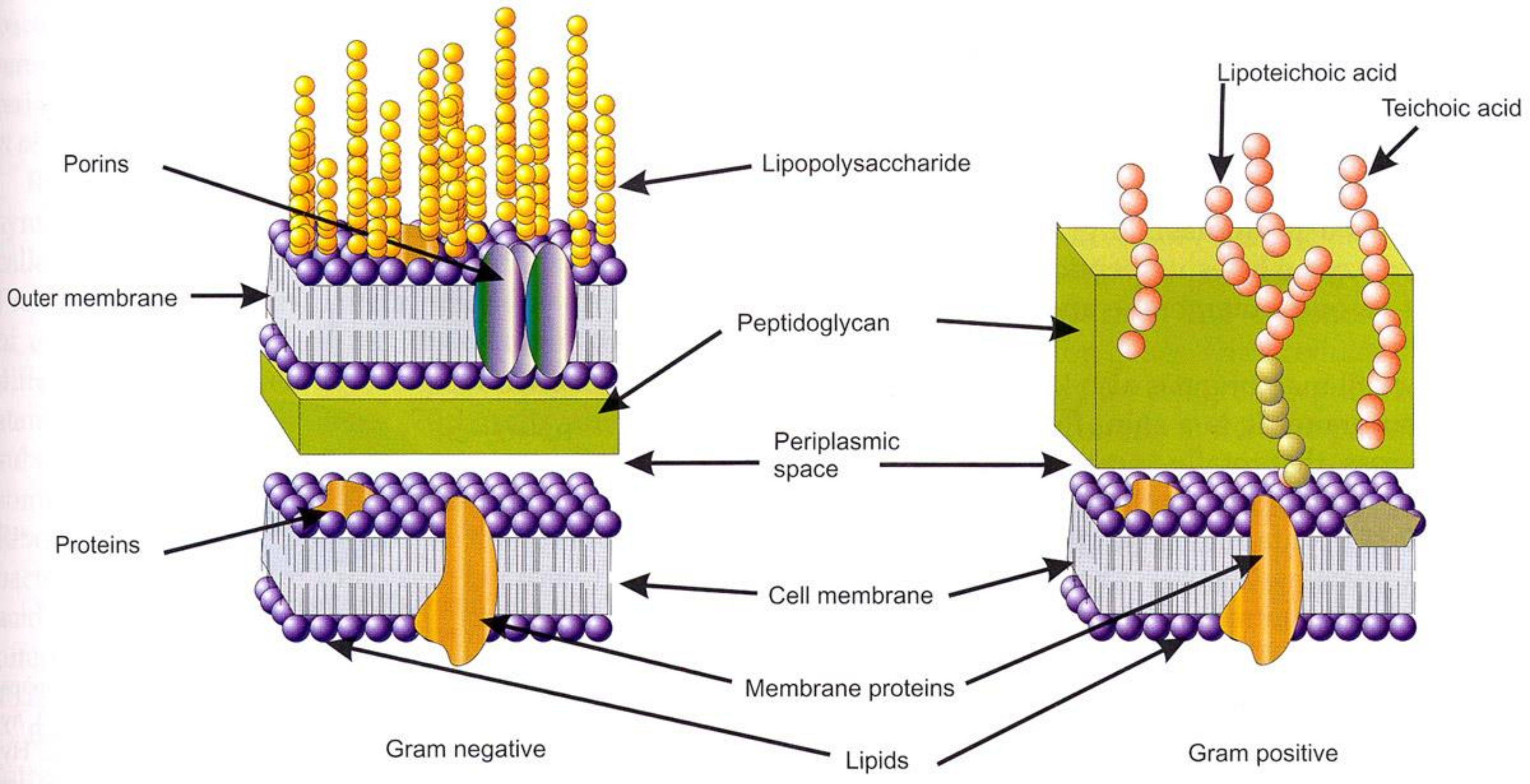
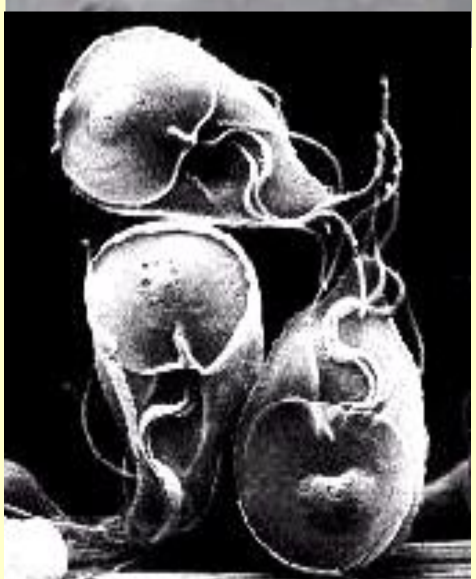



**FIGURE 2.12** Comparison of gram-positive and gram-negative bacterial cell walls.

# Different life stages of *Giardia lamblia*

- Sarcomastigonora (Mastigophora)
- Cyst
  - 8-14 μm
  - 2-4 nuclei
  - thick cyst wall (0.3 μm)
- Trophozoite
  - Heart-shaped, symmetric
  - 10-18 μm long, 6-8 μm wide
  - 2 nuclei
  - 8 flagella
- Reproduction
  - Binary fission of trophozoites

# Environmentally resistant forms

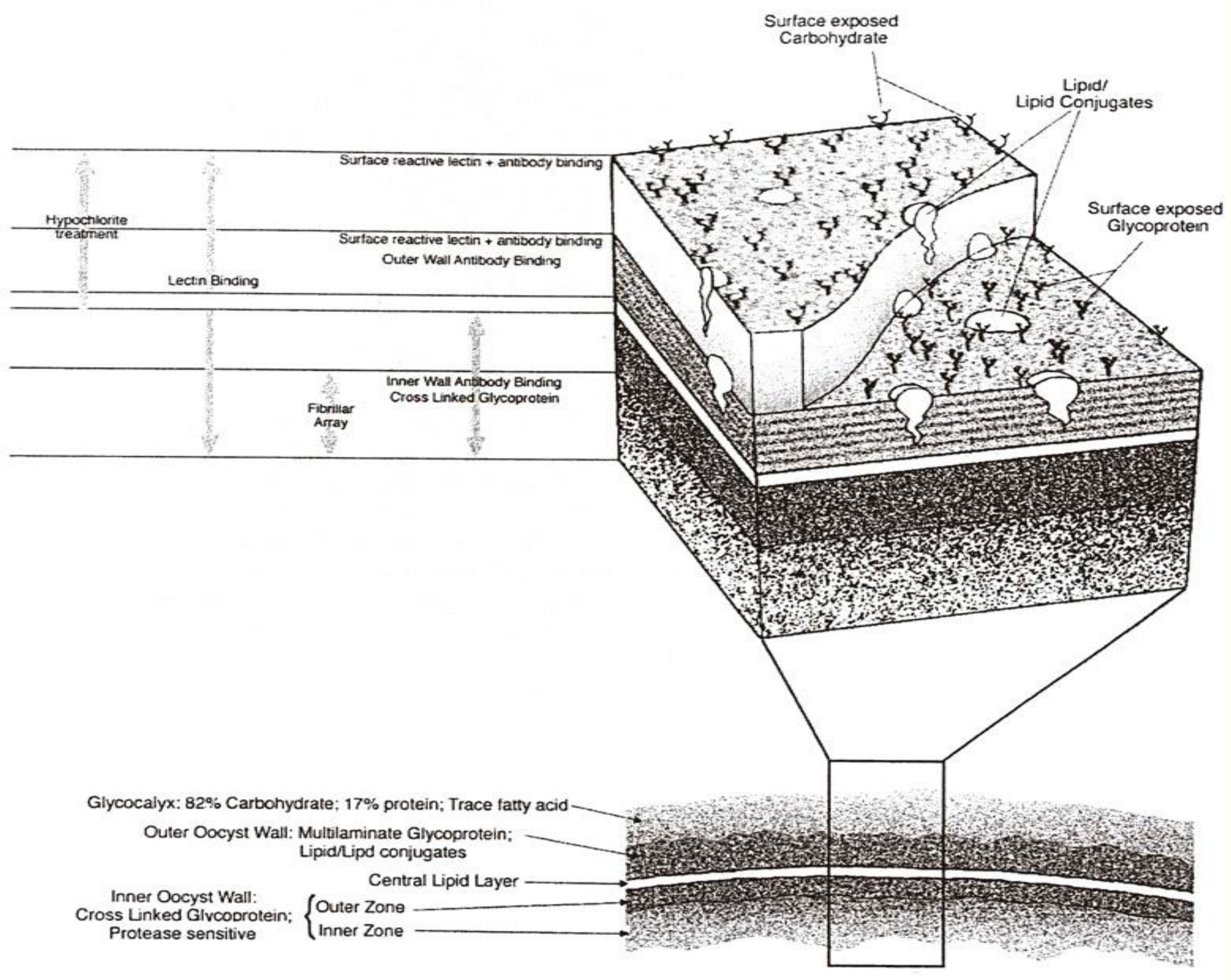
- Protozoans
  - Cysts or Oocysts
- Helmints
  - Eggs
- Bacteria
  - Spores

# Structure of *Giardia lamblia* cysts

# Surface structure of *Giardia lamblia* cysts

- An inner membrane
- A thick (0.3 µm) outer filamentous portion
- Filaments
  - 7-20 nm in diameter
  - Protein and a unique carbohydrate (ß(1-3)-N-acetyl-D-galactopyranosamine)
  - Strong interchain interaction and tightly packed meshwork
- Remarkable physical and chemical barrier against environmental stresses

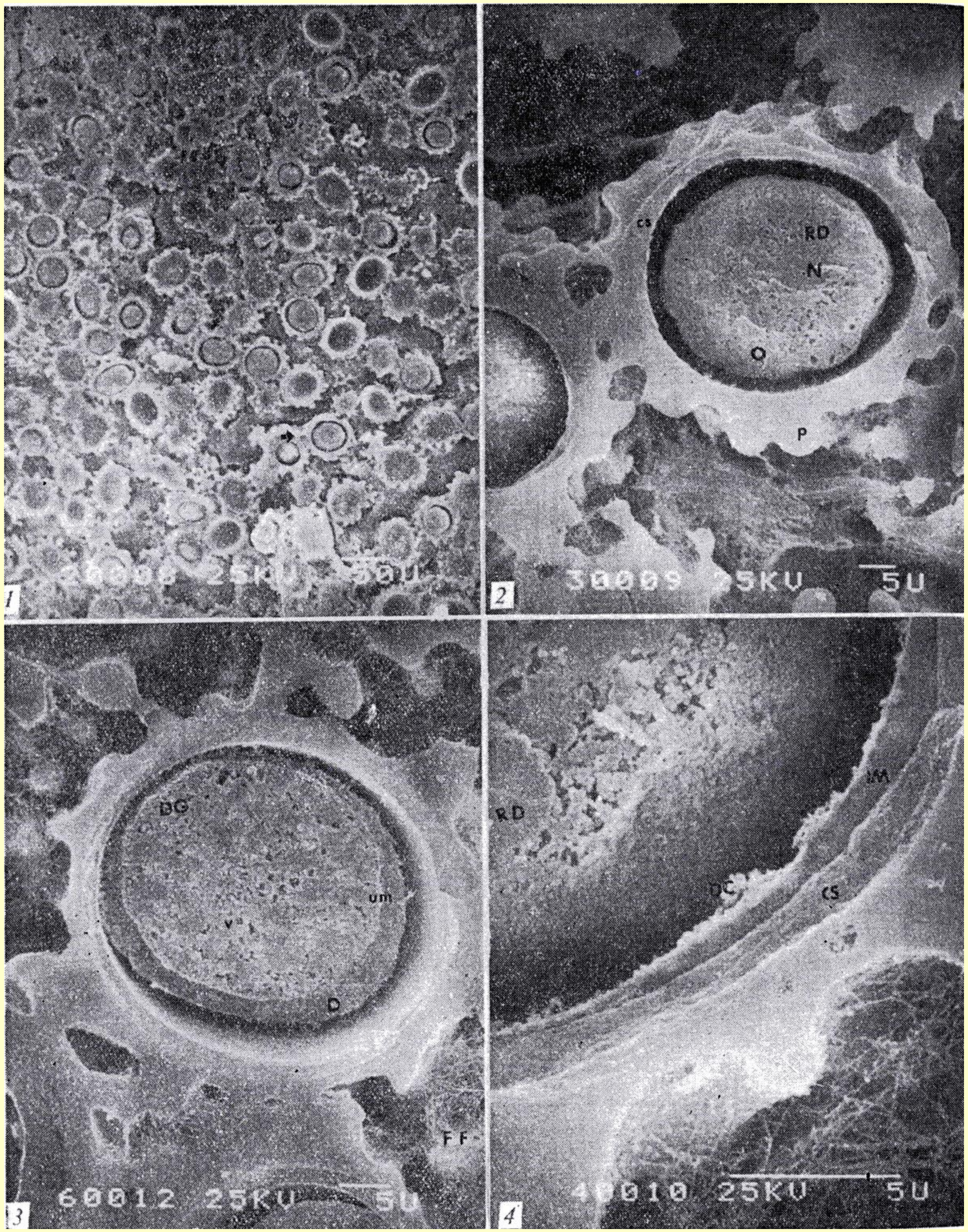
# Surface structure of *Cryptosporidium parvum* oocyst



# Surface structure of *Cryptosporidium parvum* oocyst

- Glycocalyx
  - 82 % carbohydrate, 17 % protein, and trace fatty acid
- Outer oocyst wall
  - Multilaminate glycoprotin, lipid, and lipid conjugates
- Central lipid layer
- Inner oocyst layer
  - Cross-linked glycoprotein
  - Outer and inner zone
- Remarkable physical and chemical barrier against environmental stresses

# Structure of Helminth eggs (Ascaris)



# Surface structure of Helminth eggs (Ascaris)

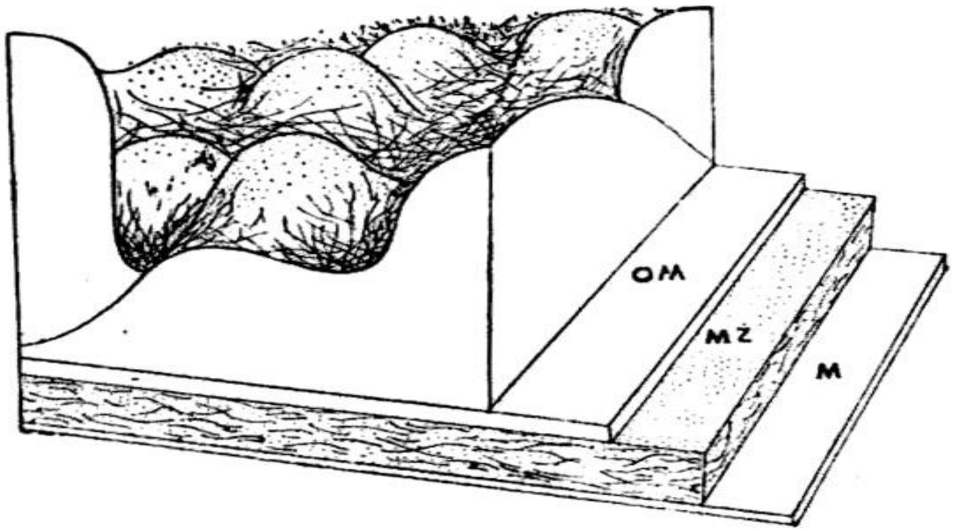
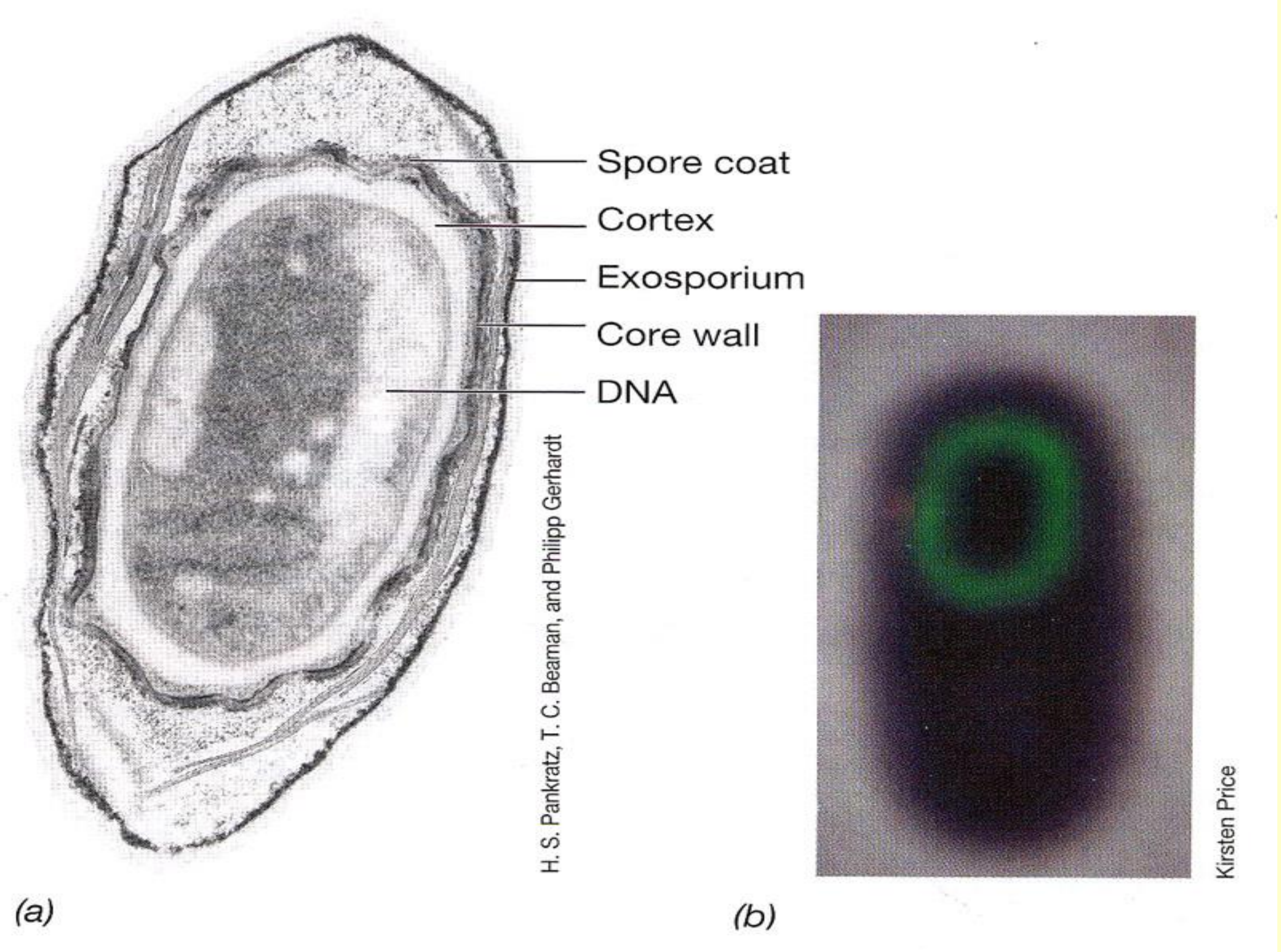



Fig 7. The A lumbricoides oocyte envelope submicroscopic structure diagram shows protein layer and chitinous shell consisting of 3 layers, inner limited membrane (IM), middle chitinous zone (MZ) and outer limited membrane (OM).

# Surface structure of Ascaris eggs

- Outer surface: protein and filamentous fibers
- Outer limited membrane
- Chitinous zone
- Inner limited membrane
- Considerable physical and chemical barrier against environmental stresses

# Structure of bacterial spores




● **Figure 4.48** **Dissecting the bacterial endospore.**

# Surface structure of bacterial endospores

- Exosporium: a thin protein cover
- Spore coat: layers of spore-specific proteins
- Cortex: loosely cross-linked peptidoglycan
- Core: core cell wall, cytoplasmic membrane, cytoplasm, nucleoid, ribosomes, and others
- Remarkable physical and chemical barrier against environmental stresses
  - Survive up to 150 $^{o}$C with dry heat
  - Extremely resistant to ultraviolet, strong acid and bases, and chemical disinfectants

# Environmental factors

# TEMPERATURE

- Most important
- Most microbes survive better at lower temperature
  - Some bacteria experience “cold injury” or “cold shock” at low temperature; VBNC
  - Some microbes grow better at higher temperatures
    - *Salmonella enteritis* (pasteurized wastewater sludge), *Leigionella spp.* (home heating systems)

# pH

- Most microbes survive better near neutral pH (pH 5-9: typical of environmental waters)
- Extreme pH inactivates microbes
  - Chemically alters macromolecules, disrupts enzyme and transport functions
- Many enteric pathogens survive pH 3.0 (tolerate stomach acidity)
- Some pathogens survive pH 11 and fewer survive pH 12

| Microbe or Group | pH Growth Range |
|---|---|
| Molds | 0.2 - 11 |
| Yeasts | 1.5 – 8.5 |
| Salmonella | 3.6 – 9.5 |
| *Listeria monocytogenes* | 4.2 - 9.6 |
| *Yesinia entercolitica* | 4.2 - 9.0 |
| *Escherichia coli* | 4.3 - 9.0 |
| *Bacillus cereus* | 5.0 - 9.5 |
| *Campylobacter* | 5.0 - 9.0 |
| *Shigella* | 5.0 - 9.2 |
| *Vibrio parahaemolyticus* | 5.0 - 11 |
| *Vibrio cholerae* | 5.0 - 9.5 |
| *Clostridium perfringens* | 5.0 - 8.5 |
| *Clostridium botulinum* | 4.3 - 8.5 |

# Sunlight

- Ultraviolet radiation in sunlight inactivates microbes
  - Ultraviolet radiation: about 200 to 330 nm
  - Primary effects on nucleic acids
  - Most effective in clear water than turbid water

# Solar spectrum

# Salt and inorganic solutes

- Salts
  - Change ionic strength in water
    - Many microbes can't survive very well at high (or low) ionic strength
      - Many microbes survive poorly in seawater than in freshwater
- Inorganic solutes
  - Could be beneficial or antagonistic
    - Beneficial (Nutrient)
    - Antagonistic (Mercury, lead, silver, cadmium, etc. are antimicrobial)

# Particulates and dissolved organics

- Particulates
  - Could be beneficial or antagonistic
    - May protect pathogens (Mineral clays)
    - Toxic to microbes (aluminum, heavy metals)
- Dissolved organics
  - May protect pathogens
    - Absorb UV radiation
    - React with oxidants
  - Harmful to pathogens
    - Promote activity of natural microbial population
    - Proteolytic enzymes/proteases
    - Nucleases
    - Amylases (degrade carbohydrates)
    - Antibiotics/antimicrobials: many produced naturally by microbes
    - Oxidants/oxides