Microbes as Agents of Infectious Disease

- Normal Flora
- Virulence and Pathogenicity
- Toxicity vs. Invasiveness

WE ARE NOT ALONE!

"We are outnumbered. The average human contains about 10 trillion cells. On that average human are about **10 times as many microorganisms**, or 100 trillion cells...As long as they stay in balance and where they belong, [they] do us no harm…In fact, many of them provide some important services to us. [But] most are opportunists, who if given the opportunity of increasing growth or invading new territory, will cause infection."

- Sullivan (1989)

Take Home Message:

Bacterial Cells ~10^{14} cells/body

Eukarya Cells ~10^{13} cells/body

Normal Flora helps maintain our health

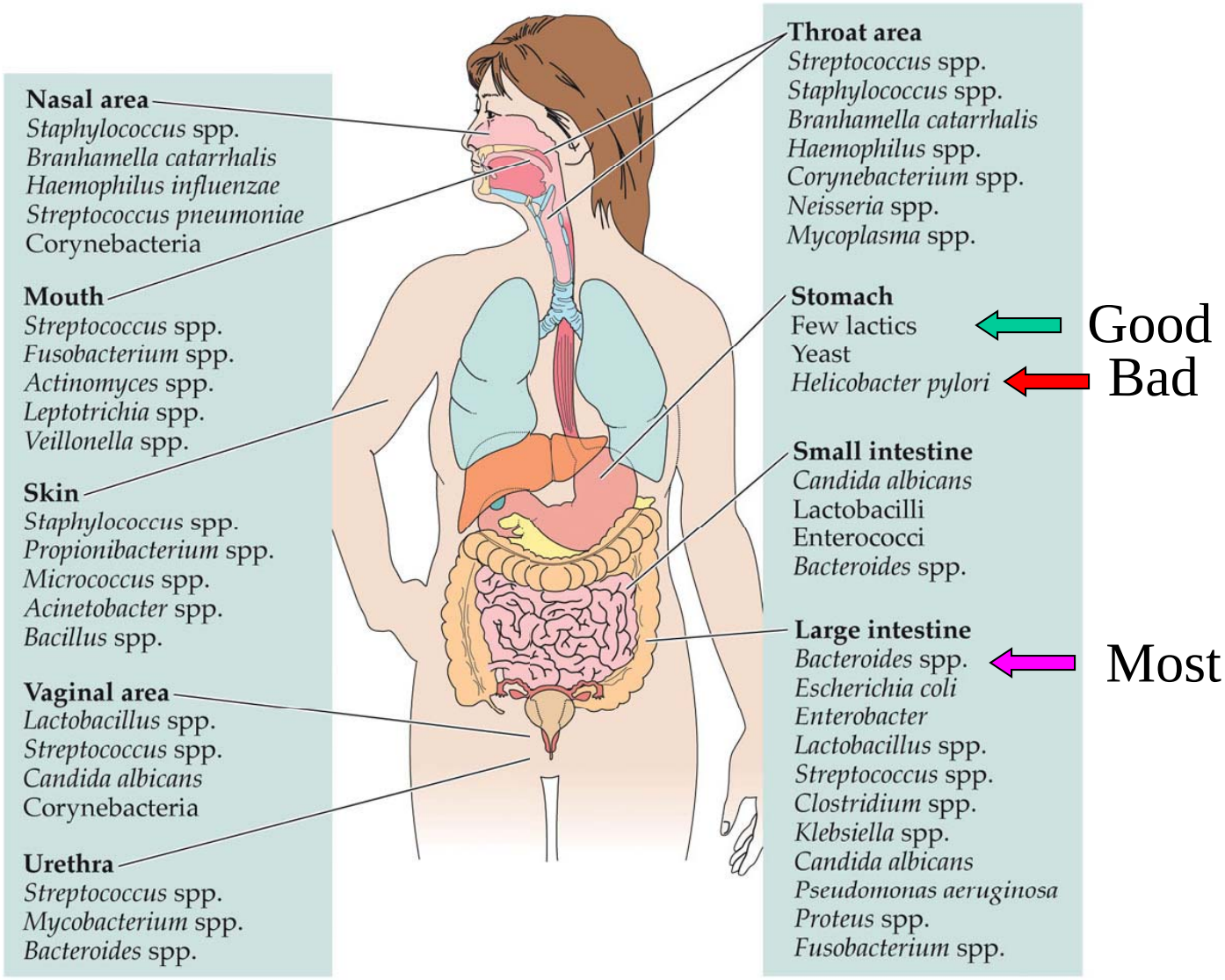
- Provides vitamins & nutrients
- Detoxify many compounds
- Prevent colonization of pathogens

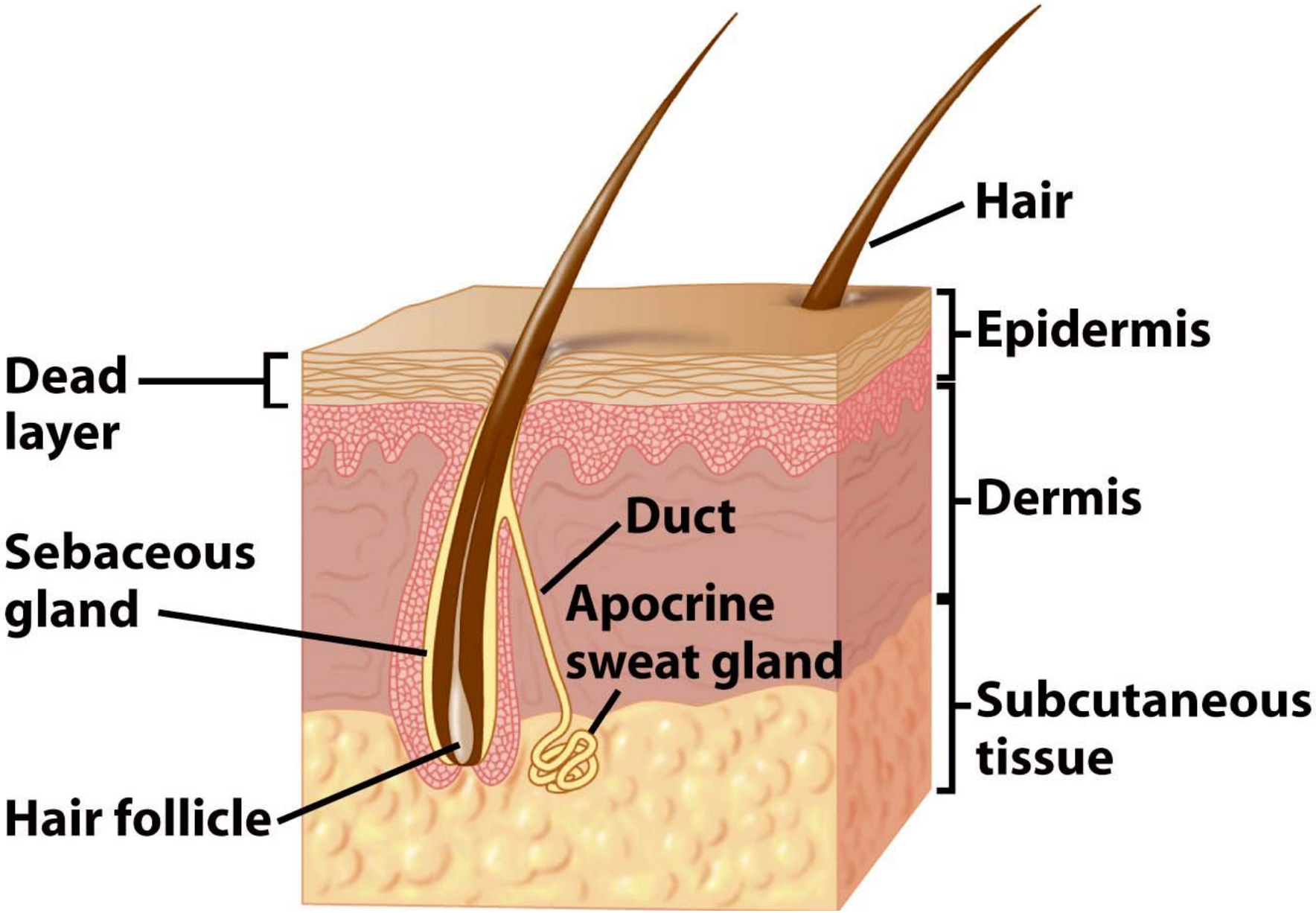
Table 21.1 **Representative genera of microorganisms in the normal flora of humans**

Anatomical site	Genera[a]
Skin	*Acinetobacter, Corynebacterium, Enterobacter, Klebsiella, Malassezia (f), Micrococcus, Pityrosporum (f), Propionibacterium, Proteus, Pseudomonas, Staphylococcus*
Mouth	*Streptococcus, Lactobacillus, Fusobacterium, Veillonella, Corynebacterium, Neisseria, Actinomyces, Geotrichum (f), Candida (f), Capnocytophaga, Eikenella, Prevotella,* spirochetes (several genera)
Respiratory tract	*Streptococcus, Staphylococcus, Corynebacterium, Neisseria, Haemophilus*
Gastrointestinal tract	*Lactobacillus, Streptococcus, Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, Clostridium, Escherichia, Klebsiella, Proteus, Enterococcus, Staphylococcus*
Urogenital tract	*Escherichia, Klebsiella, Proteus, Neisseria, Lactobacillus, Corynebacterium, Staphylococcus, Candida (f), Prevotella, Clostridium, Peptostreptococcus, Ureaplasma, Mycoplasma, Mycobacterium, Streptococcus, Torulopsis (f)*

[a] This list is not meant to be exhaustive, and not all of these organisms are found in every individual. Some organisms are more prevalent at certain ages (adults vs. children). Distribution may also vary between sexes. Most of these organisms can be opportunistic pathogens under certain conditions. Several genera can be found in more than one body area. (f)–fungi.

Normal human microflora




Hair
Epidermis
Dead layer
Dermis
Duct
Sebaceous gland
Apocrine sweat gland
Subcutaneous tissue
Hair follicle

Skin:

Resident Microbes:

Most are Gram (+)

Staphylococcus

Micrococcus

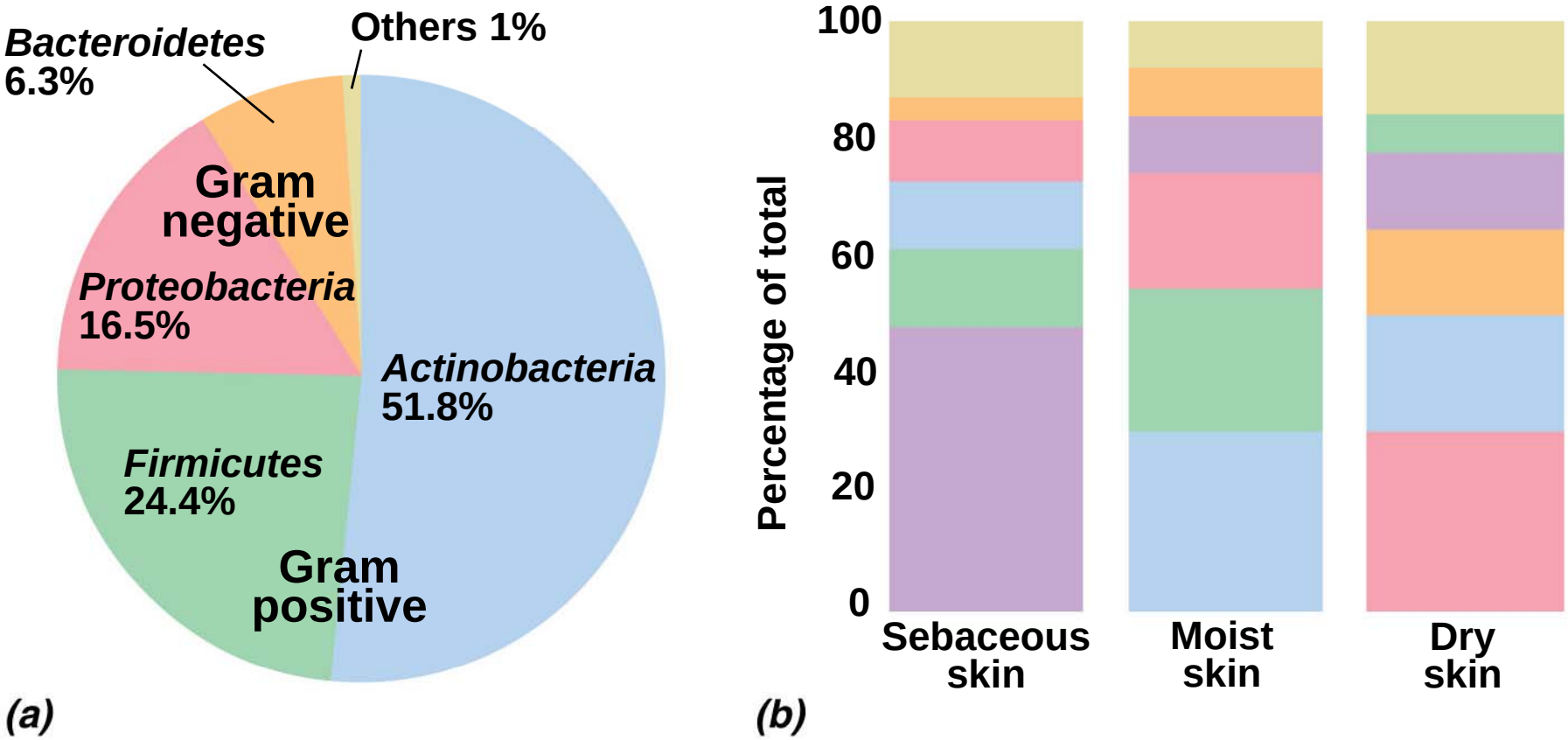
Actinobacteria

Environmental Conditions: Hostle

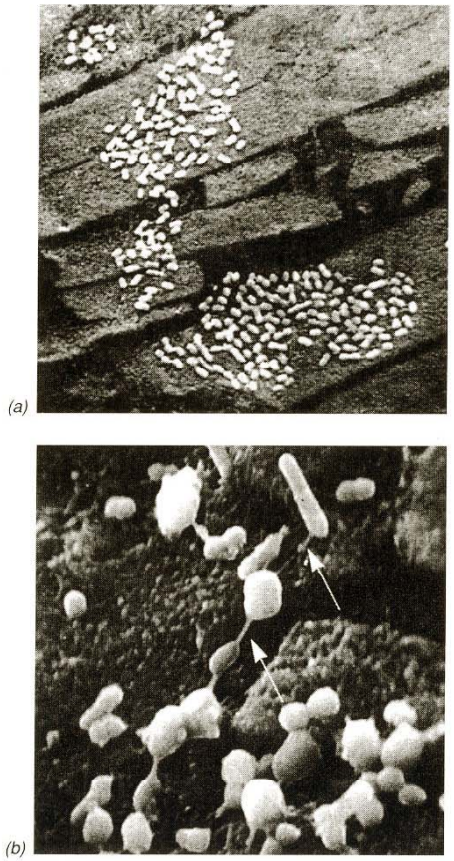
- **High Salt**
- **Low pH**
- **Dry**

Figure 27.3

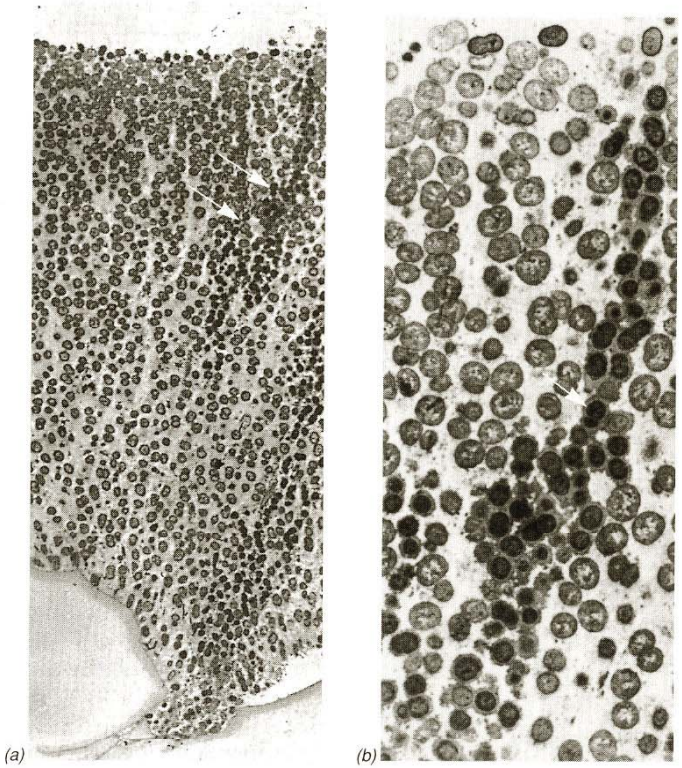
Normal skin microflora. *(a) Analysis of the skin microbiome from 10 healthy human volunteers* detected 19 bacterial phyla. Four phyla were predominant. *(b) Composite populations of Bacteria from the* same volunteers, divided according to sebaceous, moist, and dry skin microenvironments.



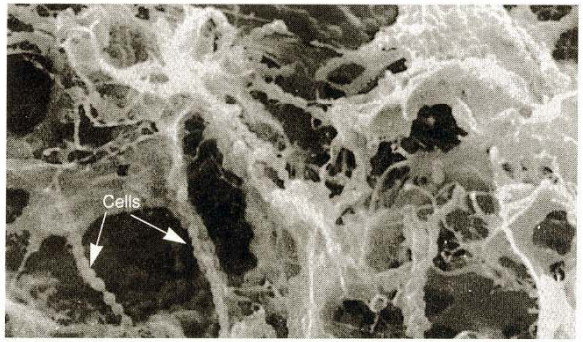
Dental Plaque Bacteria



Tooth Colonies

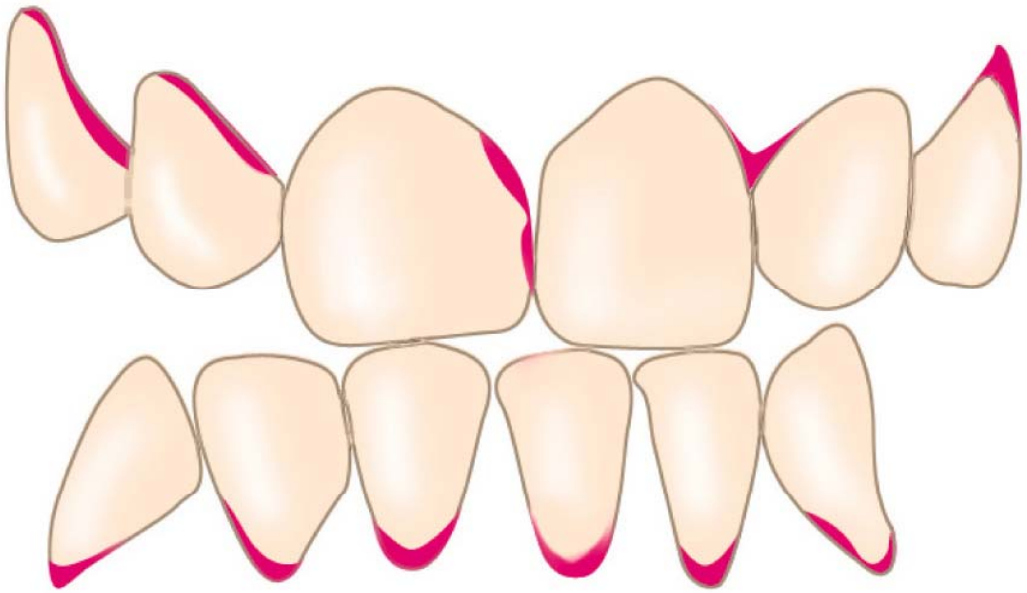


Plaque Cross Section

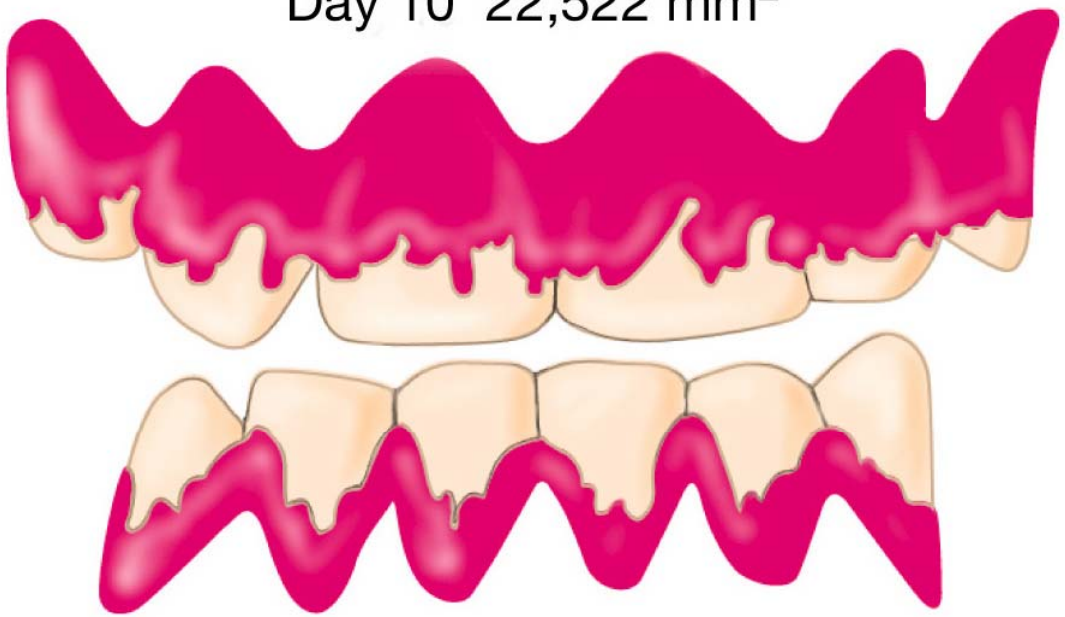


Streptococcus mutans

Day 1 1436 mm^2



Day 10 22,522 mm^2



Mouth:

Resident Microbes:

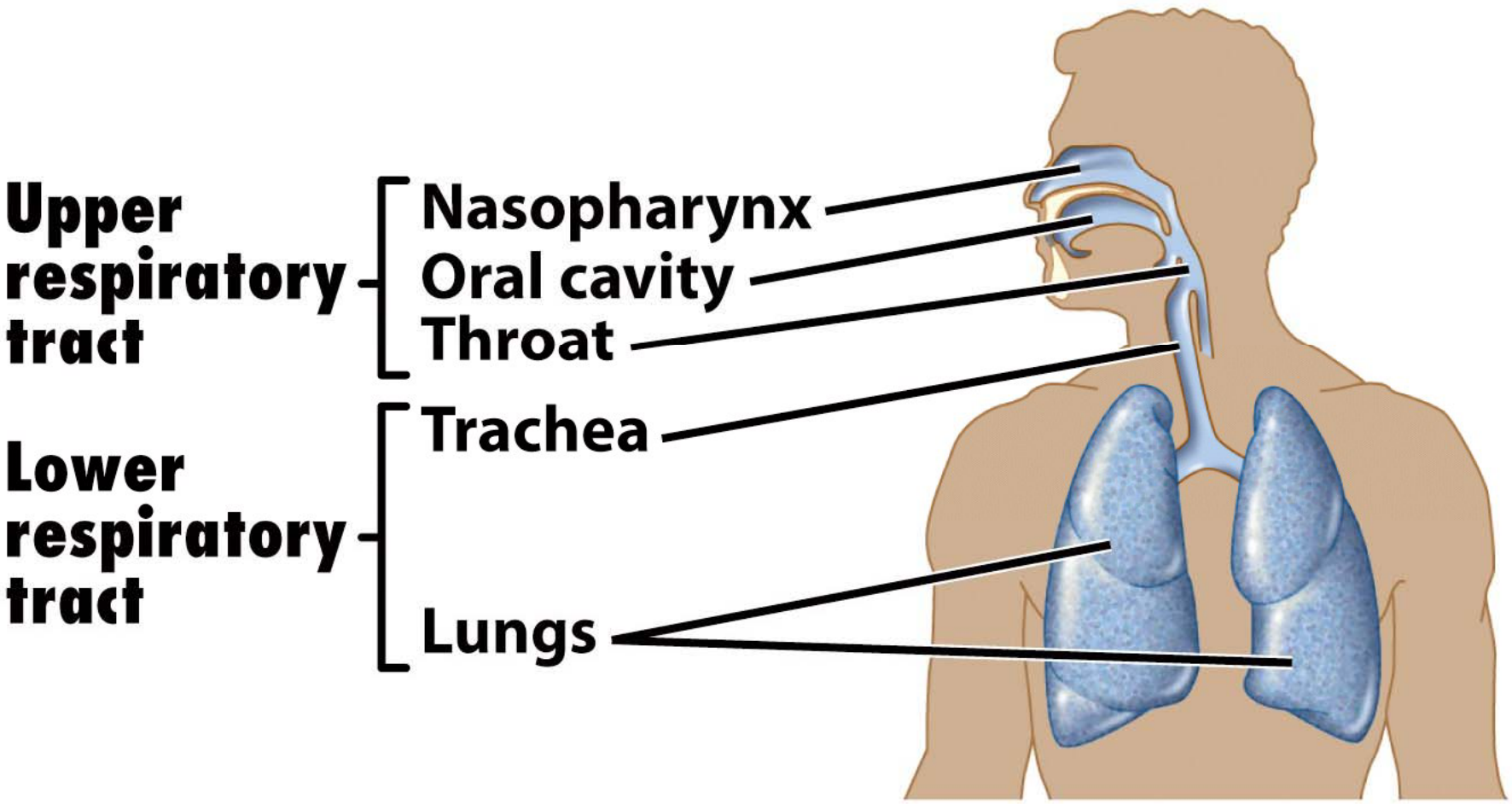
Gram (+): *Streptococcus & Lactobacilli*

Gram (-): obligate anaerobes

Spirochetes: *Borrelia*

Environmental Conditions: More Favorable

- **Moist, though contains lysozyme**
- **Lots of polysaccharides**
- **Lots of amylase & protease**


Upper respiratory tract
Nasopharynx
Oral cavity
Throat
Lower respiratory tract
Trachea
Lungs

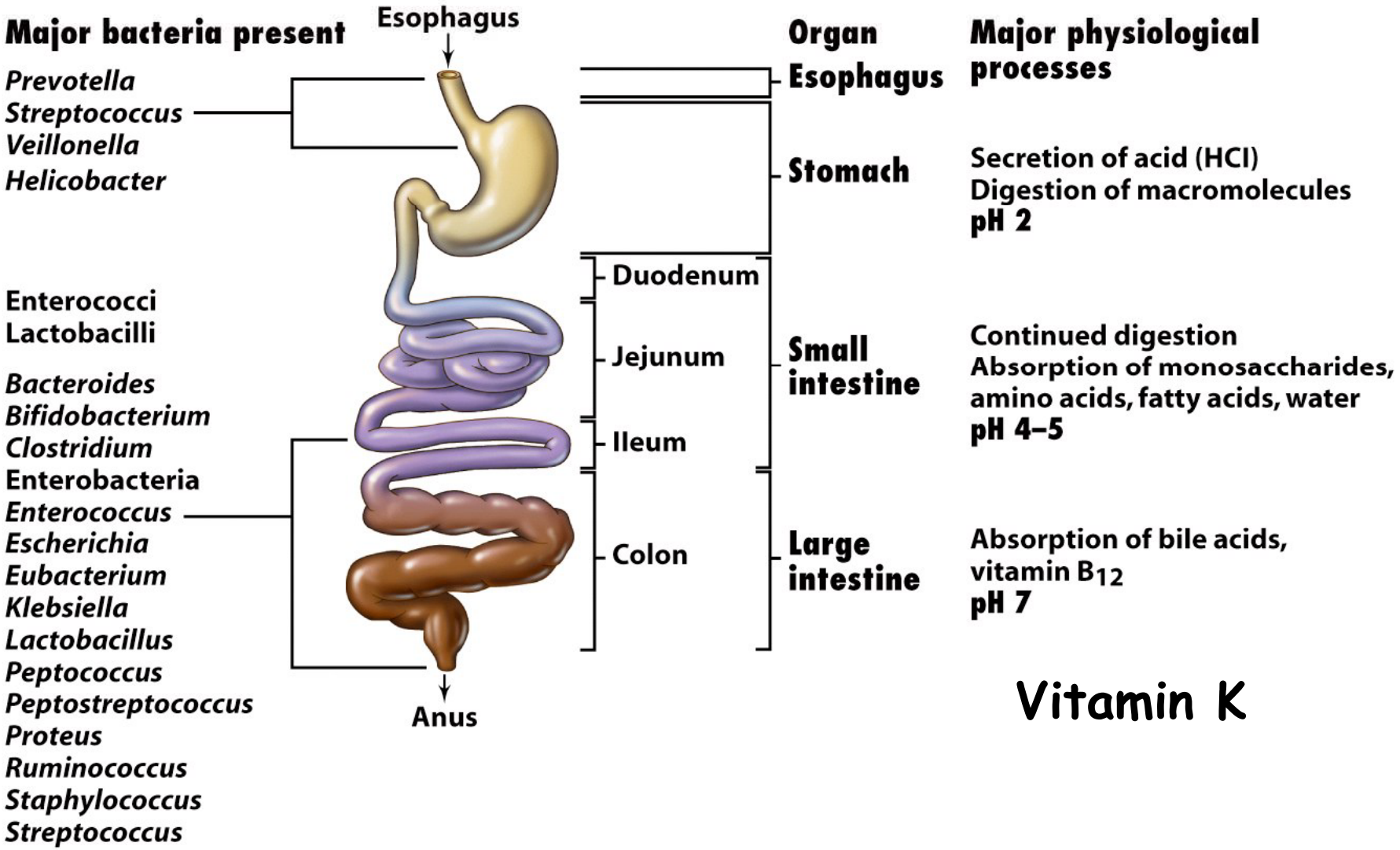
Respiratory Tract:

Resident Microbes: Upper Only

Gram (+): *Streptococcus & Staphylococcus*

Environmental Conditions:

- **Mucous membranes**
- **Others compete with potential pathogens**


Major bacteria present
Prevotella
Streptococcus
Veillonella
Helicobacter
Enterococci
Lactobacilli
Bacteroides
Bifidobacterium
Clostridium
Enterobacteria
Enterococcus
Escherichia
Eubacterium
Klebsiella
Lactobacillus
Peptococcus
Peptostreptococcus
Proteus
Ruminococcus
Staphylococcus
Streptococcus
Esophagus
Anus
Organ
Esophagus
Stomach
Duodenum
Jejunum
Ileum
Small intestine
Colon
Large intestine
Major physiological processes
Secretion of acid (HCl)
Digestion of macromolecules
pH 2
Continued digestion
Absorption of monosaccharides, amino acids, fatty acids, water
pH 4–5
Absorption of bile acids, vitamin B_{12}
pH 7

Vitamin K

G.I. Tract:

Stomach: Hostle, pH ~2

- Gram (+): *Lactobacilli & Streptococcus*
- Gram (-): *Helicobacter pylori*

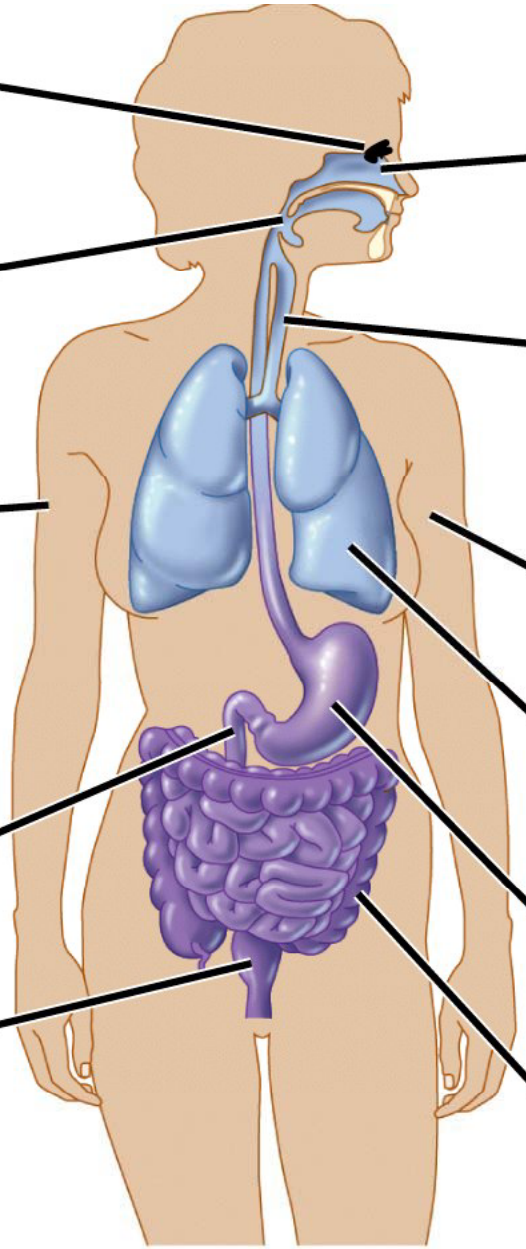
Small Intestine: Gradient in pH

- low pH: *Lactobacilli*
- neutral: *Enterococcus*

Large Intestine: Moist and pH ~7

- 10^{11} to 10^{12} bacteria/g wet wt feces
- #1 is *Bacteroides vulgatus* at ~15%
- *E. coli* is only ~0.03%
- Methanogens can also be detectable

Physical, chemical, & anatomical barriers to infection



Lysozyme in tears and other secretions dissolves cell walls

Normal flora compete with pathogens

Skin is a physical barrier, produces antimicrobial fatty acids, and its normal flora inhibit pathogen colonization

Rapid pH change inhibits microbial growth

Flushing of urinary tract prevents colonization

Removal of particles including microorganisms by rapid passage of air over cilia in nasopharynx

Mucus, cilia lining trachea suspend and move microorganisms out of the body

Blood proteins inhibit microbial growth

Mucus and phagocytes in lungs prevent colonization

Stomach acidity (pH 2) inhibits microbial growth

Normal flora compete with pathogens

Virulence and Pathogenicity

Pathogen: A parasitic organism that causes damage to, or disease in its host.

Pathogenicity: The ability to cause disease.

Virulence: The relative degree or intensity of pathogenicity.

Virulence is determined by the five following characteristics of the pathogen →

Invasiveness: The ability of the organism to spread to adjacent tissues or other tissues.

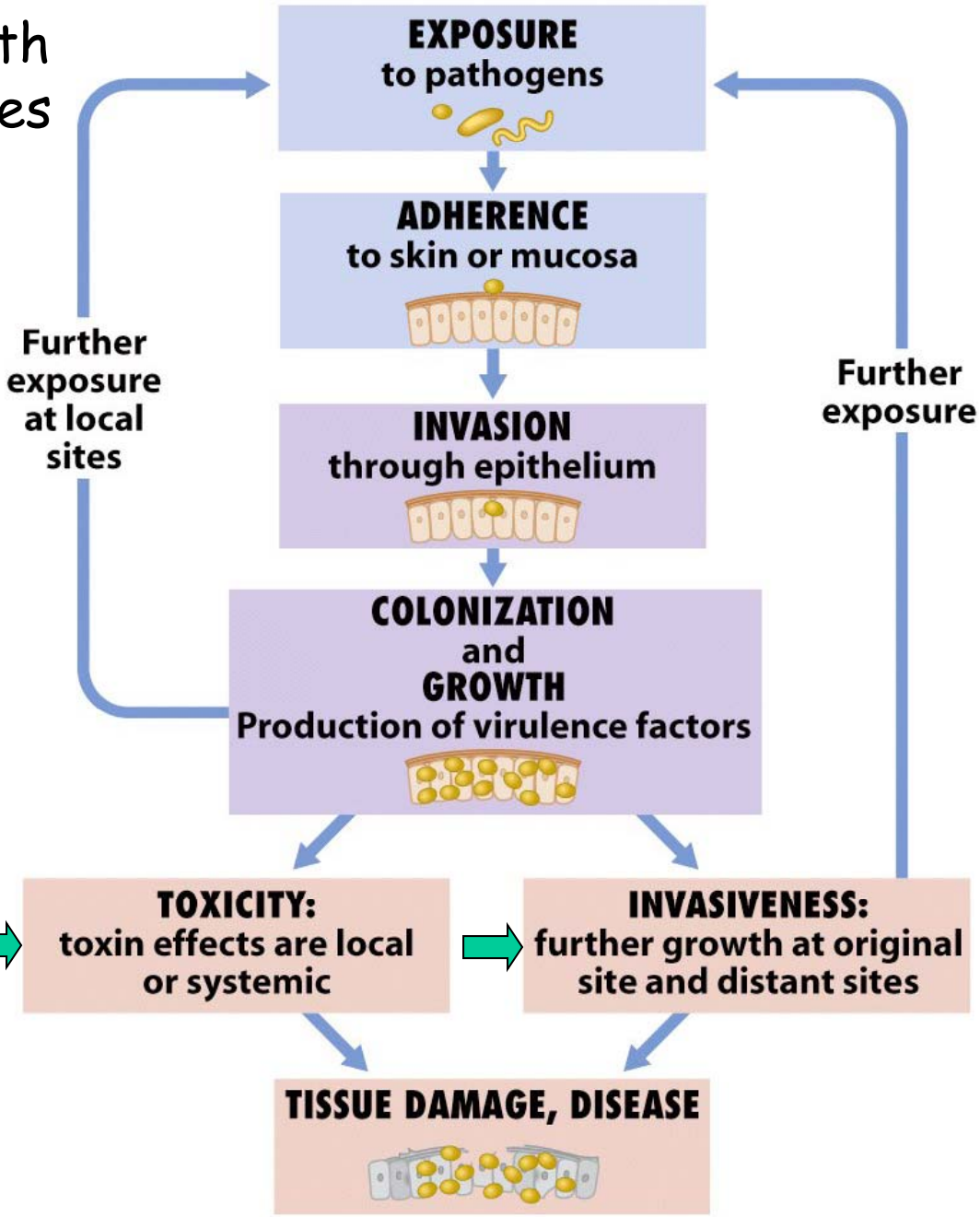
Toxigenicity: The ability of the organism to produce toxic products that cause disease and/or damage in the host.

Infectivity: The ability of the organism to establish a focal point of infection through growth.

Pathogenic potential: The degree that the pathogen causes morbid symptoms.

Hypersensitivity: Host's innate sensitivity to pathogen.

The presence or even growth of microbes on the host does not always lead to disease.



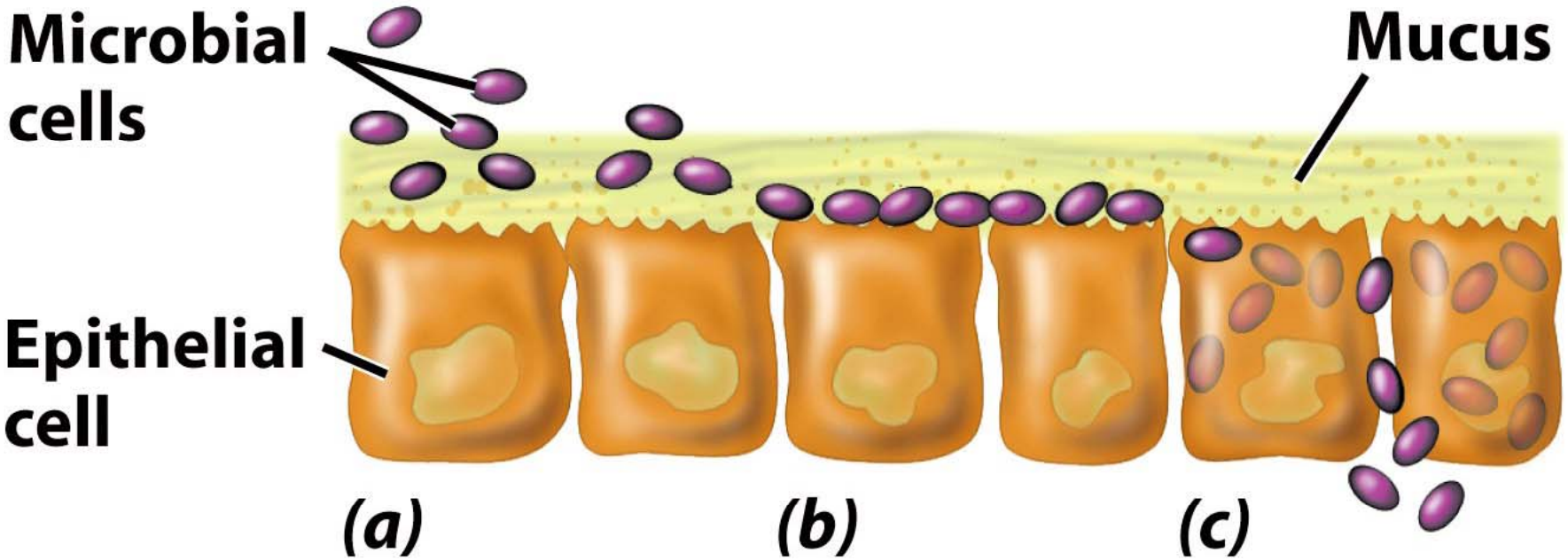
These two are key factors to the success or failure of a potential pathogen to cause disease!

Determinants of Infectious Disease

To produce an infectious disease, a pathogen must be able to:

1. initially be transported to the host
2. adhere to, colonize or invade the host
3. grow, multiply, or complete its life cycle in the host
4. initially evade host defense mechanisms
5. damage the host by mechanical and/or chemical means

In the end it is – Numbers (of bacteria) that make you sick!



Loose association

Adhesion

Invasion

Microorganisms and mechanisms of **Pathogenesis**

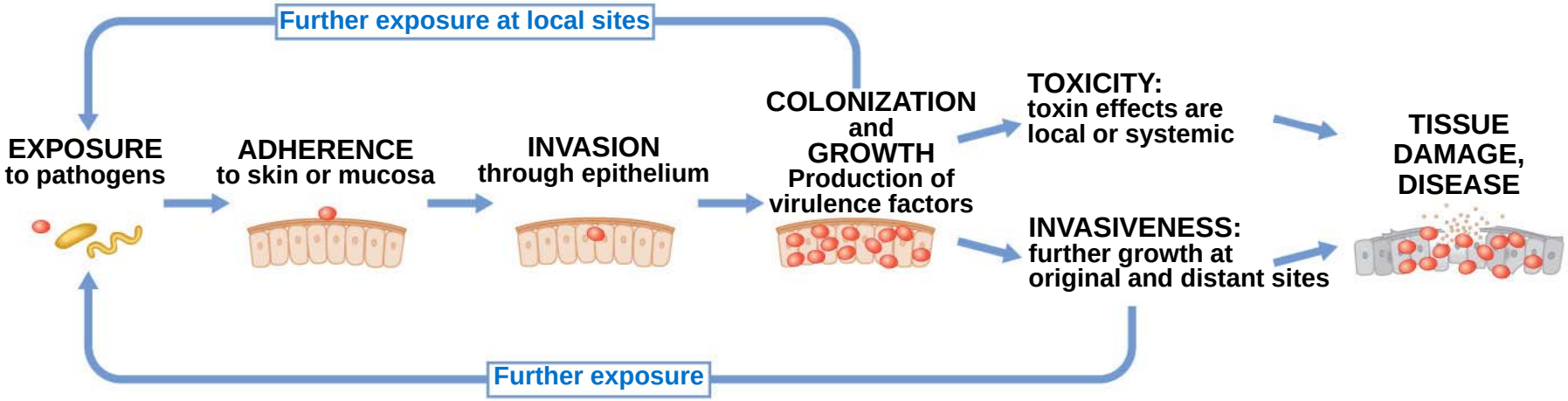
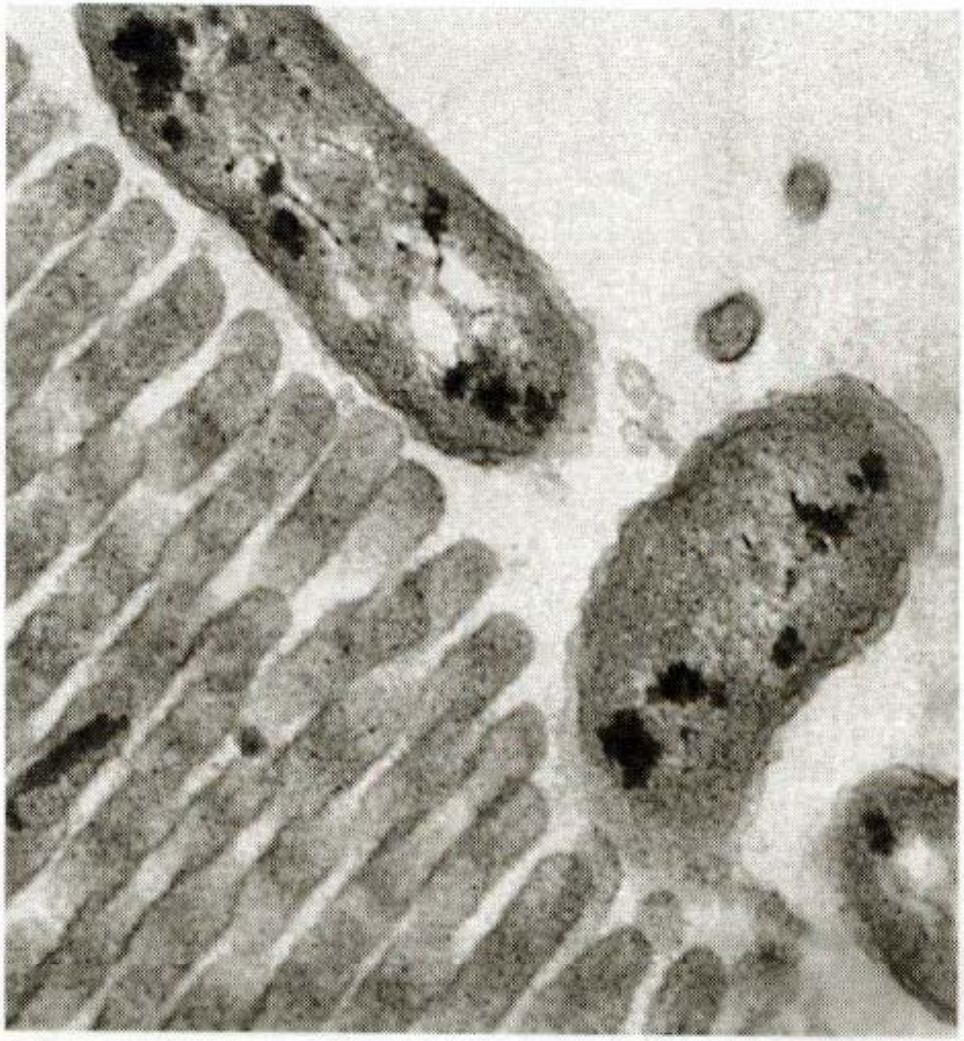
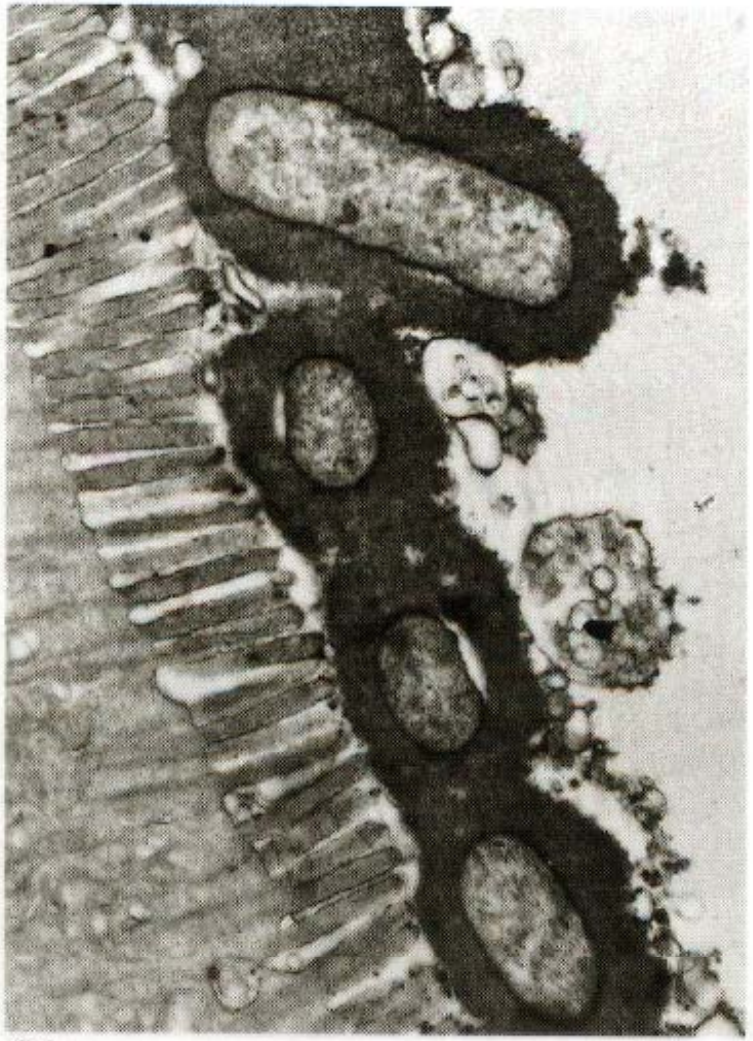


Figure 27.13

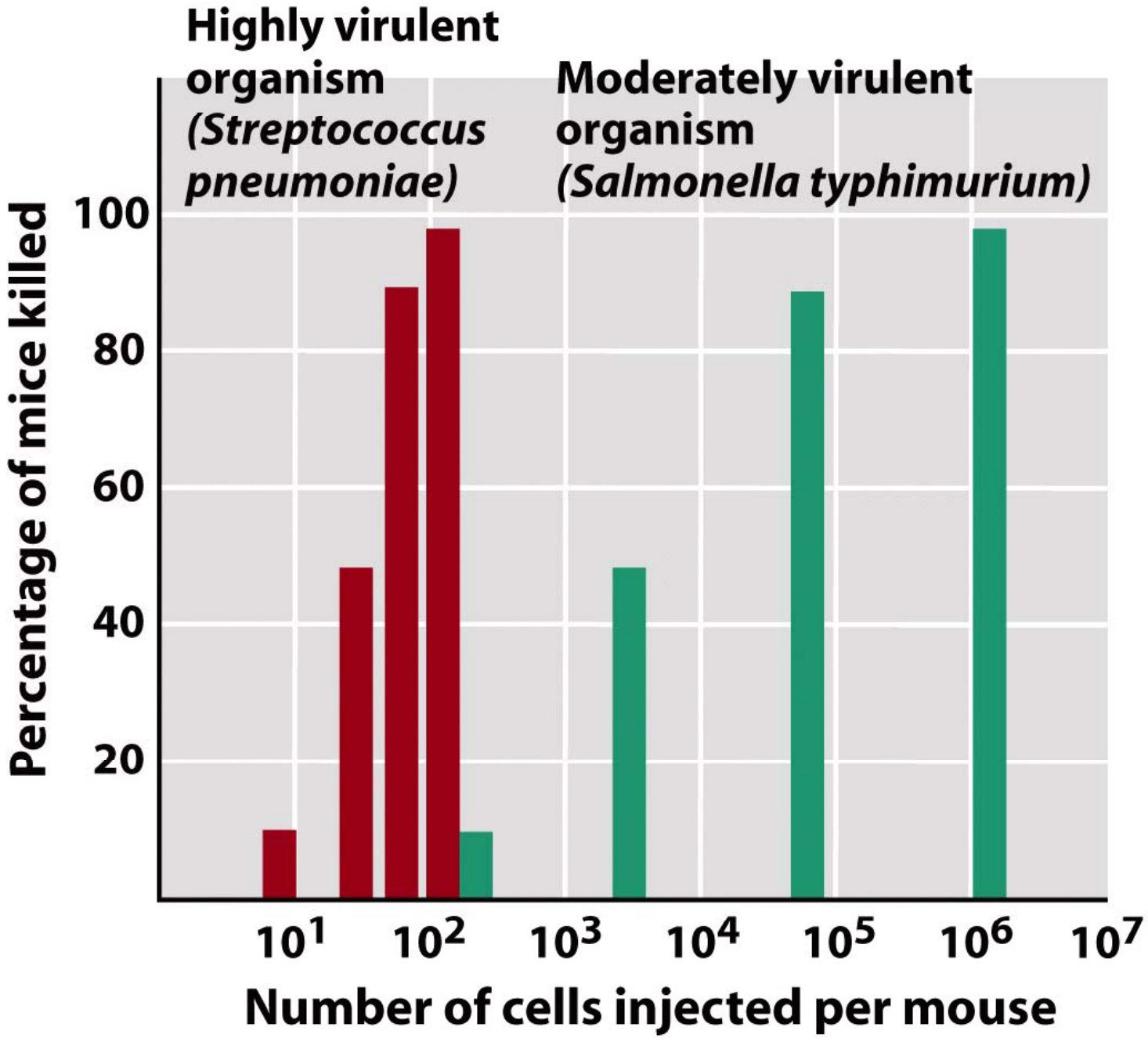
Adherence of microorganisms

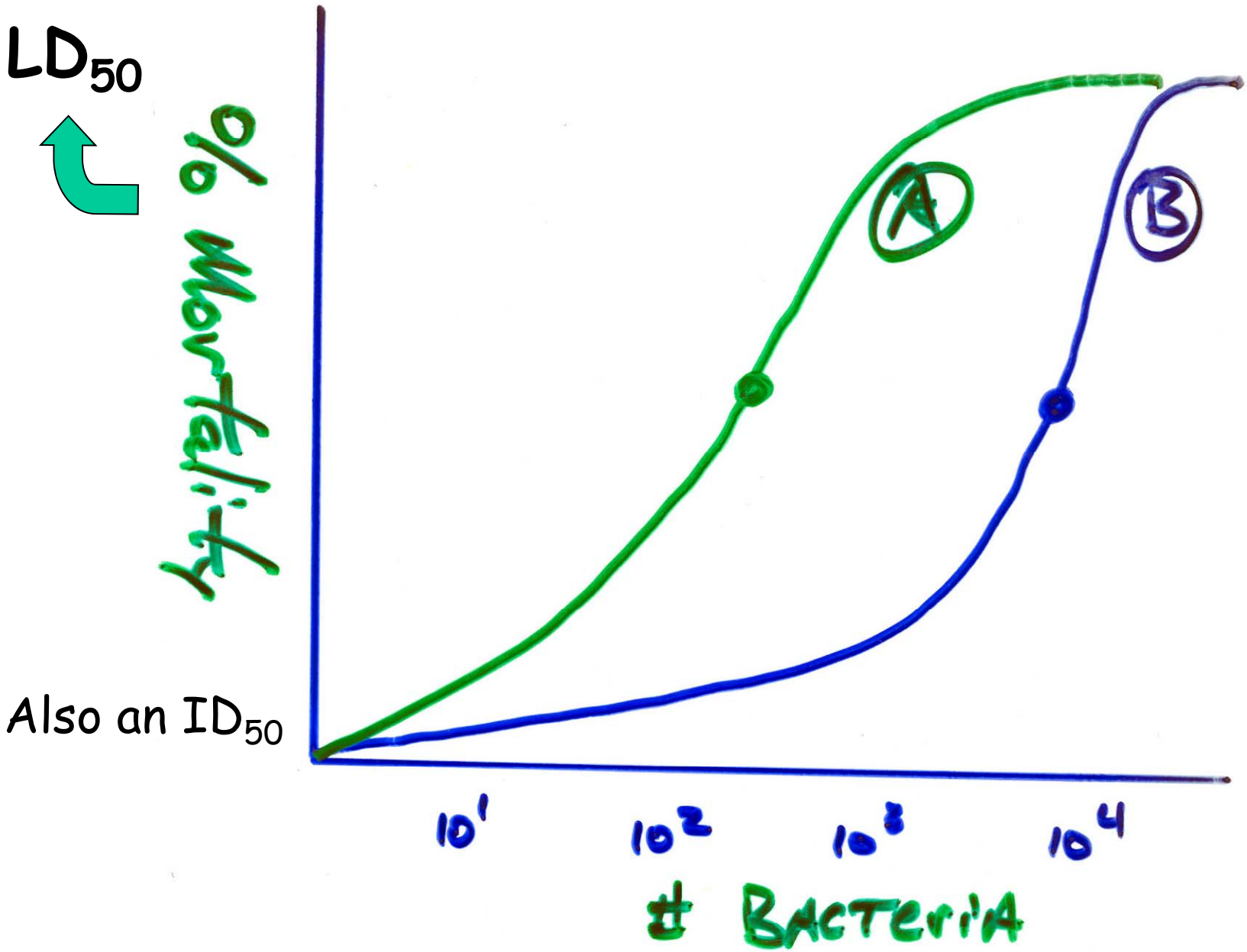


(a) Entero-toxic *Vibrio cholerae*



(b) Entero-invasive *E. coli*


Highly virulent organism (Streptococcus pneumoniae)
Moderately virulent organism (Salmonella typhimurium)
Percentage of mice killed
100
80
60
40
20
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Number of cells injected per mouse



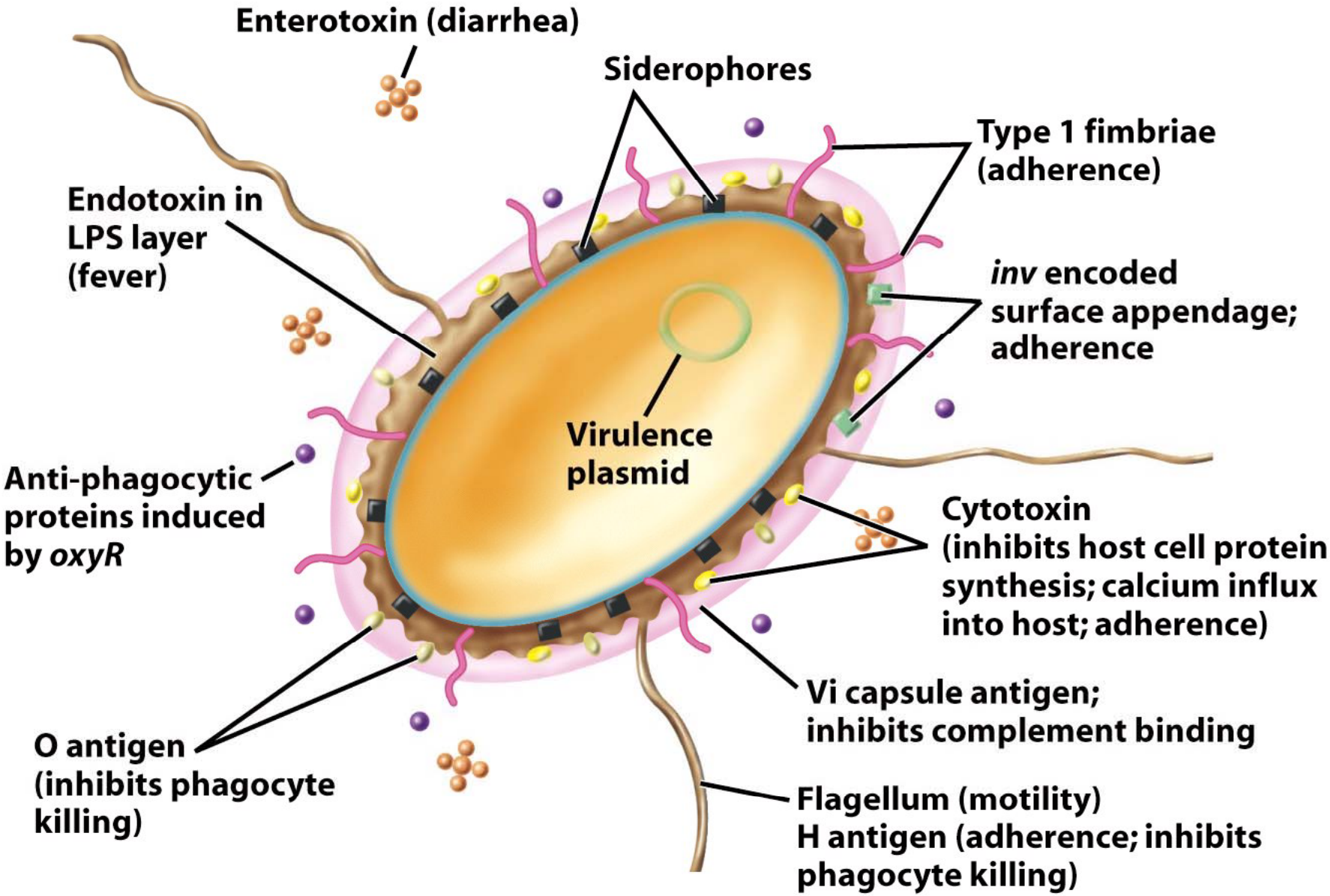
Also an ID_{50}

Bacterium A is more virulent than bacterium B

Table 27.6 *Tissue specificity in infectious disease*

Disease	*Tissue infected*	*Organism*
Acquired immunodeficiency syndrome (AIDS)	T helper lymphocytes	Human immunodeficiency virus (HIV)
Botulism	Motor end plate	*Clostridium botulinum*
Cholera	Small intestine epithelium	*Vibrio cholerae*
Dental caries	Oral epithelium	*Streptococcus mutans, S. sobrinus, S. sanguiins, S. mitis*
Diphtheria	Throat epithelium	*Corynebacterium diphtheriae*
Gonorrhea	Mucosal epithelium	*Neisseria gonorrhoeae*
Influenza	Respiratory epithelium	Influenza A and influenza B virus
Malaria	Blood (erythrocytes)	*Plasmodium* spp.
Pyelonephritis	Kidney medulla	*Proteus* spp.
Spontaneous abortion (cattle)	Placenta	*Brucella abortus*
Tetanus	Inhibitory interneuron	*Clostridium tetani*

Virulence factors in *Salmonella*



Adherence Factors:

Table 26.2 Adherence factors involved in attachment of organisms to host cells

Adherence Factor	Example
Fimbriae (adhesion proteins)	*Proteus mirabilis*—urinary tract infections
	Neisseria gonorrhoeae—attach to urinary epithelia
	Salmonella—attach to intestinal epithelia
	Streptococcus pyogenes—M protein attaches to epithelia
Capsule (glycocalyx)	*Streptococcus mutans*—dextrans attach to teeth
	Streptococcus salivarius and *S. sanguis*—attach to tongue epithelia
Teichoic acids Lipoteichoic acids	*Staphylococcus aureus*—attach to nasal epithelia

Virulent Factors: Invasiveness

Table 26.3 Some enzymes produced by pathogenic bacteria that promote invasion of the host

Enzyme	Organism	Function
Collagenase	*Clostridia*	Breaks down collagen in connective tissue
Coagulase	*Staphylococcus aureus*	Clot formation around point of entry protects from host defenses
Elastase	*Pseudomonas aeruginosa*	Disrupts membranes
Hyaluronidase	*Streptococcus* *Staphylococcus* *Clostridium*	Hydrolyzes hyaluronic acid–intercellular cement
Lecithinase	*Clostridia*	Disrupts phosphatidylcholine in membranes
Streptokinase	*Staphylococcus* *Streptococcus*	Digests fibrin clots

- Also considered cytolytic toxins!

Virulent Factors: Plasmids

Table 26.4 **Virulence factors that are generally encoded in plasmids**

Organism	Factor	Disease
Escherichia coli	Enterotoxin	Diarrhea
Clostridium tetani	Neurotoxin	Tetanus
Staphylococcus aureus	Coagulase enterotoxin	Boils/skin infections, food poisoning
Streptococcus mutans	Dextransucrase	Tooth decay
Agrobacterium tumefaciens	Tumor	Crown gall
Staphylococcus spp.	Antibiotic resistance	Various

Virulent Factors: Antiphagocytic

Table 26.5 Antiphagocytic factors produced by bacteria and their mode of action

Factor	Action
Leukocidins	Specific lytic agent for leukocytes including phagocytes
Hemolysins	Form pores in host cells including macrophages. Streptolysin O affects sterols in membranes. Streptolysin S is a phospholipase
Capsules (glycocalyx)	Long polymers of carbohydrate—physically prevents engulfment
Fimbriae	(1) Bind to surface components of phagocytes, prevent close contact, and phagocytosis may not occur (2) Phase variation—a change in the antigenic composition

➡ Also considered cytolytic toxins!

Exotoxins:

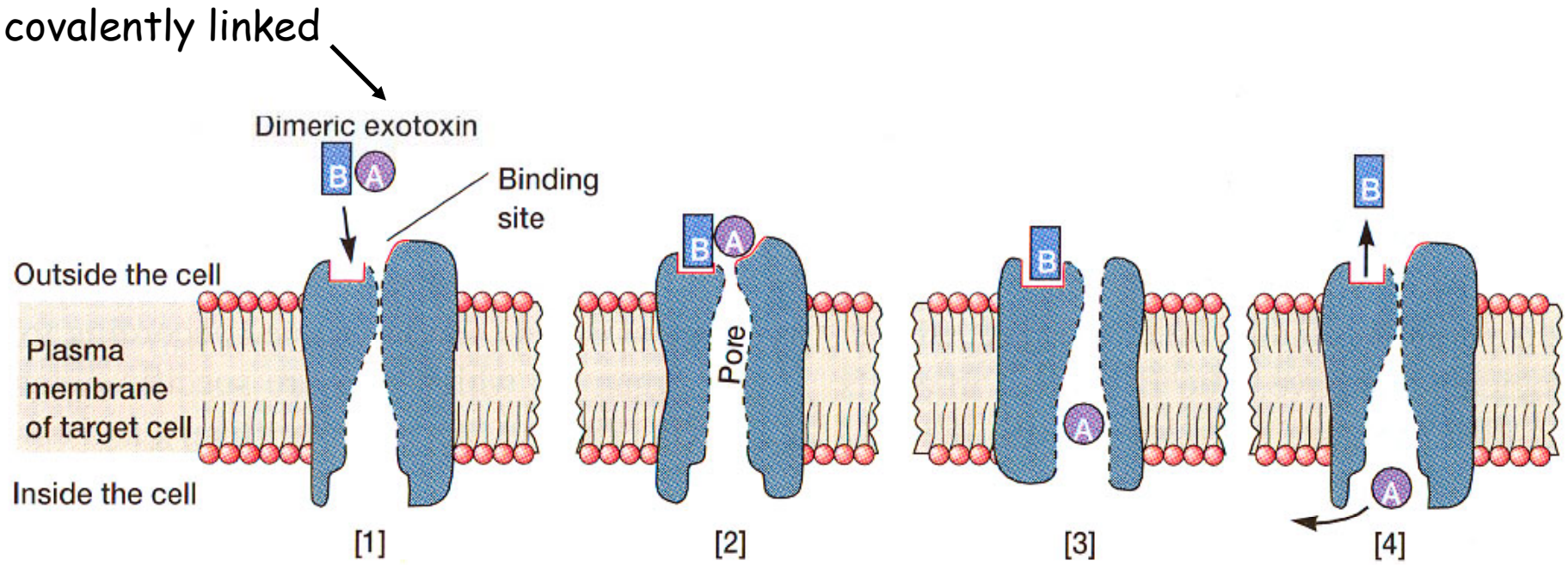
Extracellular toxic proteins released by pathogens.

1. **Cytolytic toxins** cause cell lysis.

2. **Superantigen toxins** stimulate the immune system.

3. **A-B toxins** where one part binds to surface receptor and the second enters and impacts cellular function.

A-B exotoxins and their cellular entry



Staphylococcal alpha-toxin is Cytolytic

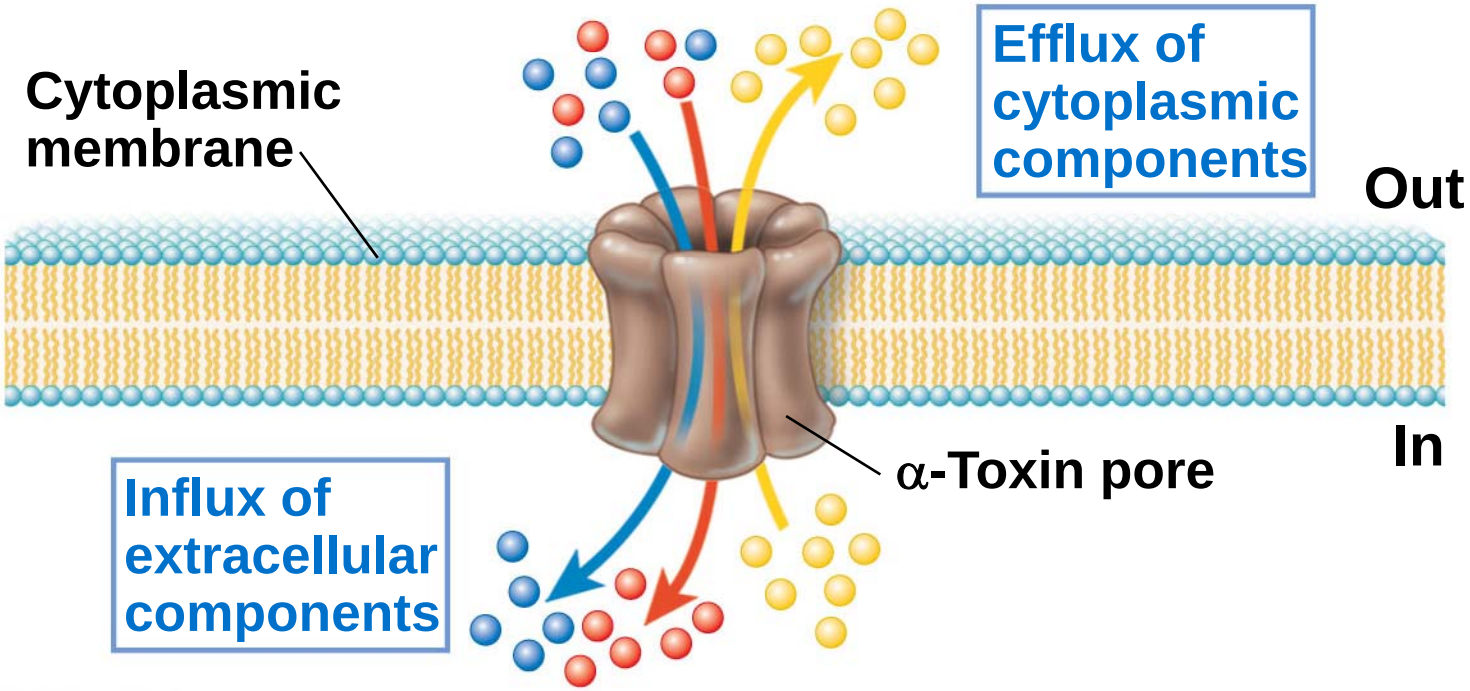
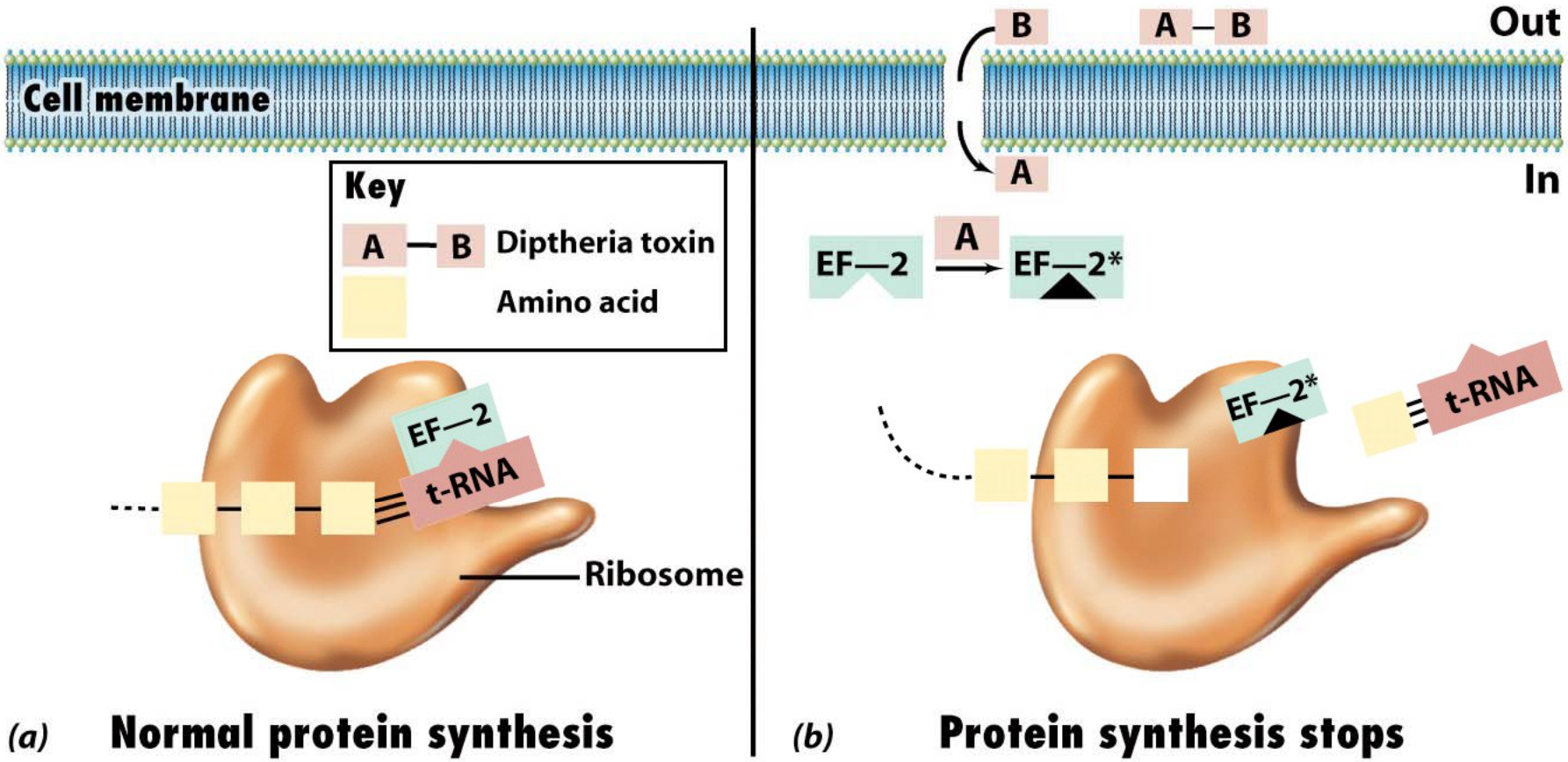


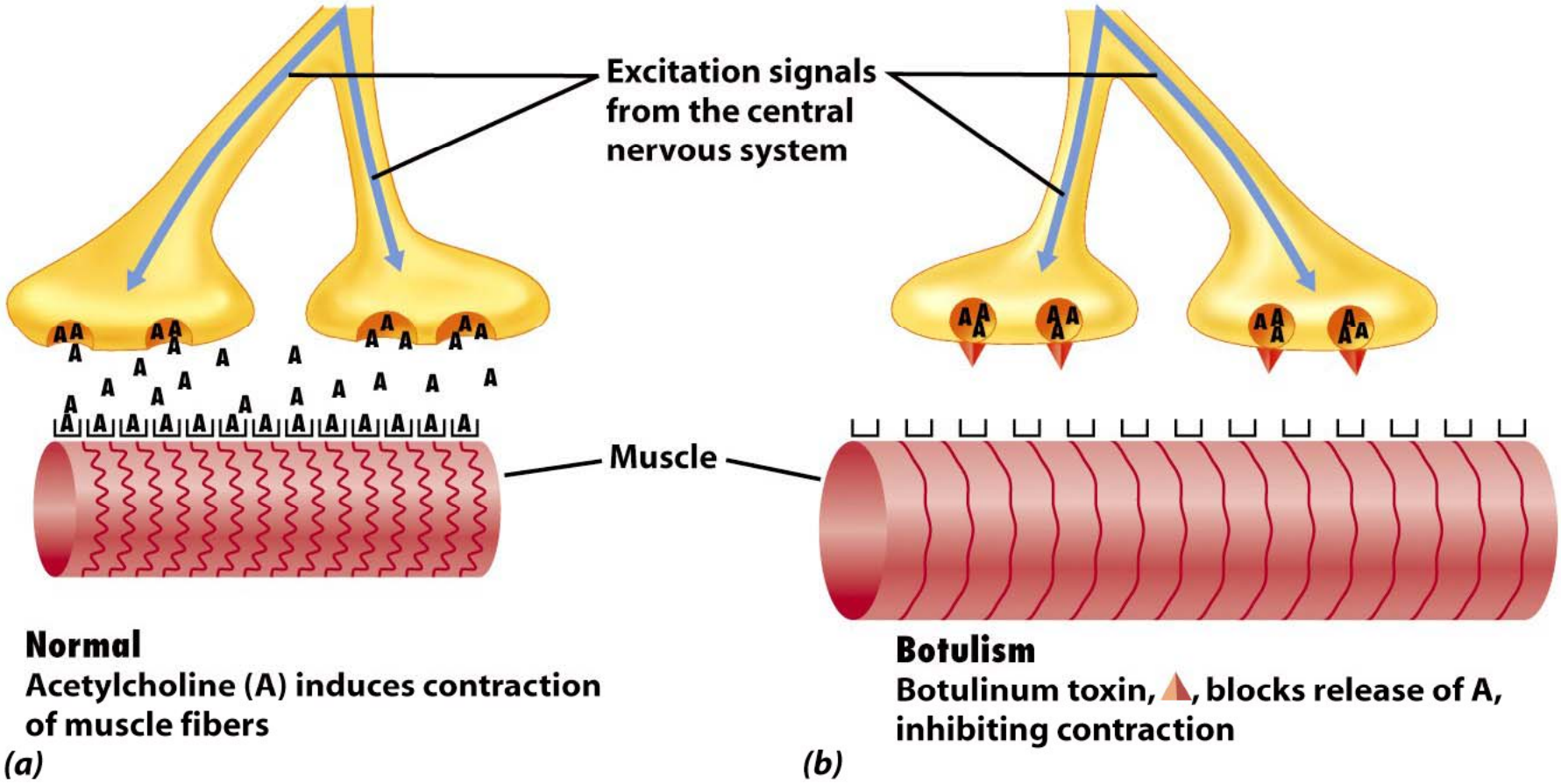
Figure 27.20

Action of diptheria toxin from *Corynebacterium diphtheriae*

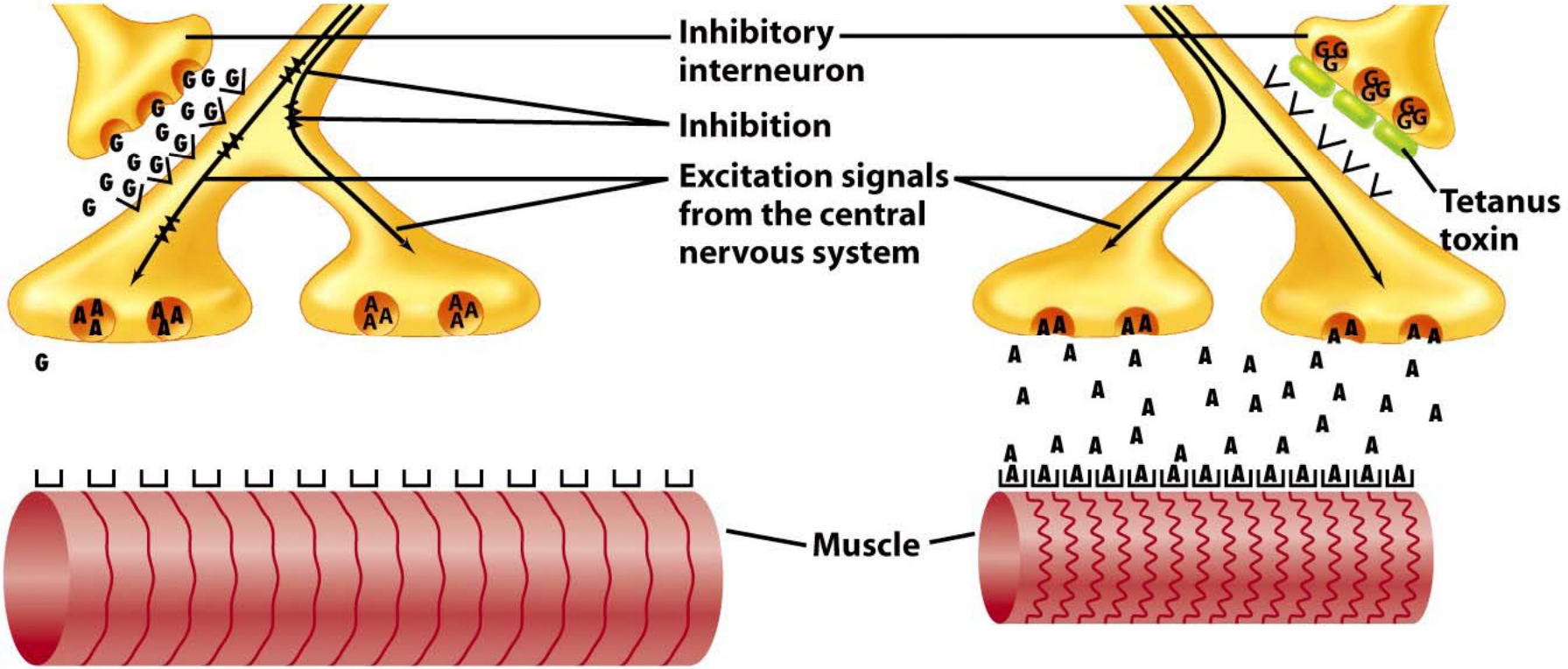


- ADP-ribosylates EF-2
- only takes one to kill cell

Action of botulinum toxin from *Clostridium botulinum*



Action of tetnus toxin from *Clostridium tetani*



Normal
Glycine (G) release from inhibitory inter-neurons stops acetylcholine (A) release and allows relaxation of muscle

(a)

Tetanus
Tetanus toxin binds to inhibitory interneurons, preventing release of glycine (G) and relaxation of muscle

(b)

Enterotoxins:

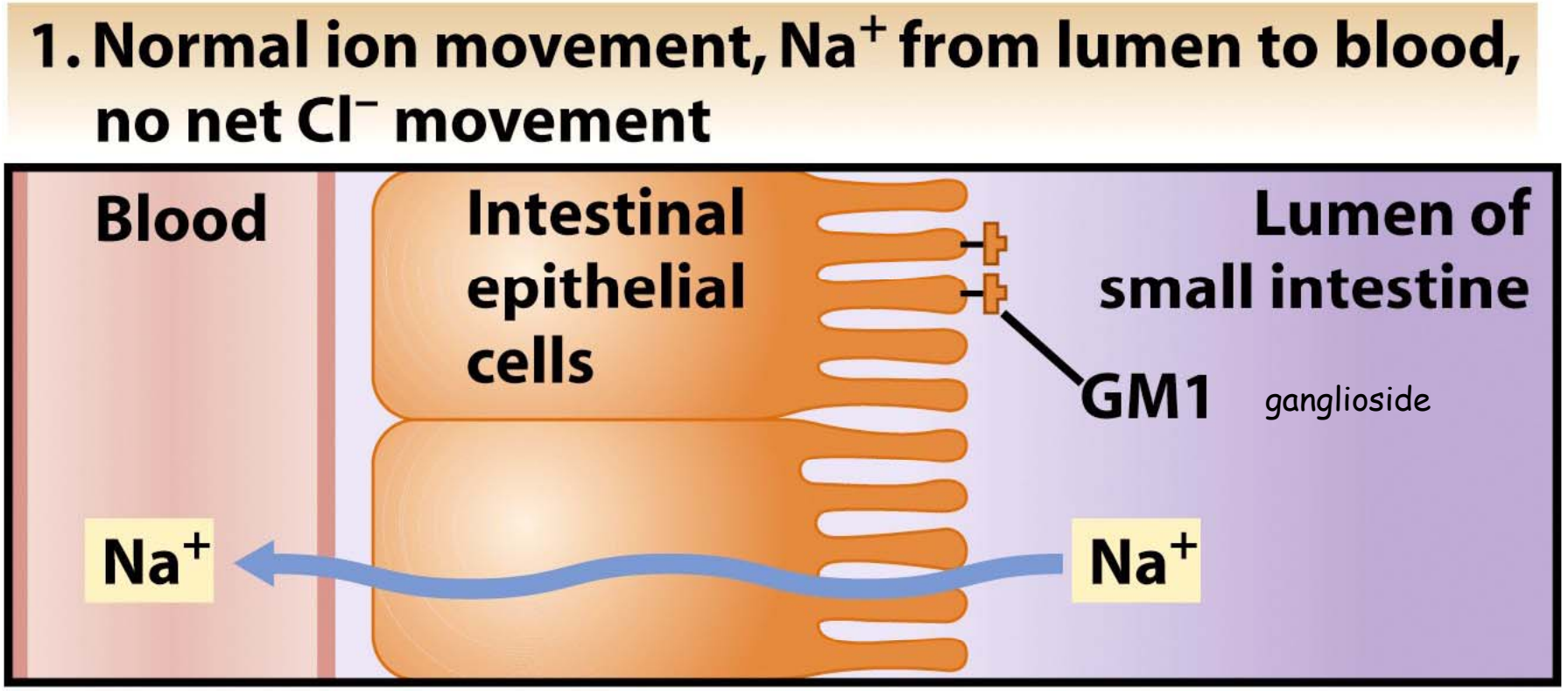
Exotoxins that specifically affect the small intestine.

1. Generally cause massive secretion of fluid.

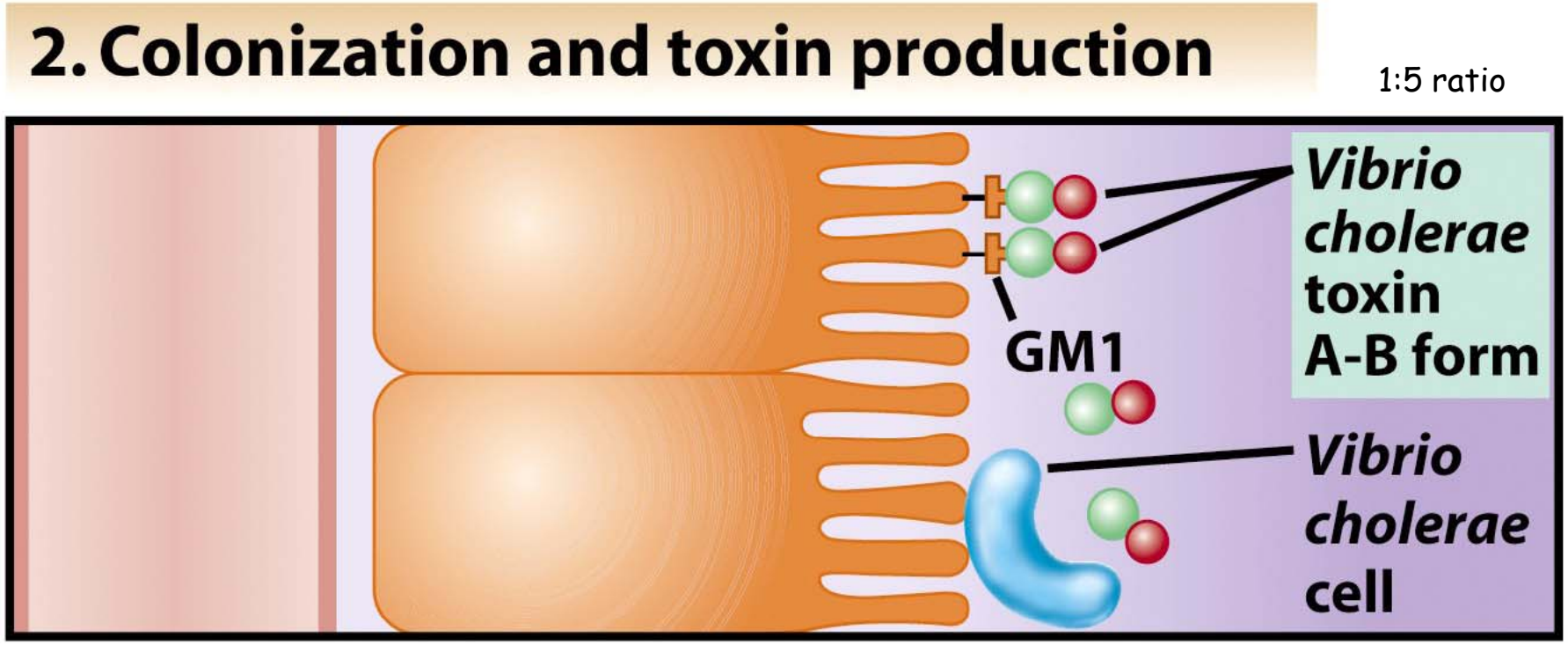
2. Leads to vomiting and/or diarrhea.

3. Often associated with food poisoning.

Action of cholera enterotoxin

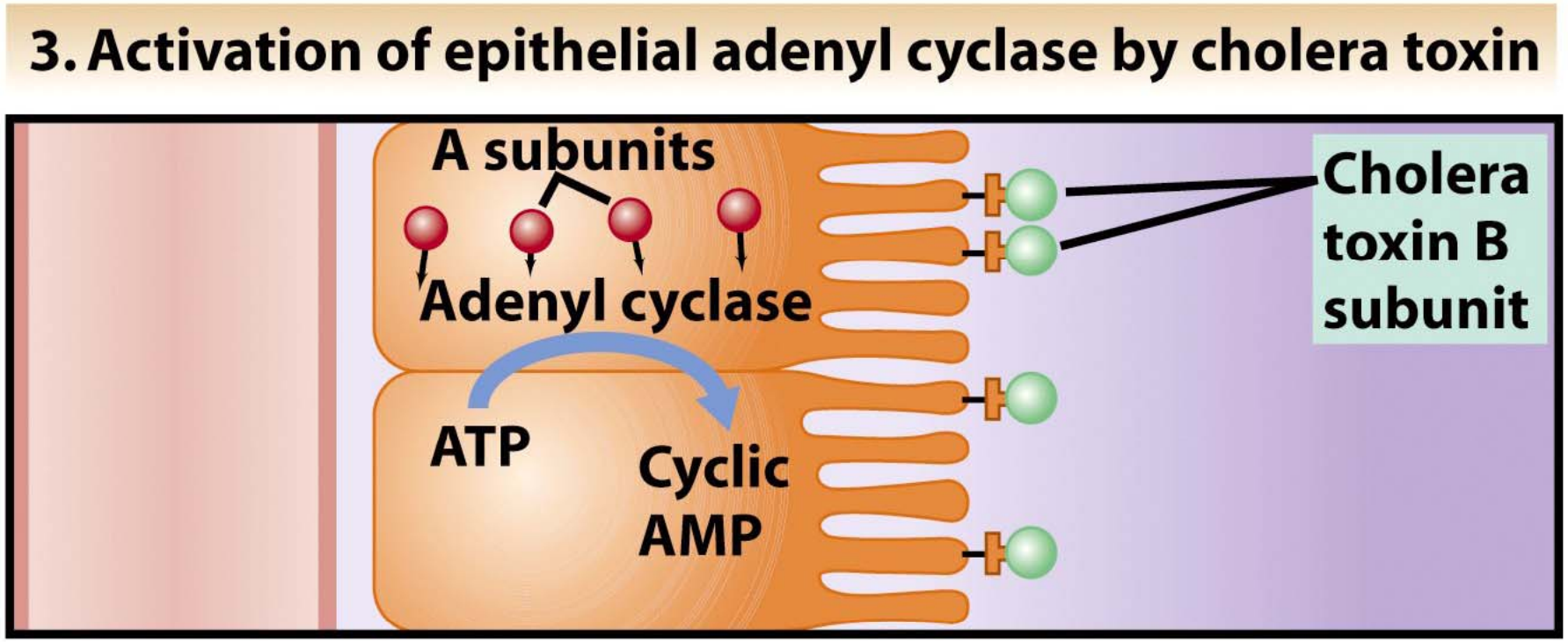


Action of cholera enterotoxin



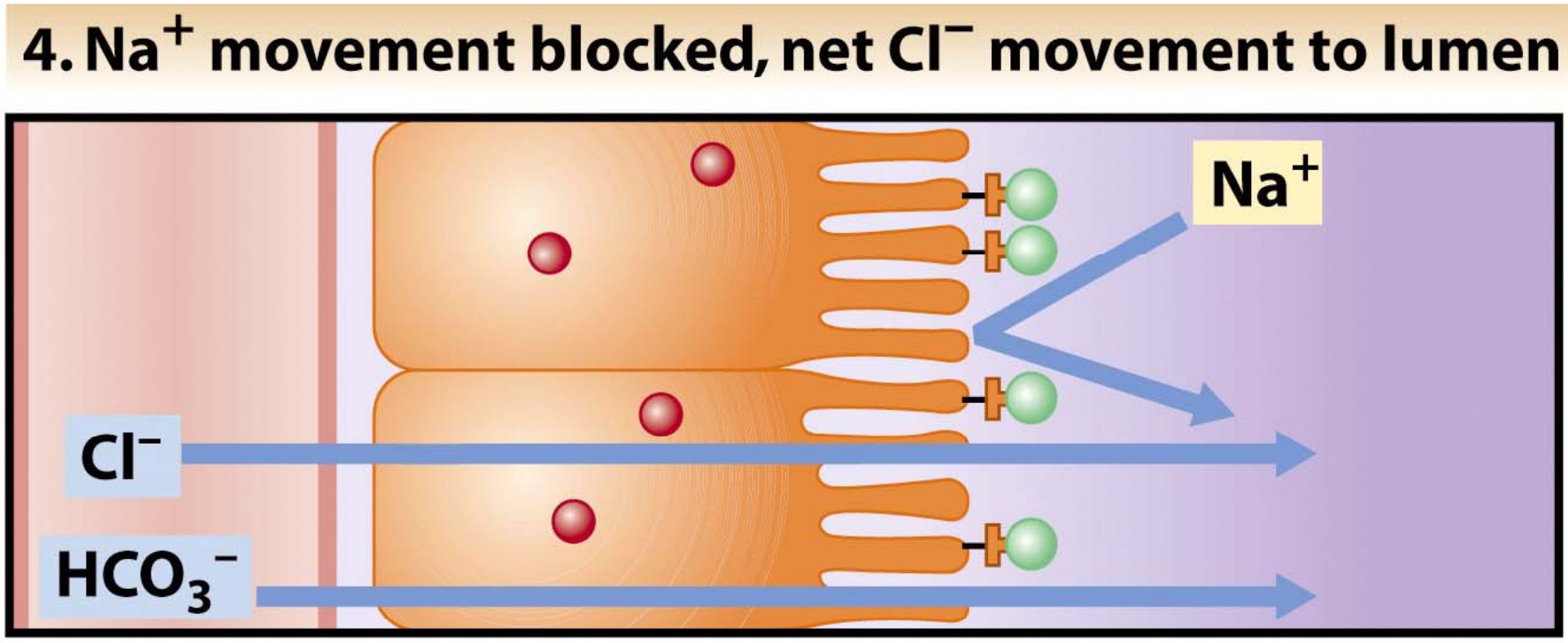
Purified "B" blocks process

Action of cholera enterotoxin



Activates this reaction!
(Causing sodium influx blockage)

Action of cholera enterotoxin



Action of cholera enterotoxin

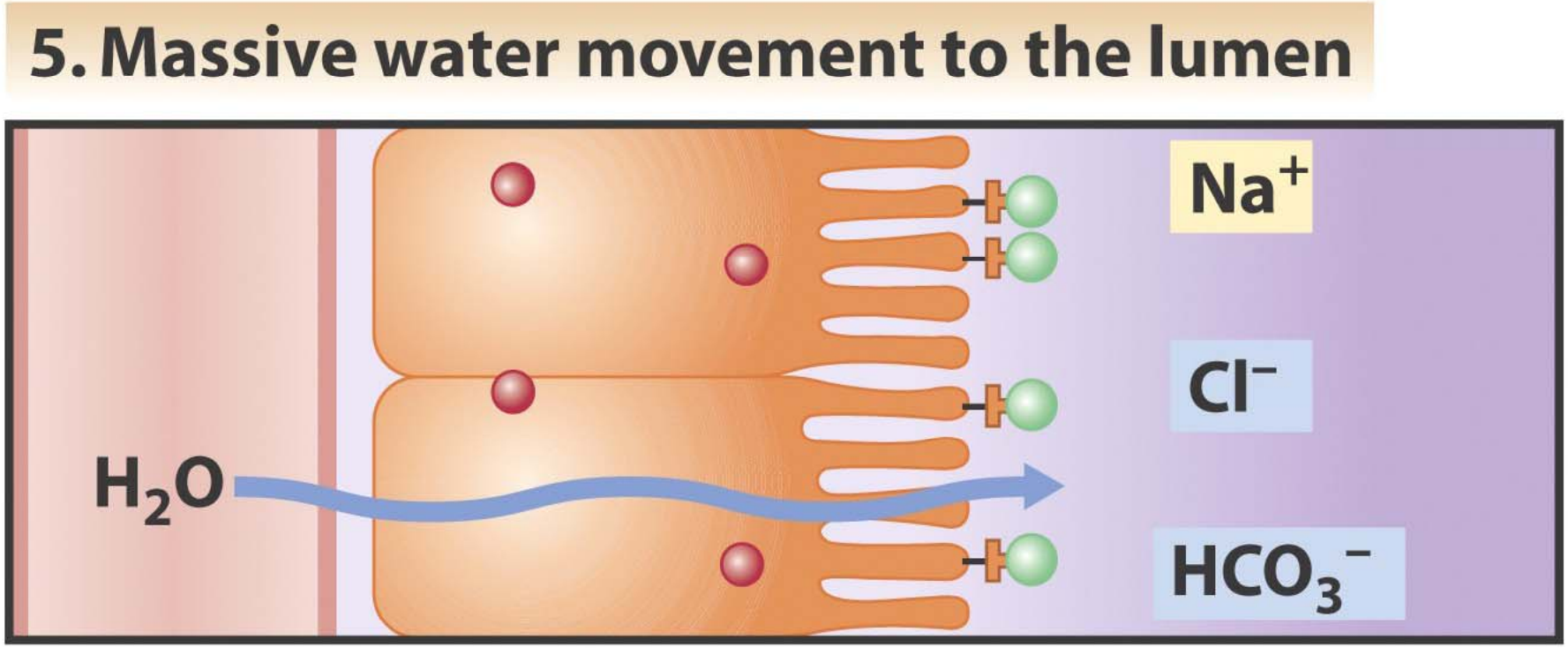


Table 26.6 Characteristics of exotoxins and endotoxins

Exotoxins	Endotoxins
Heat labile 60°C to 80°C	Heat stable
Immunogenic	Weakly immunogenic
Cause no fever	Cause fever
Can be lethal at low concentrations	Toxic at high doses
Different genera produce different toxins	Similar regardless of source
Released by live bacterium	Released on lysis of bacterium
Inactivated by chemicals that affect proteins	Not generally harmed by chemicals that affect proteins

Rem: Lipid A region of LPS

Table 26.7 **Some exotoxins produced by bacteria** *(Part 1)*

Exotoxin	Producing Organism	Disease	Effect
Diphtheria toxin	*Corynebacterium diphtheriae*	Diphtheria	Inhibits protein synthesis; affects heart, nerve tissue, liver
Botulism toxin	*Clostridium botulinum*	Botulism	Neurotoxin; flaccid paralysis
Perfringens toxin	*Clostridium perfringens*	Gas gangrene	Hemolysin, collagenase, phospholipase
Erythrogenic toxin	*Streptococcus pyogenes*	Scarlet fever	Capillary destruction
Pyrogenic toxin	*Staphylococcus aureus*	Toxic shock syndrome	Fever, shock
Exfoliative toxin	*Staphylococcus aureus*	Scalded skin	Massive skin peeling
Exotoxin A	*Pseudomonas aeruginosa*	— (~ Diphtheria)	Inhibits protein synthesis

Table 26.7 **Some exotoxins produced by bacteria** *(Part 2)*

Exotoxin	Producing Organism	Disease	Effect
Pertussis toxin	*Bordetella pertussis*	Whooping cough	Stimulates adenyl cyclase
Anthrax toxin	*Bacillus anthracis*	Anthrax	Pustules; blood poisoning
Enterotoxin	*Escherichia coli*	Diarrhea	Water and electrolyte loss
Enterotoxin	*Vibrio cholerae*	Cholera	Water and electrolyte loss
Enterotoxin	*Staphylococcus aureus*	"Staph" food poisoning	Diarrhea, nausea
Enterotoxin	*Clostridium perfringens*	Food poisoning	Permeability of intestinal epithelia
Neurotoxin	*Clostridium tetani*	Tetanus	Rigid paralysis

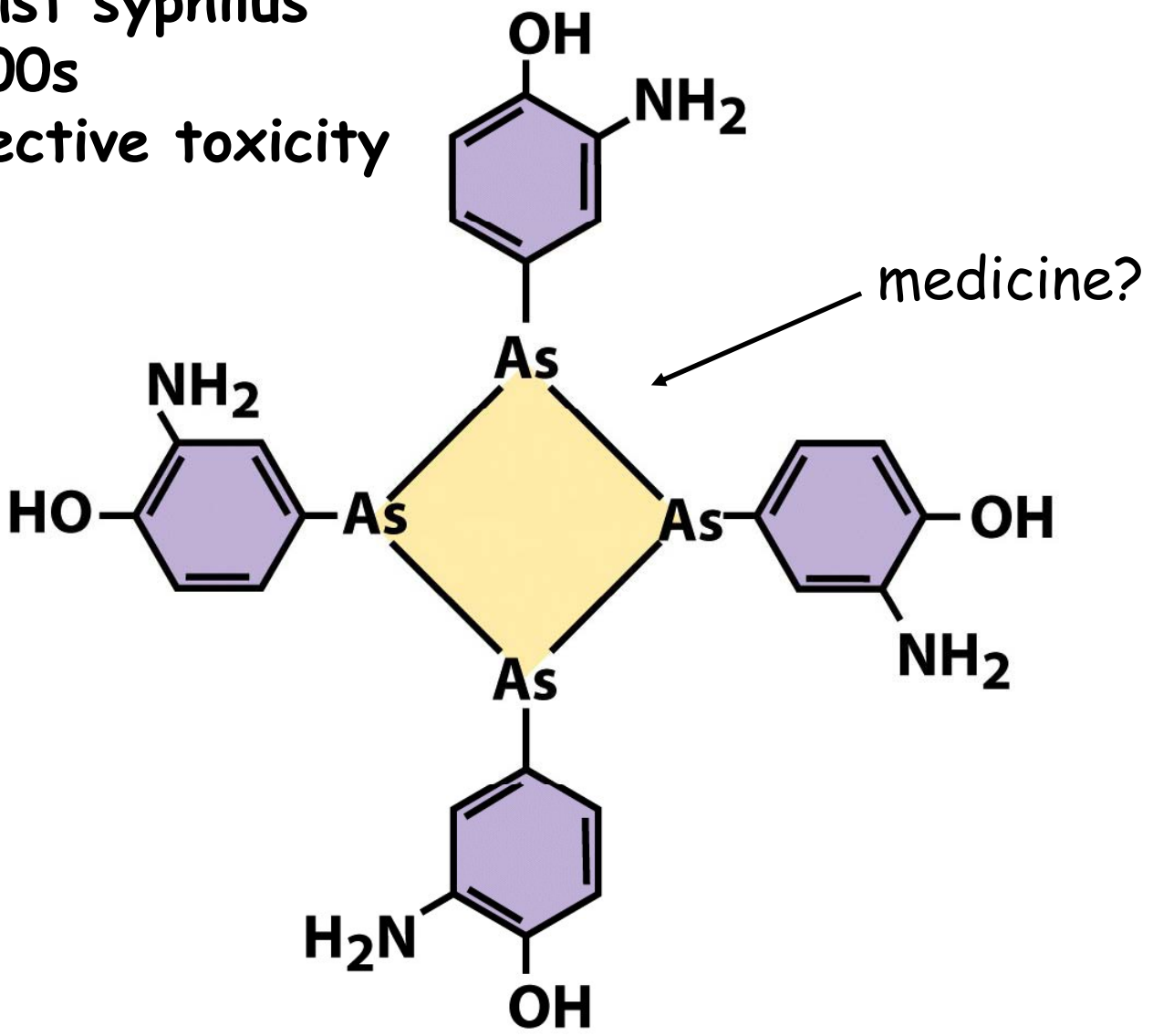
Classification of Antibiotics:

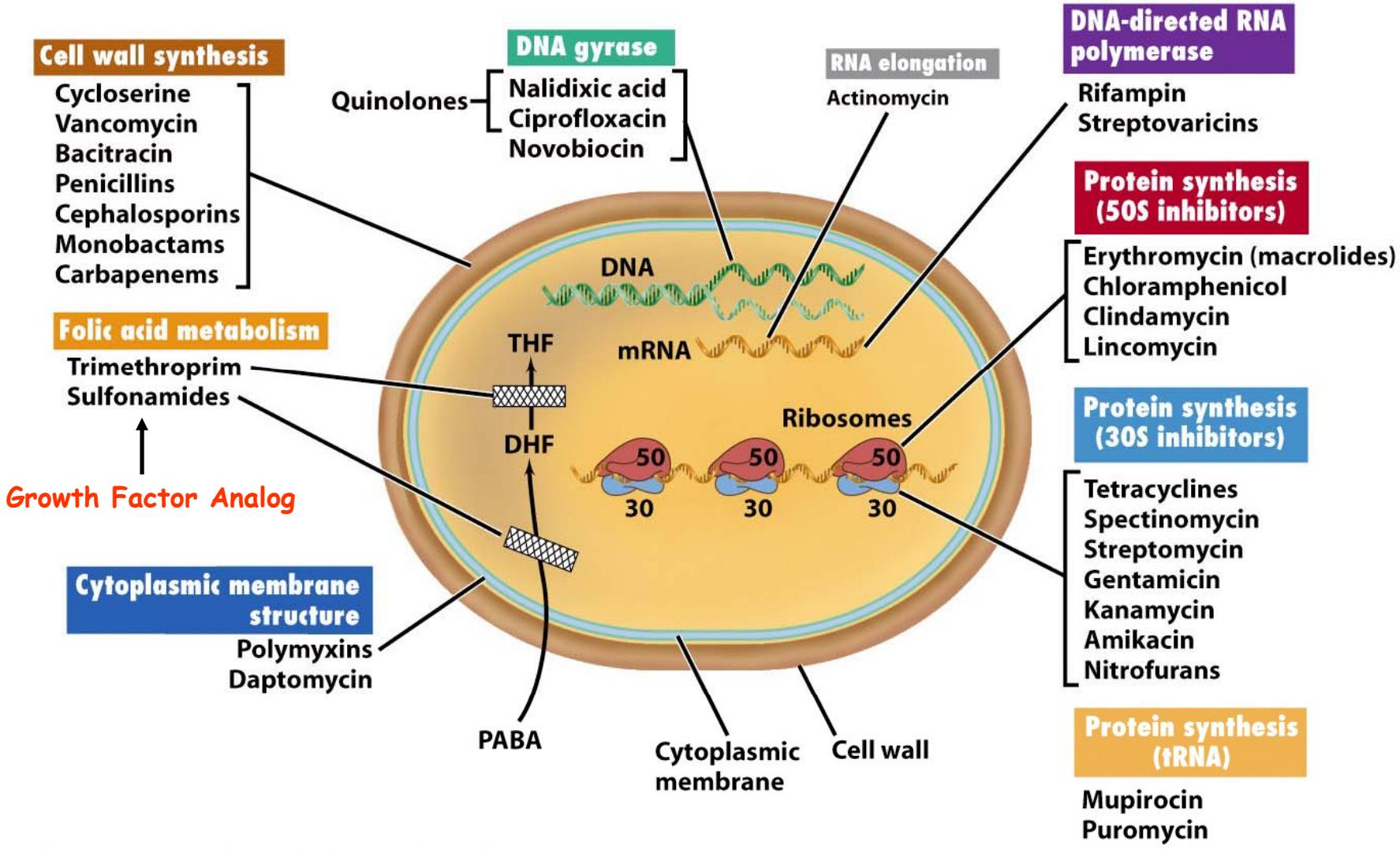
1. Inhibit growth – "stat"
   Kill bacterium – "cide"

2. Broad and Narrow spectrum

3. Production Types:
   - Natural
   - Synthetic
   - Semi-synthetic

Salvarsan: The first magic bullet
Works against syphilus
Ehrlich, 1900s
Idea of selective toxicity




Cell wall synthesis
Cycloserine
Vancomycin
Bacitracin
Penicillins
Cephalosporins
Monobactams
Carbapenems
DNA gyrase
Quinolones
Nalidixic acid
Ciprofloxacin
Novobiocin
RNA elongation
Actinomycin
DNA-directed RNA polymerase
Rifampin
Streptovaricins
Protein synthesis (50S inhibitors)
Erythromycin (macrolides)
Chloramphenicol
Clindamycin
Lincomycin
DNA
mRNA
THF
DHF
Ribosomes
50
30
Folic acid metabolism
Trimethroprim
Sulfonamides
Growth Factor Analog
Protein synthesis (30S inhibitors)
Tetracyclines
Spectinomycin
Streptomycin
Gentamicin
Kanamycin
Amikacin
Nitrofurans
Cytoplasmic membrane structure
Polymyxins
Daptomycin
PABA
Cytoplasmic membrane
Cell wall
Protein synthesis (tRNA)
Mupirocin
Puromycin

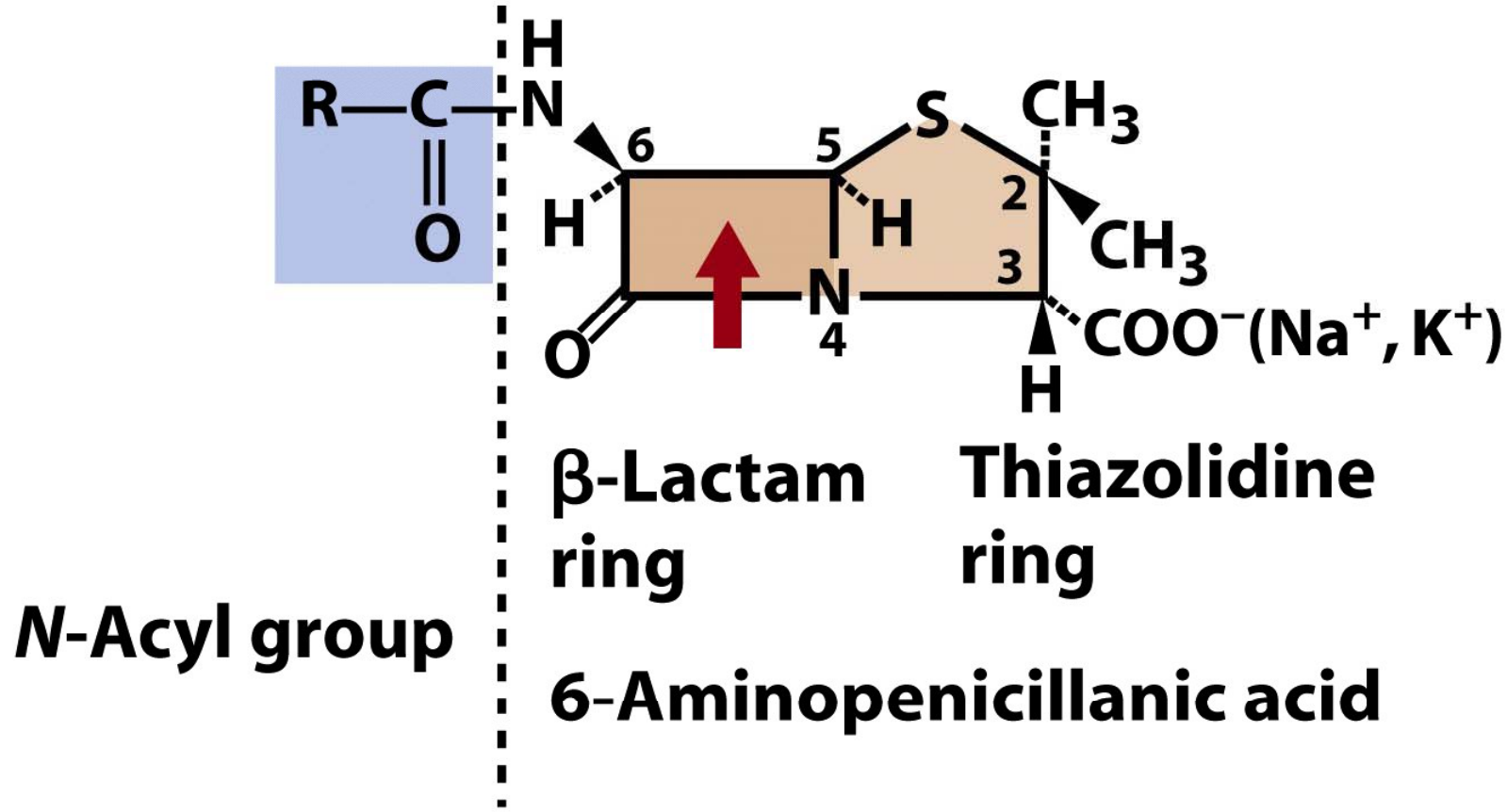
Antibiotics Affecting Replication, Transcription, & Translation

DNA replication:
Nalidixic Acid & Novobiocin – Inhibits DNA gyrase

Transcription:
Rifampin – Beta subunit of RNA polymerase
Actinomycin – DNA binding, blocks elongation

Translation:
Streptomycin – Blocks initiation on SSU of ribosome
Chloramphenicol – Blocks elongation on LSU via peptide bond
Tetracycline – Blocks elongation SSU
Cycloheximide – Eucarya ribosome specific
Diptheria Toxin – EF blocker; both Archaea and Eucarya

How to build a better mouse trap: Penicillin
A β-lactam antibiotic



Inhibits transpeptidation of peptidoglycan chains
Forms the old 1-2-punch with autolysins

Mechanisms of Antibiotic Resistance

1. Lacks structure antibiotic inhibits:
   Mycoplasms lack a typical cell wall.

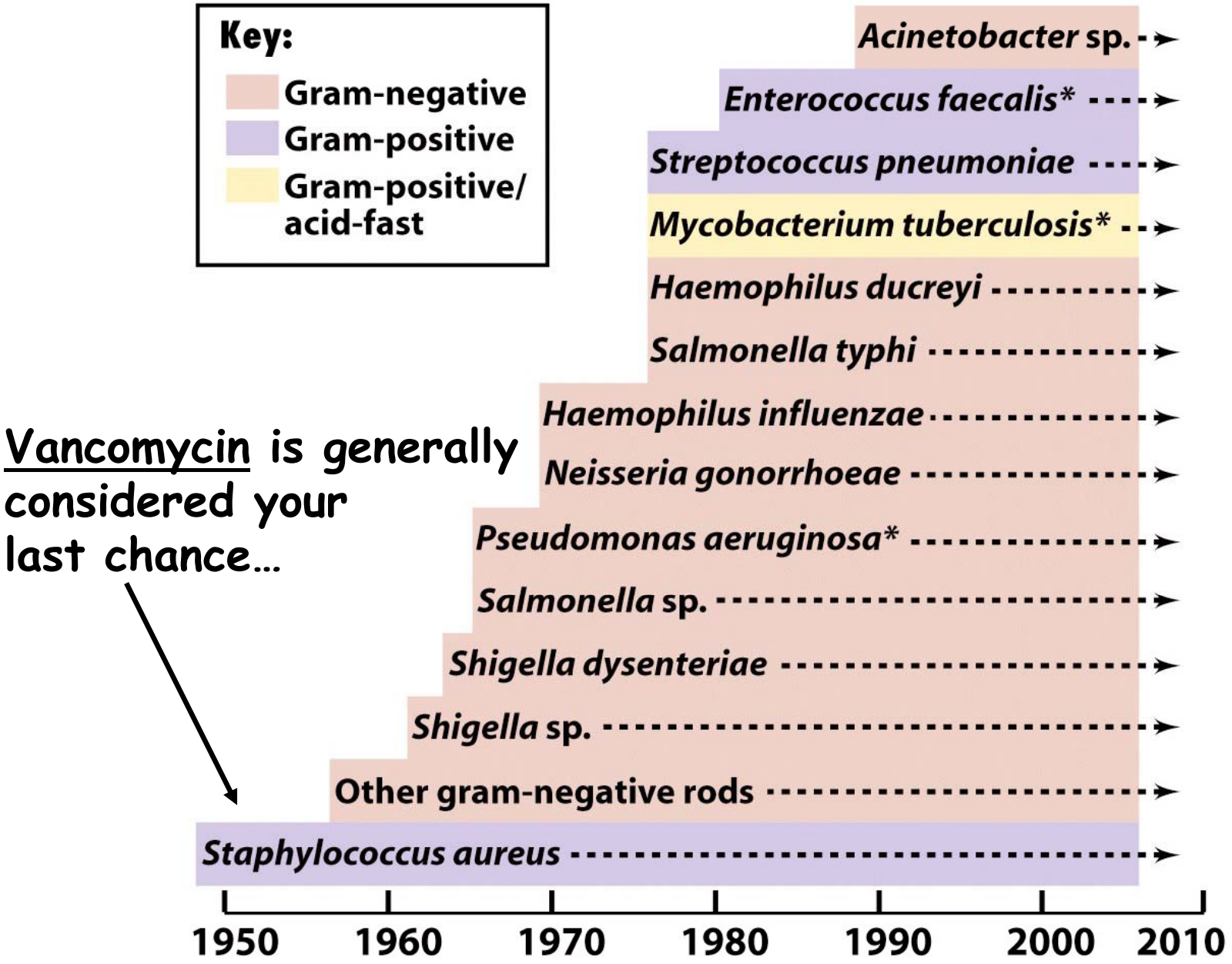
2. Impermeable to the antibiotic:
   Gram - bacteria impermeable to penicillin G.

3. Alteration of antibiotic:
   β-lactamase degrades antibiotic e.g., springs open the mouse trap.

4. Modifies the target of the antibiotic.

5. Genetically modifies the pathway that the antibiotic affects.

6. Efflux of the antibiotic:
   Tetracycline gets pumped back out of the cell.



Vancomycin is generally considered your last chance…

*symbol indicates that some multi-drug resistant strains of these organisms are now untreatable with known antimicrobial drugs.

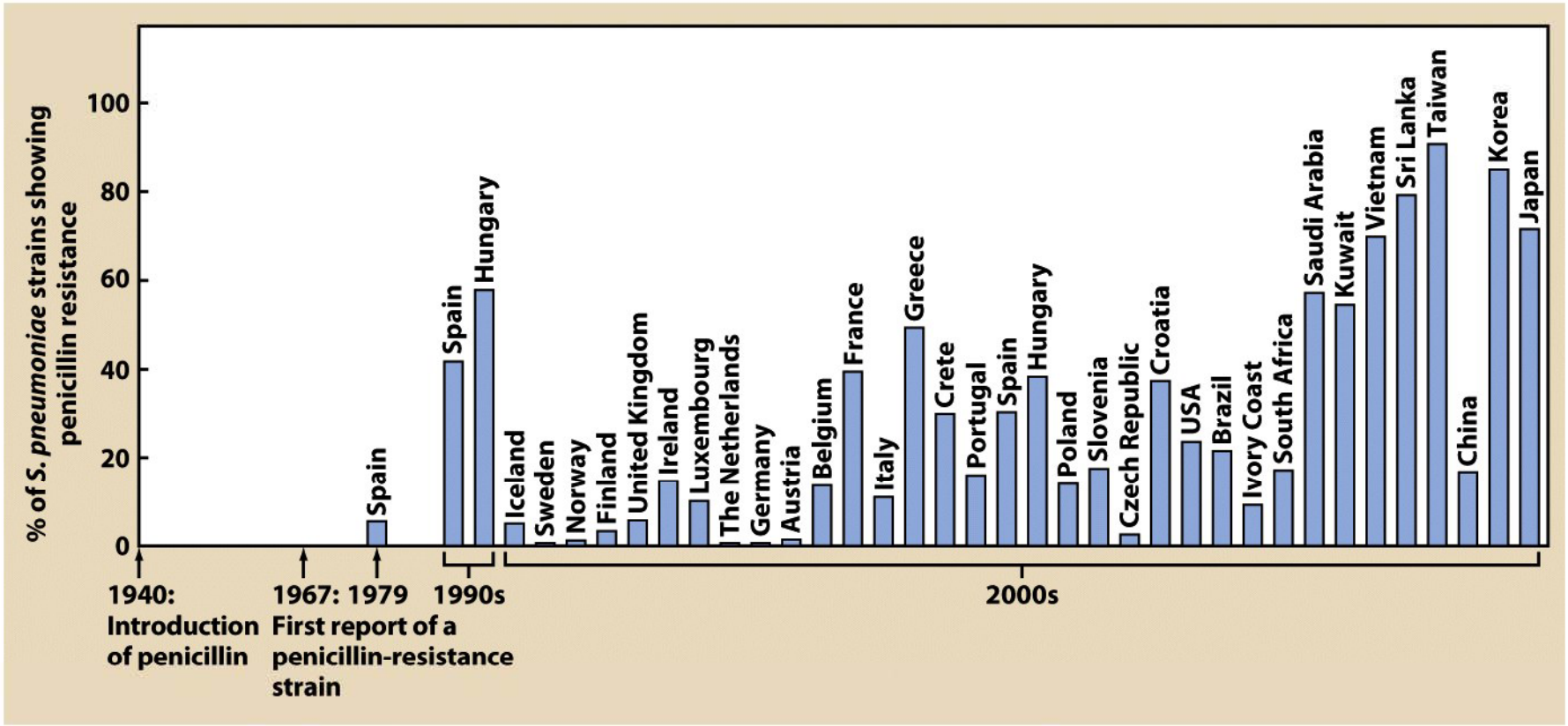


Figure 27.18 Microbiology: An Evolving Science