Bacteriology

Biology of bacteria

Bacterial genetics

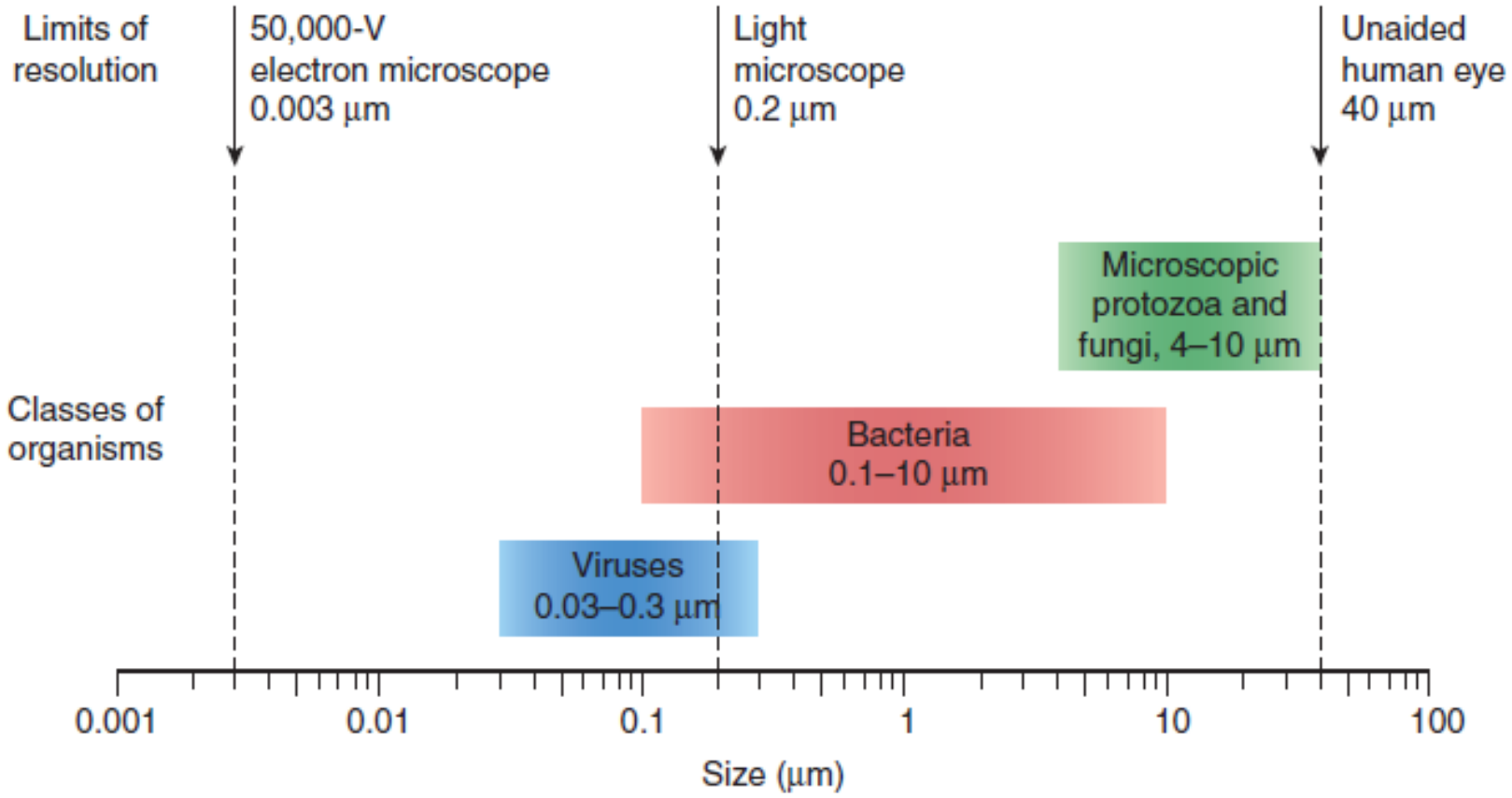
Pathogenesis of infectious diseases

Antibiotics

Sterilization and disinfection

Microbiology

science that studies organisms that cannot be seen with the unaided human eye (these are organisms that live in other cells - bacteria and viruses; but also those that have macroscopic forms during life - parasites, fungi)



History of microbiology

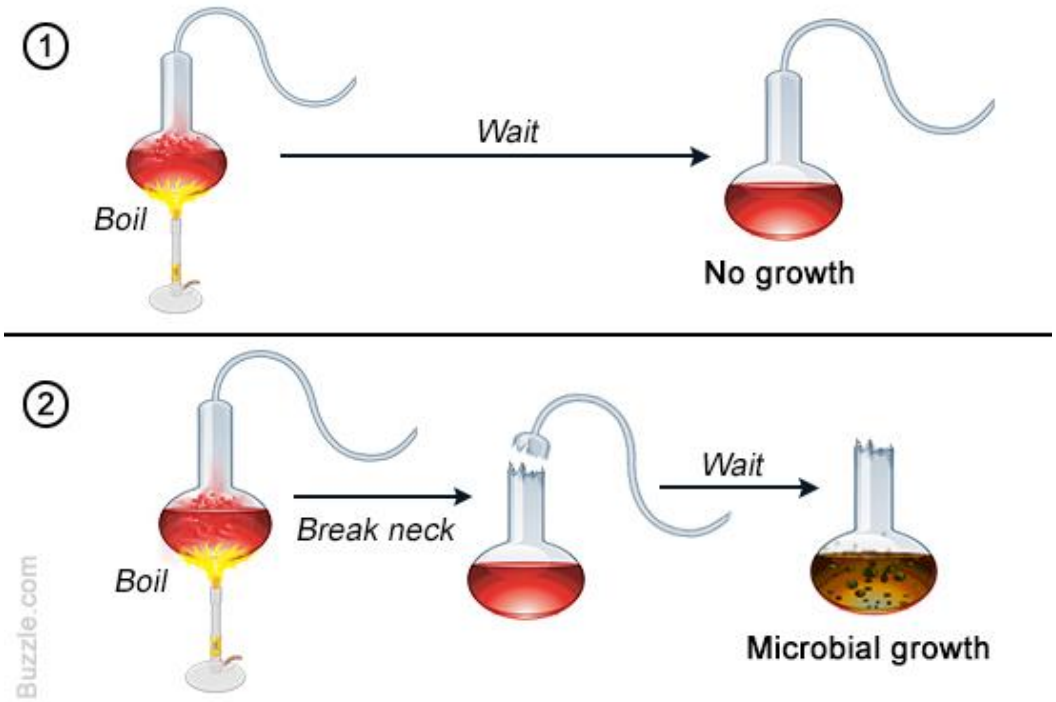
Anton van Leeuwenhoek (1632–1723) constructed a microscope and was the first to describe microroorganisms (invisible to the naked eye).

History of microbiology

Lazaro Spalanzani (1729–1799) described that cooking substrates kills microorganisms, and that microorganisms grow on substrates only if the substrates are exposed to air.

History of microbiology

Louis Pasteur (1822–1895) performed experiments that supported the germ theory that microorganisms cause most diseases.



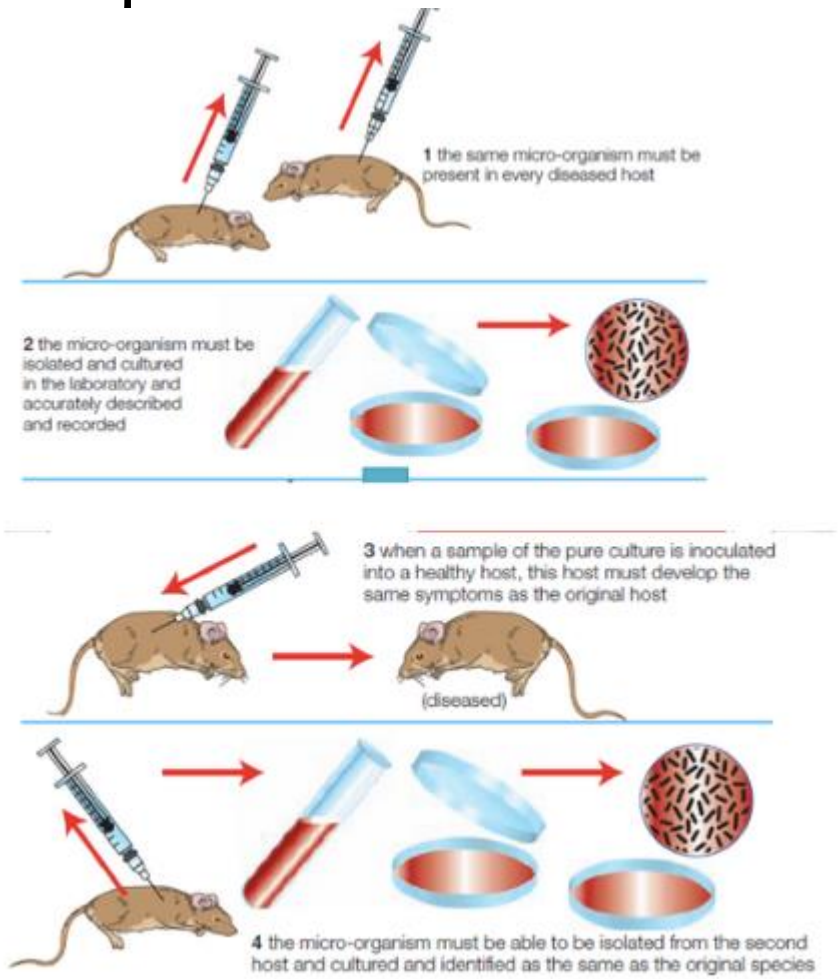
History of microbiology

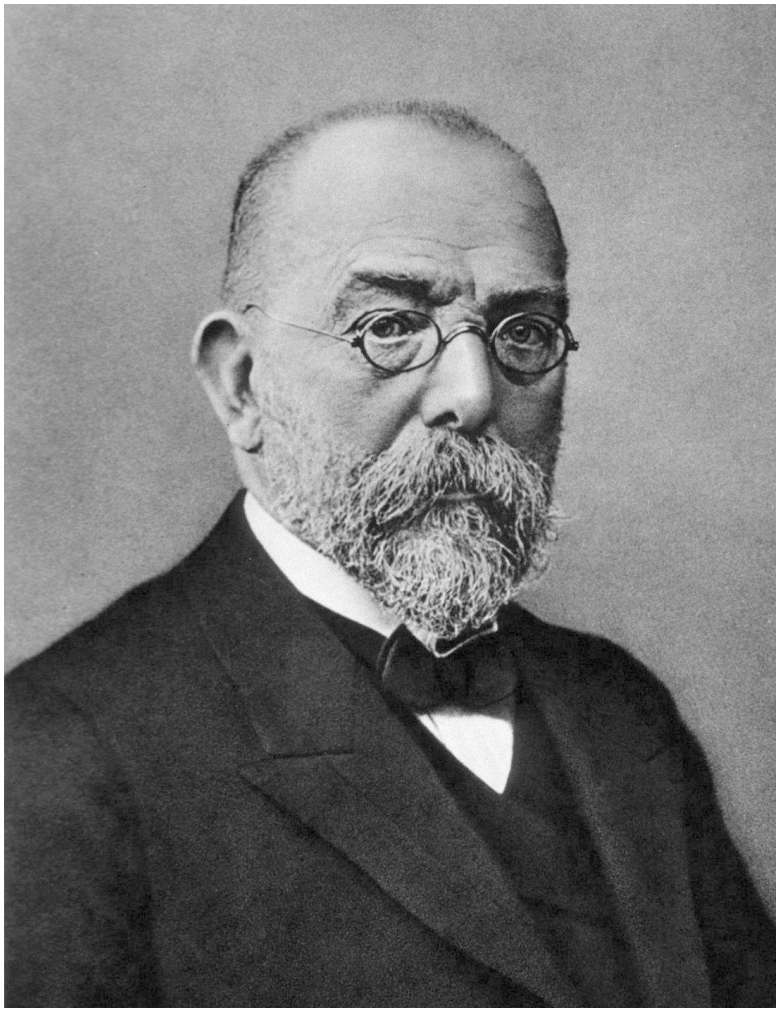
Ferdinand Julis Kon (1828–1898) classified bacteria into 4 groups on the basis of shape and described the transition of the vegetative form of Bacillus to the form of endospores under adverse conditions.

History of microbiology

Robert Koch (1843–1910) experimentally proved that microorganisms cause diseases, he discovered Mycobacterium tuberculosis

Koch's postulates





Bacterial cell biology

PROKARYOTIC AND EUKARYOTIC PATHOGENS

- Bacteria belong to the prokaryotes, whereas fungi, protozoa, and worms are eukaryotes.

- Prokaryotes lack nuclei and other internal membrane bound organelles. They do not carry out endocytosis and are incapable of ingesting particles or liquid droplets. Prokaryotes differ from eukaryotes in important biochemical details, such as the composition of their ribosomes and lipids. Prokaryotes are usually haploid, with a single chromosome and extrachromosomal DNA
- Most eukaryotes have a diploid phase and many chromosomes.

- Differences in organization between prokaryotes and eukaryotes have important consequences for the way they synthesize certain macromolecules. Lack of a nuclear membrane allows prokaryotes to simultaneously synthesize proteins and messenger RNA (mRNA) molecules.

- **Translation** (protein synthesis) can be coupled to **transcription** and therefore begin rapidly on new mRNA chains.

TABLE 3-1 Transcription and Processing of Messenger RNA (mRNA) in Typical Prokaryotes and Eukaryotes

Characteristic	*Escherichia Coli* (Prokaryote)	Yeast (Eukaryote)
Genomic Structure		
Genome organization	Single gene copies	Single gene copies plus repetitive DNA
Chromosomes	One	Many
Ploidy	Haploid	Haploid/diploid cycle
Cytoplasmic DNA	Plasmids	Mitochondria, kinetoplasts
Colinearity of gene with mRNA	Precise sequence	Introns within gene
Regulation of Gene Expression		
Type	Operon-polycistronic mRNA	Single genes
Level	Mostly transcriptional	Often posttranscriptional regulation by protein turnover, etc.
Transcription		
Relation of transcription and translation	Coupled	Uncoupled
mRNA processing	Rare; some cleavages at double-stranded functional domains	Poly(A) at 3′ end, cap at 5′ end; splicing, sites in mRNA
mRNA stability	Unstable	Range of stability; some very stable mRNAs
Translation of mRNA		
First amino acid	Formylated methionine	Methionine
Signal for start	Ribosome binding site preceding AUG codon	Binding to 5′ end, use of first AUG codon along mRNA
Initiation factors	Three	More than six
Ribosomes	30S + 50S = 70S	40S + 60S = 80S

SMALL SIZE AND METABOLIC EFFICIENCY

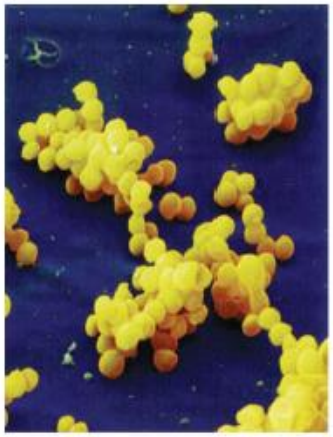
- A typical bacterium is of the order of 1 μm in diameter
- Each of us currently carries a load of some **10 to 100 trillion** bacteria in the large intestine, greatly surpassing the number of eukaryotic cells

Bacterial structure

Bacteria are the smallest microorganisms, capable of independent existence

The size of bacteria that have medical significance is from 0.1 to 10 μm.

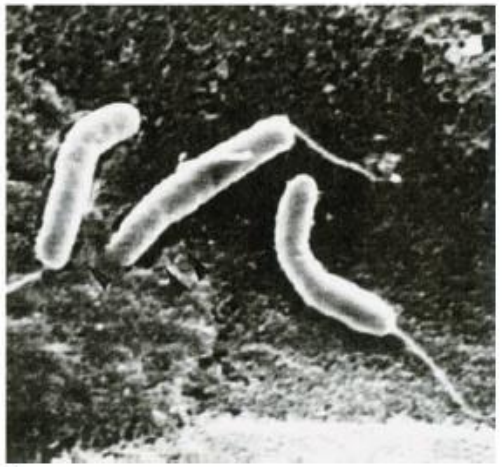
The main morphological forms of bacteria are: **cocci, bacilli, vibrios and spirals**


A

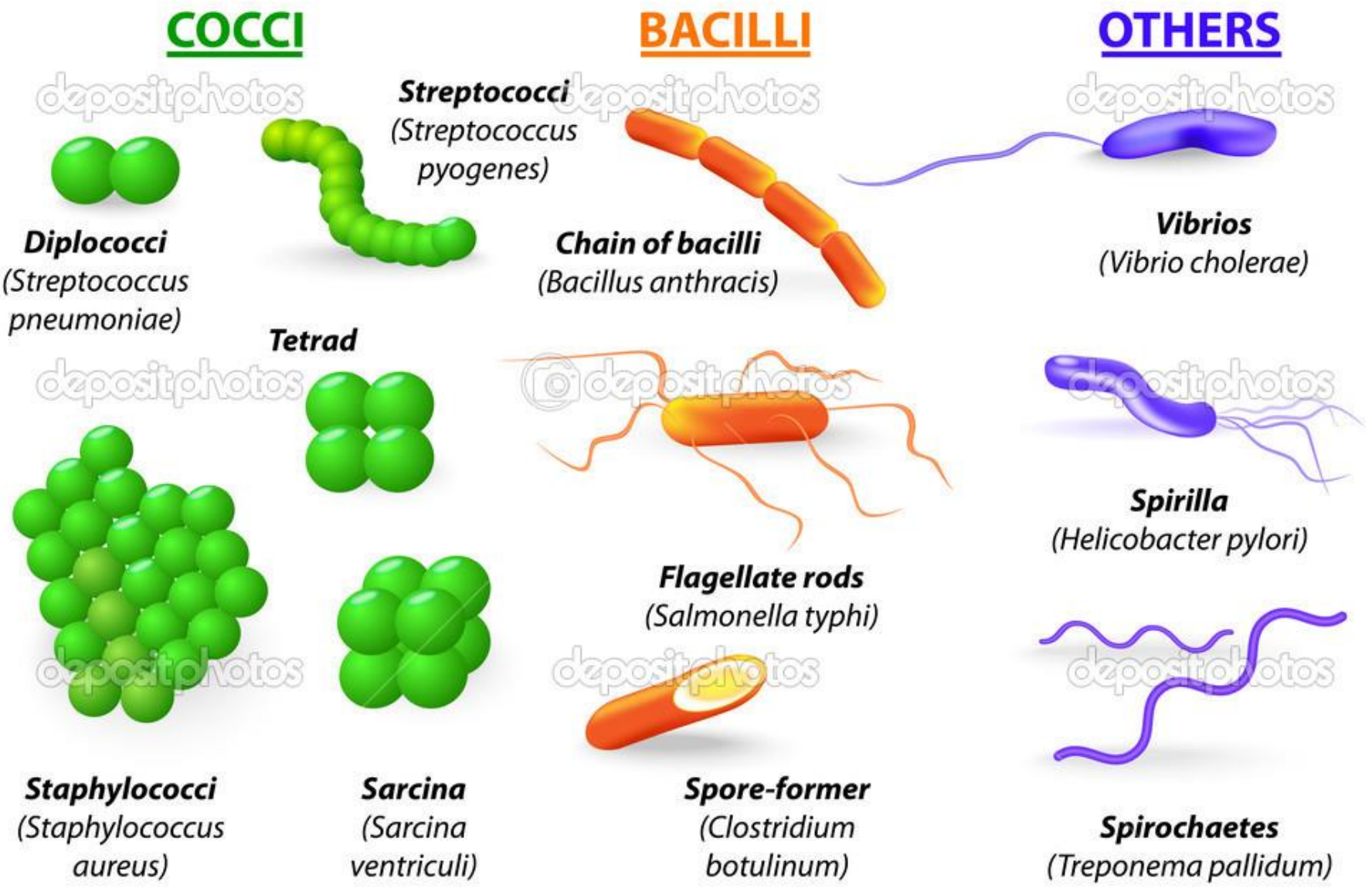

B


C


D


E

The forms of bacteria



COMPLEX ENVELOPES AND APPENDAGES

- Going from the outside inward, bacteria are surrounded by a complex set of surface layers and appendages that differ in composition from species to species.

- These surface components often determine whether an organism can survive in a particular environment and cause disease.

- Like all cells, bacteria have an essential structure, the **cytoplasmic membrane**. Most bacteria also possess structures outside the membrane-namely, a **cell wall**- and some have an **outer membrane**, **flagella**, **pili**, and a **capsule**.

- Gram staining (named after Dr. H. C. J. Gram, the Danish microbiologist who devised the method in 1884) divides most bacteria into two groups, nearly equal in number and importance.

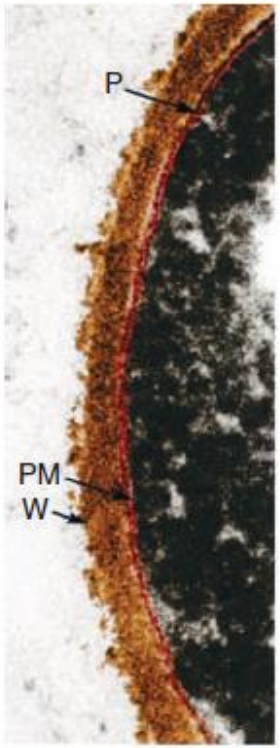
- Gram staining depends on the ability of certain bacteria (the Gram-positives) to retain a complex of a purple dye and iodine when challenged with a brief alcohol wash. Gram-negatives do not retain the dye and can therefore be counterstained after the alcohol wash with a red dye, safranin.

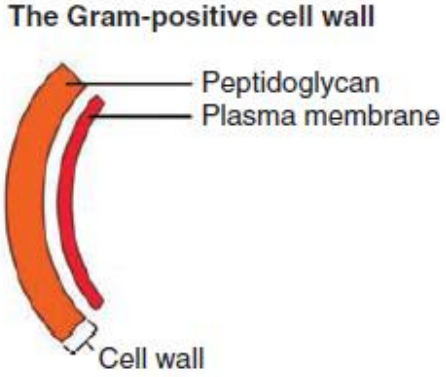
Cell wall

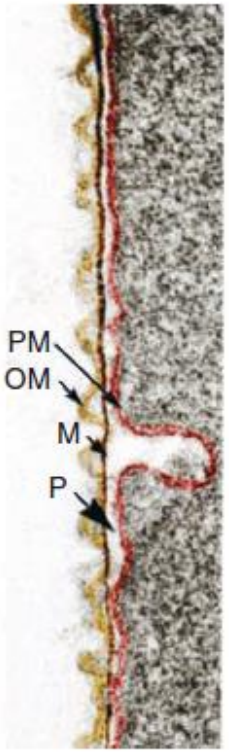
It protects bacterial cells from mechanical damage and from cell rupture due to pressure (the bacterium is hypertonic in relation to the environment), from chemical and physical agents.

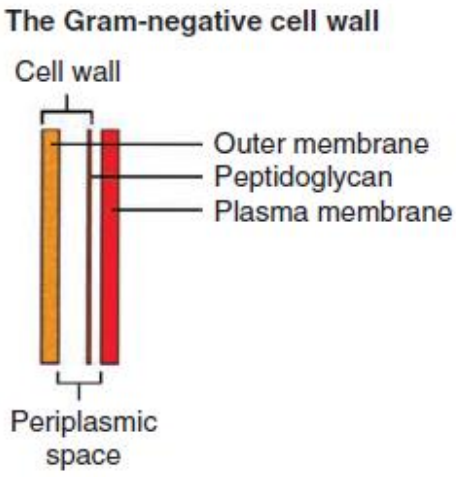
Gives shape to bacteria.

Based on Gram staining, two basic types of cell wall structure are distinguished and bacteria are divided into two groups Gram + and Gram-.









Gram-Positive Solution

- A Gram-positive bacterium protects its cytoplasmic membrane with a thick **cell wall**. The major constituent of the wall is a complex polymer **unique to bacteria**, composed of sugars and amino acids called **murein**, or **peptidoglycan**. Murein is the critical component in maintaining the shape and rigidity of both Gram-positives and Gram-negatives but plays a larger role in protecting the cell membranes of Gram-positives.

- Murein is composed of glycan (sugar) chains thatare cross-linked to one another via peptides. This polymeric fabric is wrapped around the length and width of the bacterium to form a sac of the size and shape of the organism. Depending on the shape of the murein sac, bacteria may be shaped like rods (**bacilli**), spheres (**cocci**), or helices (**spirilla**). The rigid murein corset allows bacteria to survive in media of lesser osmotic pressure than that of their cytoplasm.

- The cell wall of a Gram-positive is composed of many layers of the saclike murein. The layers are so thick that they impede the passage of hydrophobic compounds. The reason for this is that the sugars and charged amino acids of murein make the highly polar structure surrounding these cells a dense hydrophilic layer. Thus, many Gram-positives can withstand certain noxious hydrophobic compounds, including bile salts in the intestine.

- Gram-positive walls contain other unique polymers, such as **teichoic acids**, which are chains of ribitol or glycerol linked by phosphodiester bonds

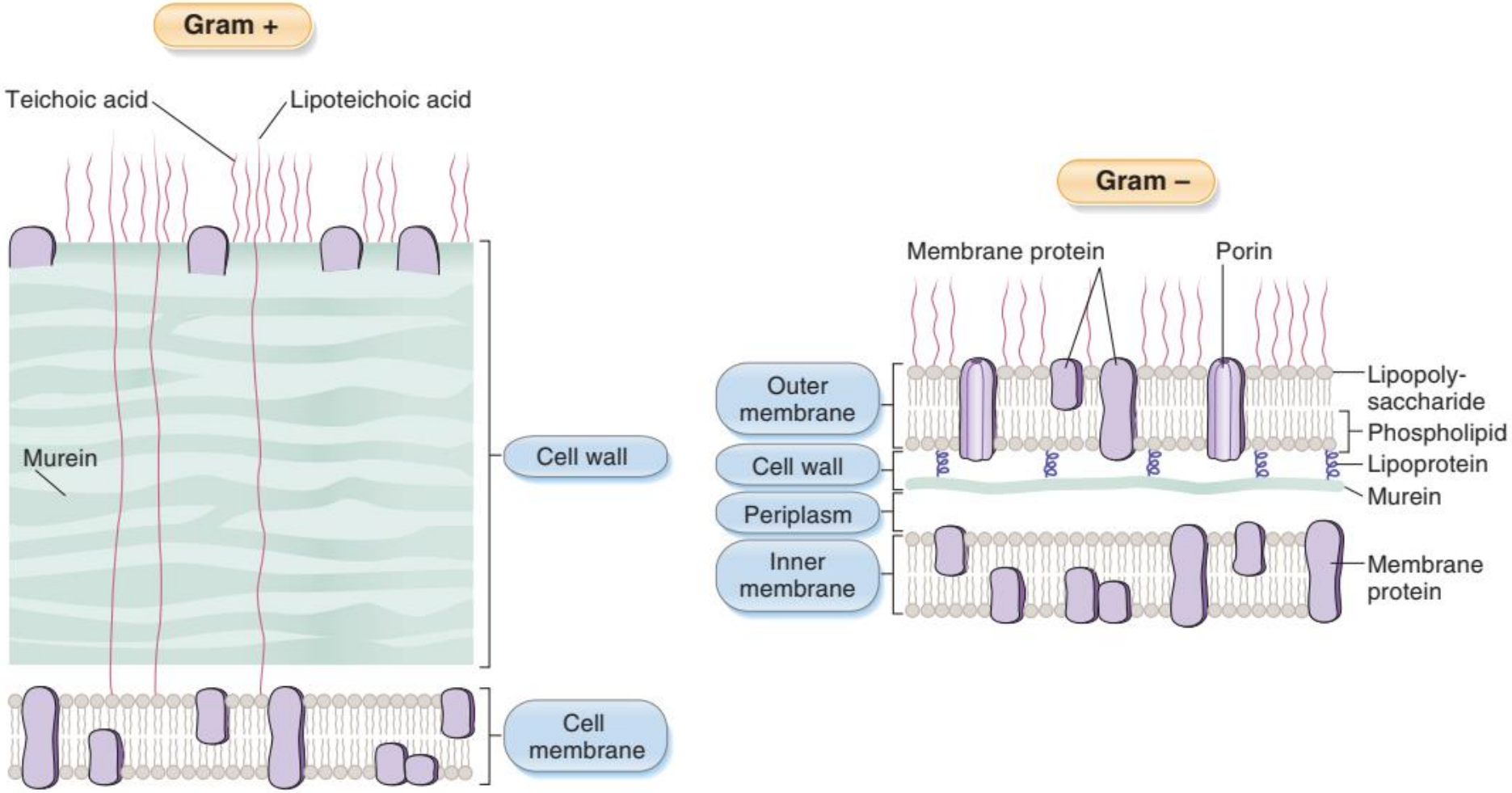


FIGURE 3-2. The envelope structure of Gram-positive (left) and Gram-negative (right) bacteria. Capsules and appendages are not shown, nor are surface proteins like the M protein of streptococci. Note the 20-fold greater amount of peptidoglycan, or murein, in the Gram-positive. The outer membrane of the Gram-negative envelope has a covering of lipopolysaccharide (LPS) molecules. The Gram-negative's outer membrane also has pores made of trimers of porin, which permit the entry of small hydrophilic molecules.

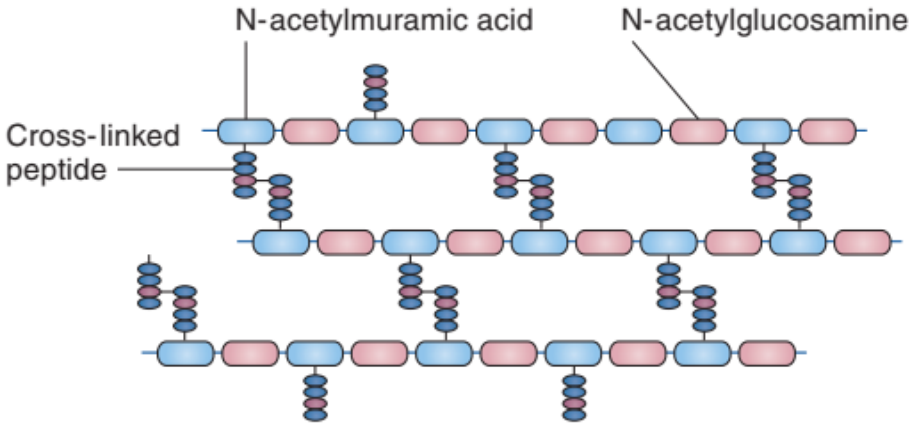


FIGURE 3-3. Structure of murein. The polysaccharide (glycan) strands consist of alternating units of *N*-acetylglucosamine and *N*-acetylmuramic acid connected to a peptide. Some of the peptides of one strand are cross-linked to those of another. Because of cross-linking, murein has a two-dimensional structure resembling a chain-linked fence.

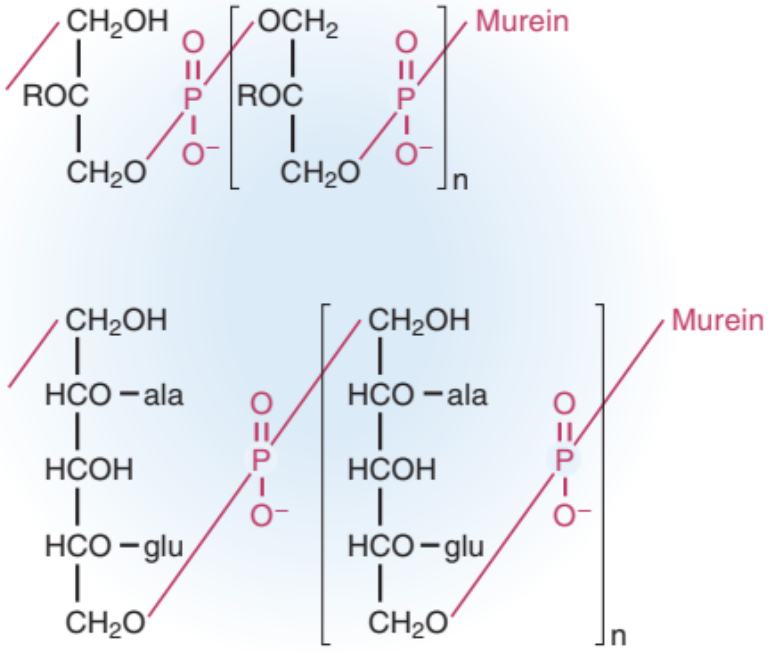


FIGURE 3-5. Teichoic acid structure. The repeating units of ribitol and glycerol teichoic acids are shown. The chains in Gram-positive organisms vary in length and amounts.

Gram-Negative Solution

- Gram-negatives build a completely different structure, an **outer membrane**, outside the murein cell wall. The outer membrane is chemically distinct from the usual biological membranes and is able to resist damaging chemicals. It is a bilayered structure, but its outer leaflet contains a unique component in addition to phospholipids. This component is a bacterial **lipopolysaccharide** (**LPS**), a complex molecule not found elsewhere in nature.

- LPS has three components:
- **Lipid A**: This lipid anchors LPS in the outer leaflet of the membrane.
- **Core**: This component consists of a short series of sugars that are nearly the same in most Gram-negative bacteria and includes two characteristic sugars, **keto-deoxyoctanoic acid** and a **heptose**.
- **O antigen**: This is a long carbohydrate chain of up to 40 sugars in length.

- The outer membrane has special **channels** that permit the passive diffusion of hydrophilic compounds like sugars, amino acids, and certain ions. These channels consist of protein molecules with holes, aptly called **porins**.

TABLE 3-2 Gram Stain and Acid-Fast Procedures

Gram Stain	Acid-Fast Stain
1. Stain with crystal violet (purple)	1. Stain with hot carbol–fuchsin (red)
2. Modify with potassium iodide	2. Decolorize with acid alcohol; only acid-fast remain red.
3. Decolorize with alcohol; only Gram-positives remain purple.	3. Counterstain with methylene blue: Acid-fast remain red; others become blue.
4. Counterstain with safranin: Gram-negatives become pink; Gram-positives remain purple.	

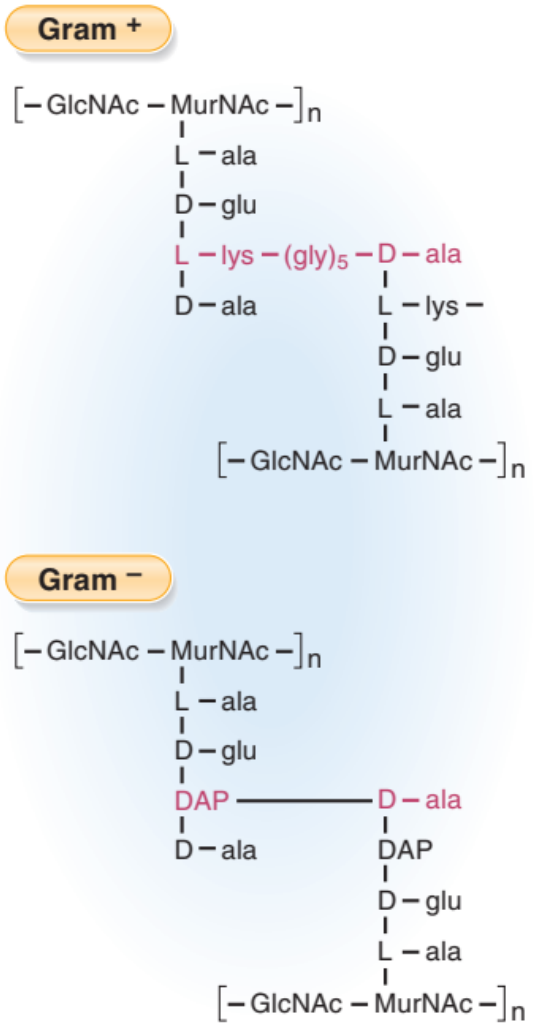


FIGURE 3-4. Typical composition of murein in Gram-positive and Gram-negative bacteria. In Gram-positives, peptide chains are cross-linked through a peptide (a pentaglycine in *Staphylococcus aureus*) between the free amino group of lysine and the terminal carboxyl group of a D-alanine residue. In the Gram-negatives, the cross-link is between diaminopimelic acid and D-alanine.

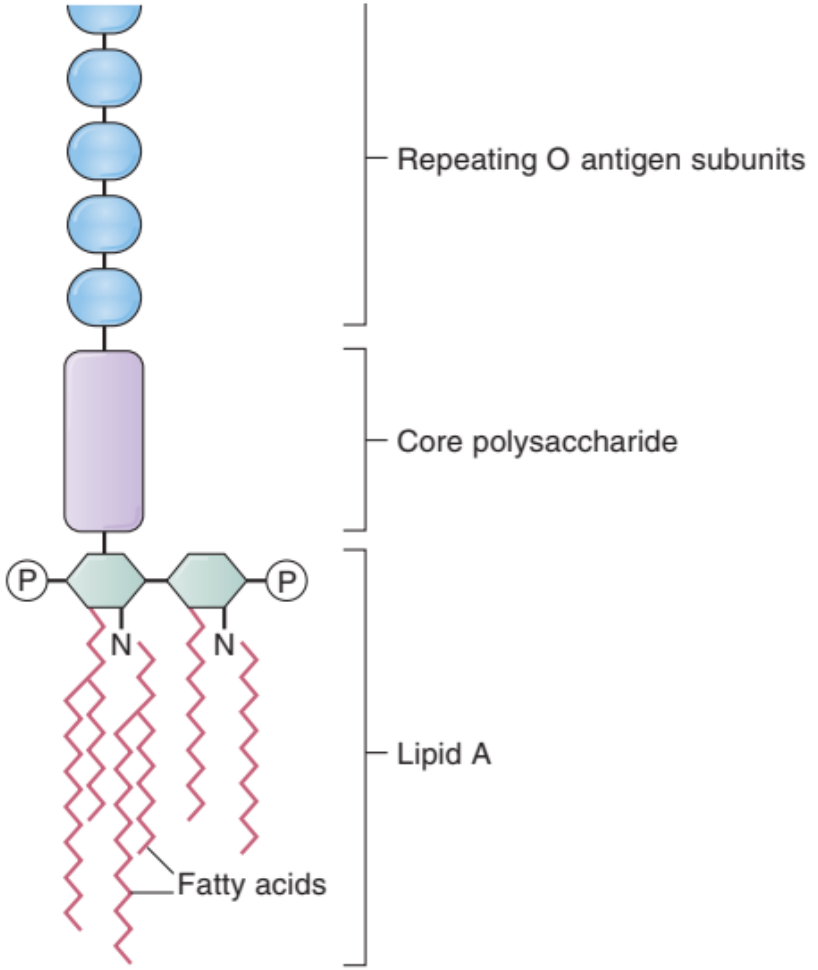
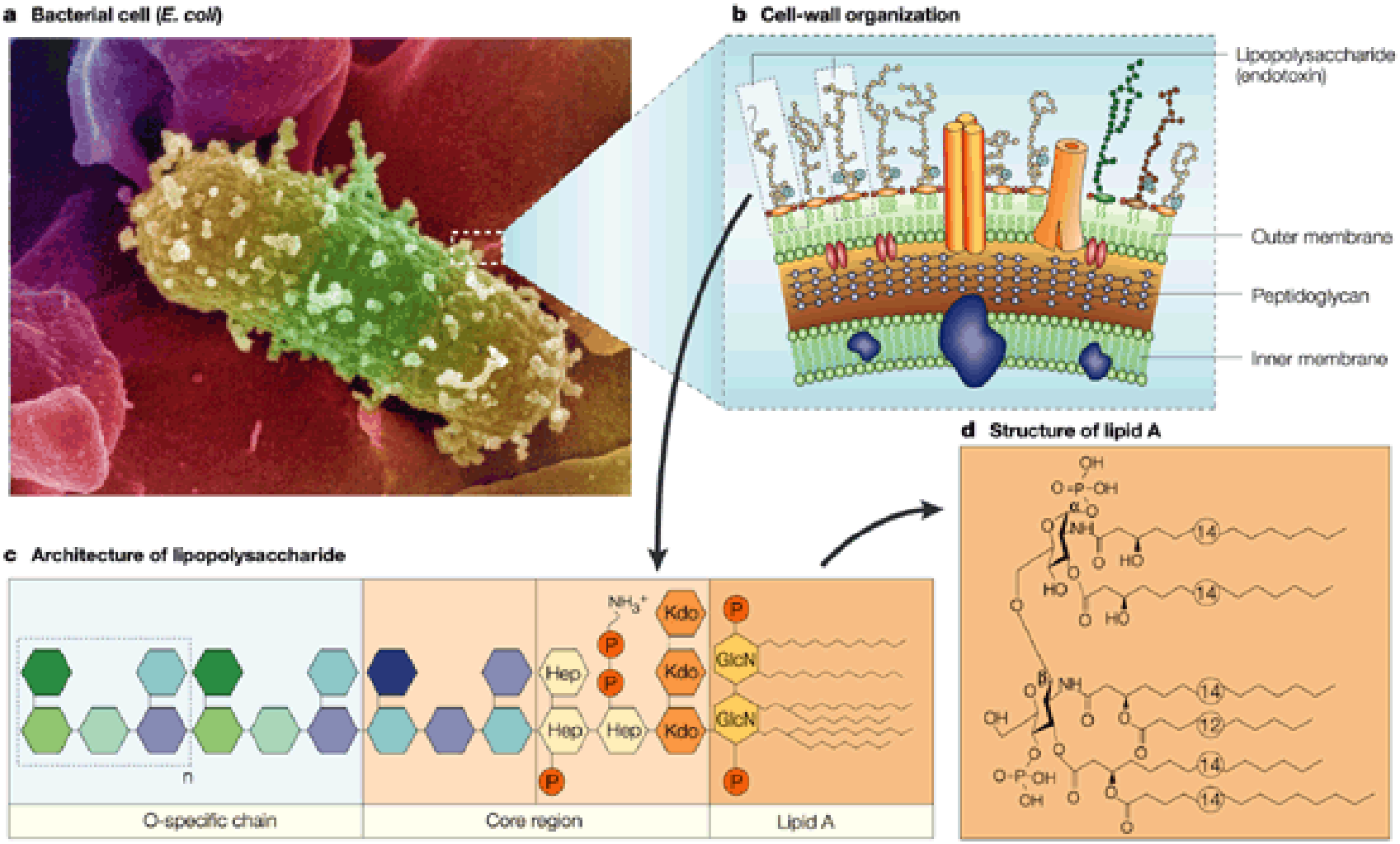


FIGURE 3-6. The structure of LPS. LPS consist of three portions. Lipid A is a phosphorylated disaccharide to which fatty acids are attached. The fatty acids vary with the organism but are always responsible for the hydrophobicity of the molecule. In a typical *Salmonella*, region I shows a characteristic series of sugars in the polysaccharide: the core polysaccharide, which consists of a variable and an invariant portion, and the O antigen, made up of repeating sugar subunits. Highly variable, the O antigen is the main reason for the different antigenic specificities among Gram-negative bacteria.

G-bacterial cell wall, LPS



Gram-Negative Solution

- Certain hydrophilic compounds that are sometimes necessary for survival are larger than the exclusion limit of porins. These larger molecules include vitamin B12, sugars larger than trisaccharides, and iron in the form of chelates. Each hydrophilic compound crosses the outer membrane by a unique permeation mechanism that uses proteins especially designed to translocate that compound.

- The dual-membrane system of Gram-negative bacteria creates a compartment called the **periplasmic space**, or **periplasm**, on the outside of the cytoplasmic or inner membrane.

- The periplasm contains so-called **binding proteins** that help soak up sugars and amino acids from the medium. It also contains enzymes that inactivate antibiotics such as penicillins and cephalosporins, the β-lactamases.

- LPS of the outer membrane is highly reactive in the host. The lipid A component has a large number of biological activities. It elicits fever and activates a series of immunological and biochemical events that lead to the mobilization of host defense mechanisms. In large doses, this compound, also known as **endotoxin**, can cause shock and even death.

Acid-Fast Solution

- A few bacterial types, notably the tubercle bacillus, have developed yet another way to confront environmental challenges to the cytoplasmic membrane. Their cell walls contain large amounts of **waxes.**

- The waxy coat is interlaced with murein, polysaccharides, and lipids. This covering enables the organisms not only to resist the action of many noxious chemicals but also to avoid being killed by white blood cells. The cost of this protection, however, is that these organisms grow very slowly.

CYTOPLASMIC MEMBRANE

- The cytoplasmic membrane of bacteria is a busy place. It assumes functions that in eukaryotic cells are divided up among the plasma membrane and intracellular organelles.

- Most critical is its role in the uptake of substrates from the medium. Bacteria take up mainly small-molecular-weight compounds and only rarely macromolecules and phosphate esters.

- The cytoplasmic membrane contains specific carrier proteins, called **permeases**, that facilitate the entry of most metabolites. In some cases, the carrier facilitates the equilibration of a compound inside and outside the cell.

- However, carrier-mediated transport usually requires the expenditure of energy.

Transport across the Cytoplasmic Membrane

- The three main versions of transport:

1. Facilitated diffusion: A substance, such as glycerol, is carried across the membrane down a concentration gradient. This mechanism does not concentrate compounds in the inside of the cells relative to the outside environment. Uptake is driven by intracellular use of the compound. The concentration of free glycerol inside cells is lowered by its phosphorylation. More glycerol is then taken up to equilibrate with the outside concentration.

2. Group translocation: Also known as phosphorylation-linked transport, this energy dependent mechanism is used to transport certain sugars. Substances transported in this manner are chemically altered in the process. The sugar binds to a specific carrier in the membrane. Subsequent enzymatic steps yield glucose-6-phosphate, which can then be further metabolized.

3. Active transport: Energy is used to drive the accumulation of substrate. A substrate- lactose— is concentrated unchanged inside the cell, which makes the transport of additional molecules energetically unfavorable. To drive the transport of lactose, the cells use energy stored in an electrochemical gradient of protons, the **proton motive force**. This gradient is generated by the extrusion of protons from the cell, resulting from the oxidation of metabolic intermediates. Lactose is accumulated intracellularly by coupling its **energetically unfavorable** transport with the **energetically favorable** reentry of protons into the relatively alkaline cytoplasm of the cell.

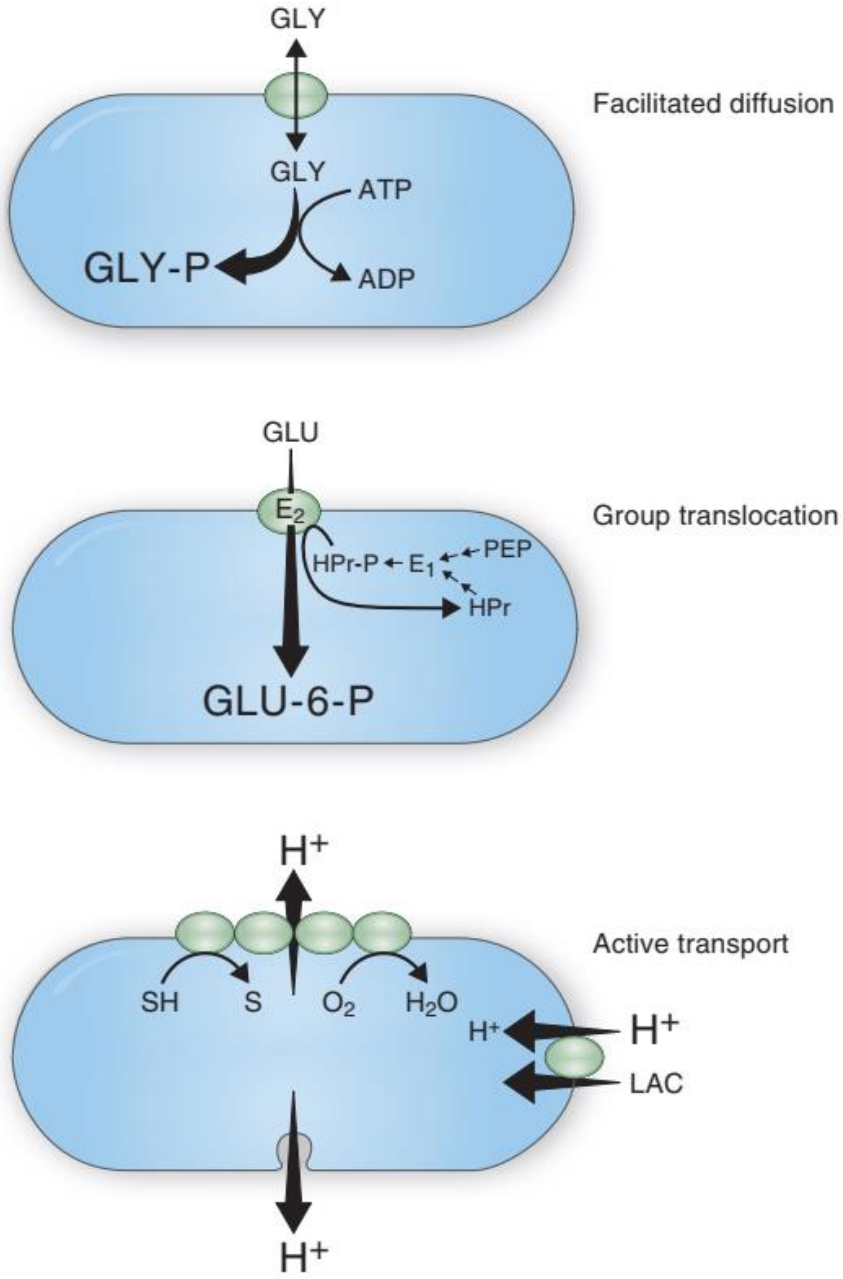
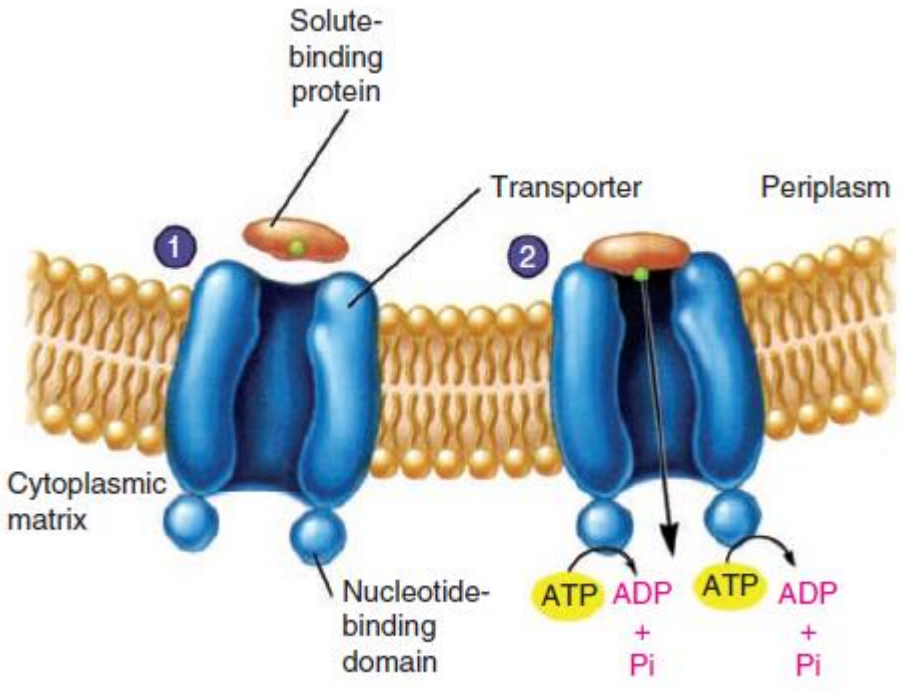
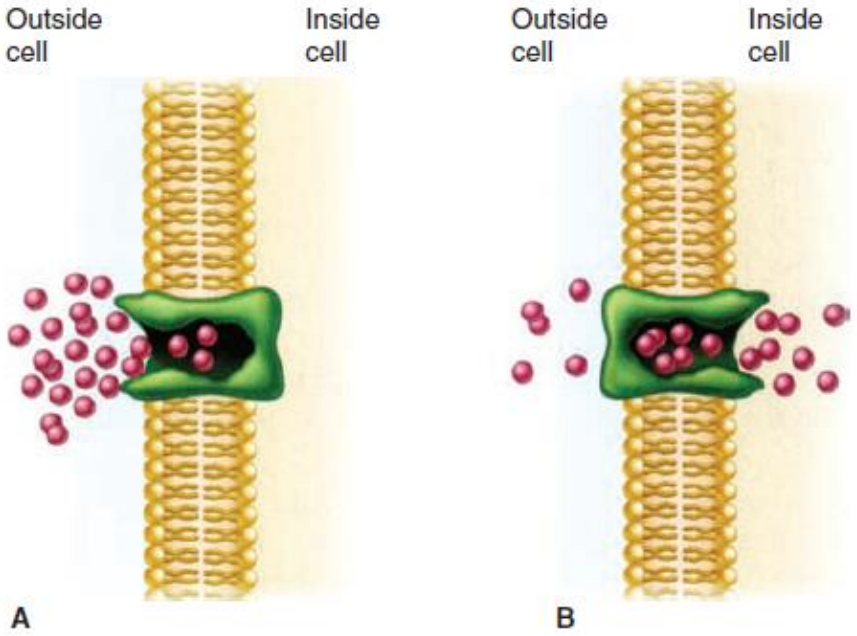


FIGURE 3-9. Mechanisms of transport. The three types of transport in *Escherichia coli* are facilitated diffusion, group translocation, and active transport with the *lac* permease.

Taking substances from the environment

- Free diffusion
- Facilitated diffusion
- Active transport
- Translocation





Transport across the Cytoplasmic Membrane

- Each type of transport system involves specific protein molecules. Some proteins aid the process by modifying or concentrating substrates in the periplasmic space of Gram-negatives. These **binding proteins** are specific for sugars, nucleotides, and so on. The periplasmic space also contains nucleotidases, nucleases, peptidases, proteases, and other hydrolytic enzymes. The actual transport process is carried out by membrane-bound carriers, or **permeases**, which are involved in all three types of transport.

Uptake of Iron

- Free iron is extremely scarce in the blood and many tissues because it is bound by proteins like transferrin or ceruloplasmin. It is essential for the growth of bacteria, and many species that inhabit the human body have developed ingenious mechanisms to obtain the amounts of this element they need for growth. They excrete chelating compounds, known as **siderophores**, that bind iron with great avidity.

Siderophores

The bacteria need iron for the growth bacteriia produces siderophores (iron-specific chelators) that capture Fe3 +; the iron-binding chelator is transported to the bacterial cell by specific active transport.

Other Functions of the Bacterial Membrane

- The cytoplasmic membrane of bacteria is also where **cytochromes** are located and **oxidative metabolism** is carried out. It thus performs the role of the mitochondria of eukaryotic cells.

- Some bacteria have also an exceptional ability to take up enormously long DNA molecules. The phenomenon was first demonstrated by genetic transformation of pneumococci and occurs among other bacteria. The mechanism of DNA uptake is less well understood, but like active transport, it depends on the proton motive force.

Core

1. Granular cytoplasm filled with ribosomes

   The basic structure of the ribosome is 50S plus 30S, total of 70S (reminiscent of eukaryotic cell ribosomes, but smaller, which allows the action of a large number of antimicrobial agents)

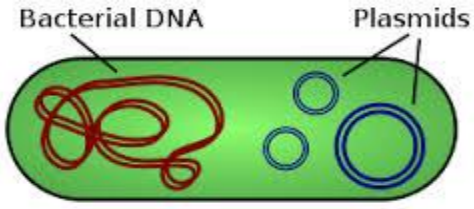
   Cytoskeleton (together with peptidoglycan gives shape to cells)

2. Nucleoid

   Double stranded helix DNA organized into a super thread, no nuclear envelope, one chromosome

3. Plasmide

   small, circular double-stranded DNA, isolated from chromosomes, replicates independently of chromosomes, encoding a number of factors that allow bacteria to cause disease



DNA AND CHROMOSOME MECHANICS

- The genome of many bacteria consists of a **single circular chromosome** of double-stranded DNA. However, some species have a linear chromosome, and others have more than one.

- Bacteria must solve a demanding topological problem in organizing their DNA because it is a long, thin molecule. If stretched out, DNA would be about 1,000 times the length of the cell.

- The DNA is coiled in a central irregular structure called the **nucleoid**.

- Roughly all that is known about the physical state of the bacterial DNA is that it is twisted into **supercoils**

DNA AND CHROMOSOME MECHANICS

- DNA replication has three stages: initiation, elongation, and termination. Replication takes place bidirectionally; that is, DNA synthesis starts at a precise place on the chromosome, the **replicative origin**, and proceeds away from it in both directions. The two moving polymerase complexes meet halfway around the chromosome.

- The timing of chromosome replication is a highly regulated process and is coupled to growth and cell division.

- In E. coli, DNA replication takes 40 minutes.

- In fast-growing cells (e.g., those dividing every 20 minutes), initiation of rounds of replication is adapted to produce new chromosomes as often as the cell divides. Since each chromosome requires 40 minutes to be synthesized, replication will initiate again on a strand long before its own replication has completed.

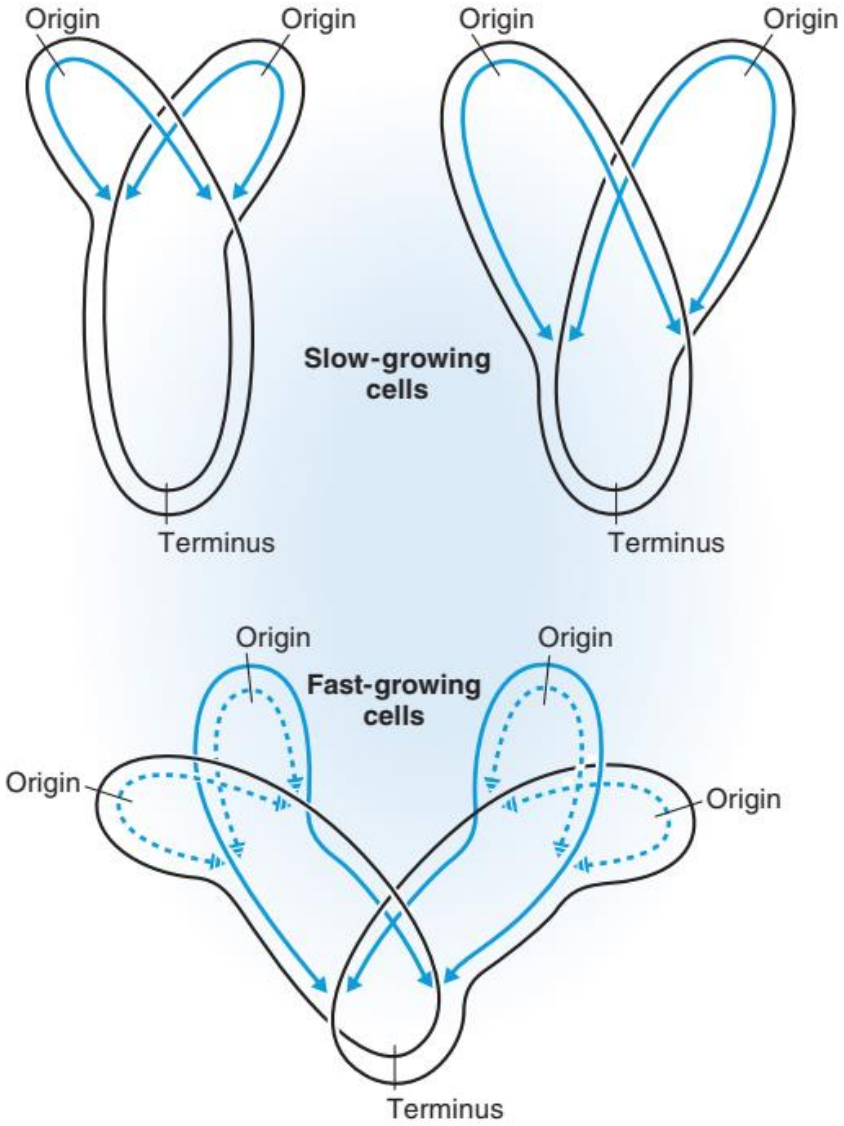
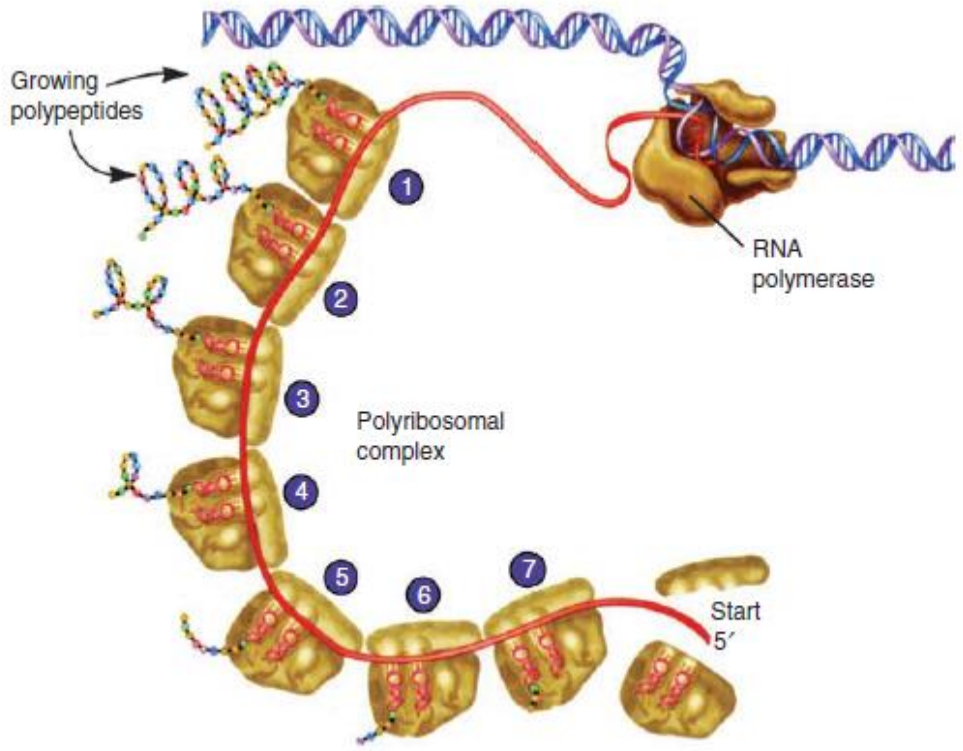


FIGURE 3-10. Replication of DNA in slow-growing and fast-growing *E. coli.* Replication begins at a specific site, the origin, and proceeds in both directions toward a terminus. The process takes about 40 minutes at 37°C. In a culture doubling every 20 minutes, the process must initiate every 20 minutes—that is, before the previous round of replication has terminated. In such cultures, the DNA is undergoing multifork replication.

Polymerization and association reactions

Transcription in bacteria differs from the process in eukaryotic cells in that all forms of bacterial RNA and mRNA, tRNA and rRNA are synthesized by the same **RNA polymerase enzyme** and that bacterial mRNA is synthesized, used and degraded very quickly within minutes at the same place.

Translation of mRNA performs simultaneously with transcription



GENE EXPRESSION: UNIQUENESS OF PROKARYOTIC RNA POLYMERASE AND RIBOSOMES

- The bacterial cytoplasm is composed largely of proteins and RNA.
- Bacterial ribosomes have smaller subunits and smaller RNA molecules than do their eukaryotic counterparts.
- The large requirement for proteins makes protein synthesis the principal biosynthetic activity of rapidly growing bacteria. A large proportion of a bacterium's energy and metabolic building blocks is devoted to the assembly of the protein-synthesizing machinery, including ribosomes and RNA polymerase.
- The rate of protein synthesis is proportional to the cellular concentration of ribosomes.

CAPSULES, FLAGELLA, AND PILI: HOW BACTERIA COPE IN VARIOUS ENVIRONMENTS

- The morphological variety of bacteria is not limited to walls and membranes. Some bacteria, but by no means all, have other exterior structures such as **capsules**, **flagella**, and **pili**. These components are dispensable; that is, they are important for survival under certain circumstances but not others.

- The **capsule** is a slimy outer coating made by certain bacteria. Under laboratory conditions the capsule is not needed, and the organisms may grow well without it. Capsules usually consist of high-molecular-weight polysaccharides that make the bacteria slippery and difficult for white blood cells to phagocytize. Pneumococci, meningococci, and other bacteria that are likely to encounter phagocytes during their infective cycle are indeed encapsulated.

Capsule

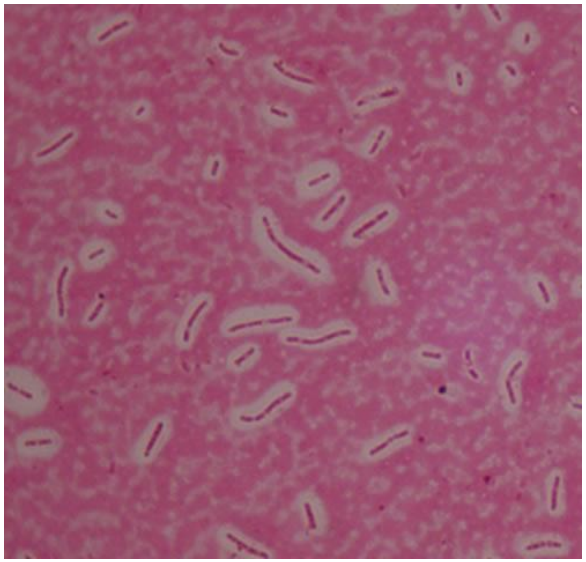
Bacterial hydrophilic envelope (optional part of the bacteria)

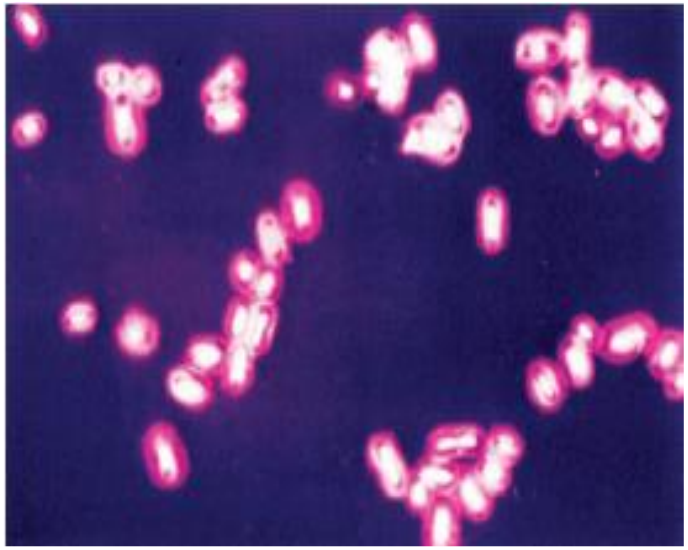
Structure: polysaccharides;

in only a few bacteria is a polypeptide

Function: protects the bacteria from the host immune system

It can be visualized with special coloring





CAPSULES, FLAGELLA, AND PILI: HOW BACTERIA COPE IN VARIOUS ENVIRONMENTS

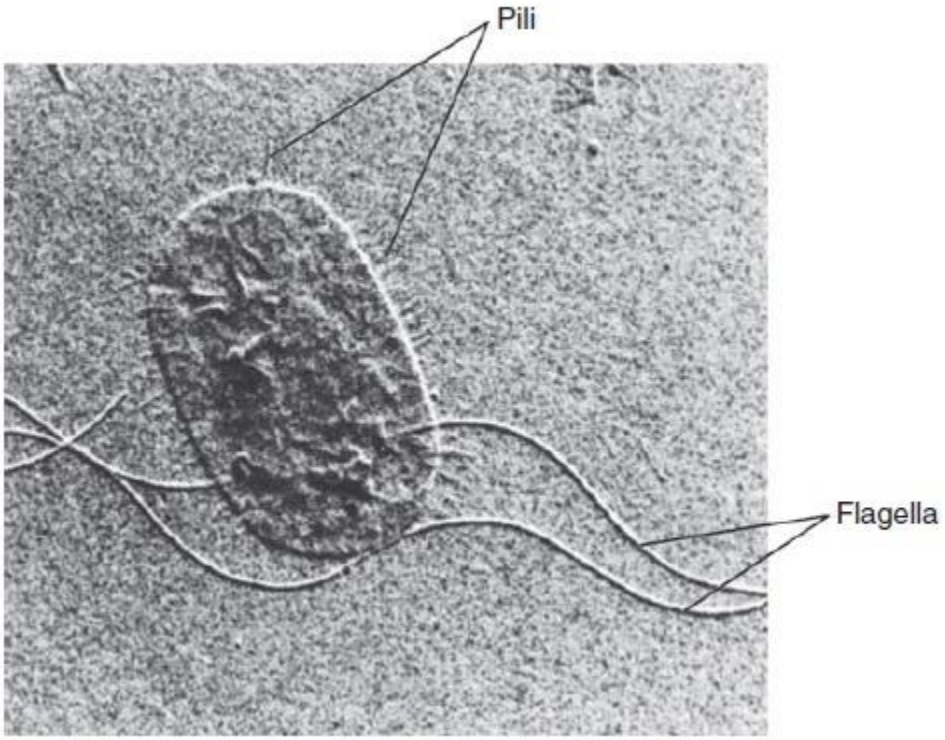
- Protruding through the surface layers of many bacteria are two kinds of filaments, **flagella** and **pili**.

- Flagella are long, helical filaments that endow bacteria with motility. Many successful pathogens are motile, which probably aids their spread in the environment and possibly in the body of the host. Depending on the species, a single bacterial cell may have one flagellum or many flagella. In some, the flagella are located at the ends of the cells (polar) and in others at random points around the periphery (peritrichous or "hairy all over"). This distinction is useful in taxonomy and in diagnostic microbiology.

- Pili (also called fimbriae) are involved in the attachment of bacteria to cells and other surfaces.

Flagellum, pili

Flagellum :
protein structures (flagellin fibers) that allow movement and classification based on flagellar antigen



Pili:
protein structures (pilin)

1. common(adhesion)
2. sex pili(conjugation)

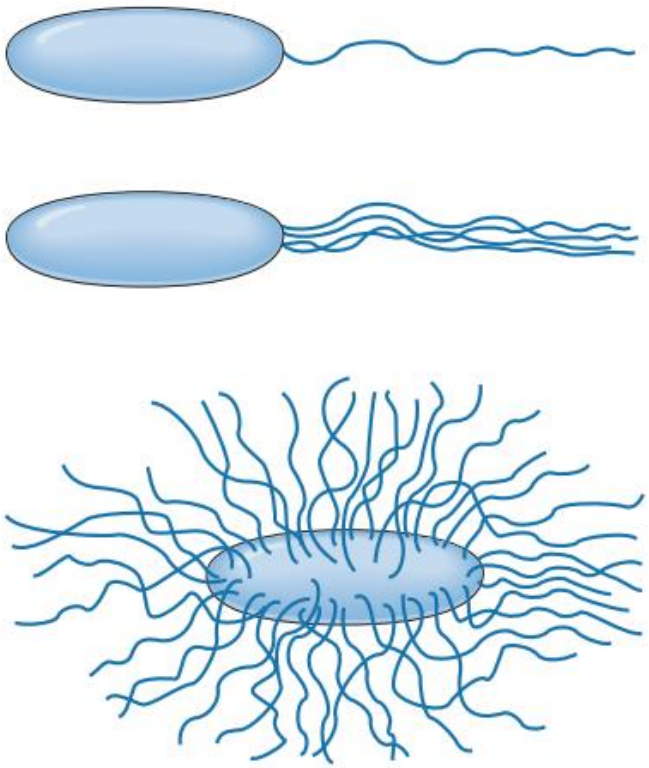


FIGURE 3-13. Arrangement of flagella in some types of bacteria.

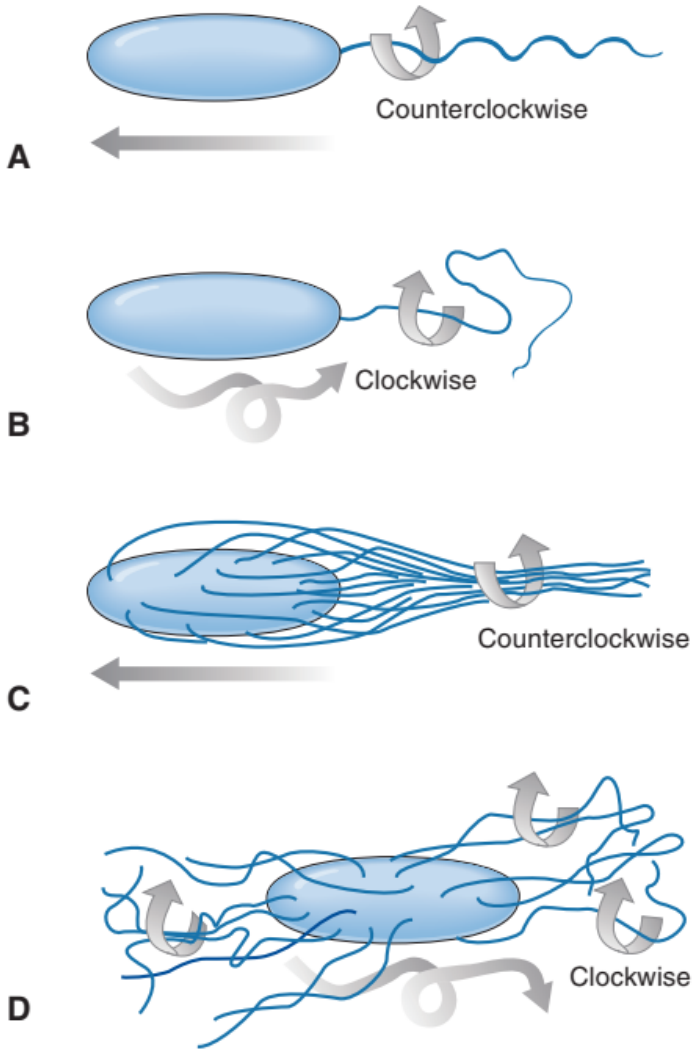


FIGURE 3-14. Flagellar arrangement and motility. A. A bacterium moving smoothly right to left when its single polar flagellum rotates counterclockwise; this is the same direction as the thread of the helix formed by the flagellin molecules in the flagellum. **B.** The same bacterium tumbles generally left to right when the flagellum rotates clockwise. **C.** With a peritrichous bacterium, counterclockwise rotation produces a coherent bundle of flagella and smooth movement. **D.** Tumbling produced by clockwise rotation is extreme.

Bacterial Chemotaxis

- The movement caused by flagella is used by bacteria for **chemotaxis**—that is, movement toward substances that attract and away from those that repel.

- Considerable research has shown that bacterial chemotaxis is based on the following sophisticated mechanism: Flagella spin around from their point of attachment at the cell surface. Each flagellum has a counterclockwise helical pitch, and when there are several on one bacterium, they array themselves into coherent bundles as long as they all rotate counterclockwise. When the flagella are arranged in these bundles, they beat together, and the bacteria swim in a straight line. However, when flagella rotate clockwise, they get in each other's way and cannot form bundles. As a result, the bacteria tumble in random fashion. The two types of motion, swimming and tumbling, account for bacterial chemotaxis.

Bacterial Adhesion and Pili

- Microbes are attracted to specific tissues. Such **tissue tropism** often involves the attachment of surface components of the organisms to specific receptors present on the cells of certain tissues. The bacterial structures most often involved in attachment are the **pili**, or fimbriae. These are protein filaments shorter than flagella and distributed, often in large numbers, over the surface of some bacteria. Bacteria that can conjugate have, in addition, specialized **sex pili**. These structures are rather different from the "common pili." They are much longer and link the donor (male) and recipient (female) cells during transfer of DNA by conjugation.

- Because of the importance of pili in adherence to the host, vaccine and drug discovery programs have been developed to block the function or production of pili as a way of controlling bacterial infection.

- Some organisms are able to change the amino acid composition of their pili and thereby put on a succession of disguises that enables them to outflank the immune system. This process is referred to as **antigenic variation**.

NUTRITION AND ENERGY METABOLISM

- Bacteria survive and grow in a large variety of habitats. Whatever the habitat, all bacteria must synthesize cellular constituents in a coordinated manner to grow. The required building blocks must either be provided at suitable levels in the medium or be synthesized in proper amounts by the organisms themselves.

- Based on their nutritional requirements, bacteria can be divided into two large groups:
- In one are the **photosynthetic** or **chemosynthetic** bacteria that subsist on carbon dioxide and minerals, using either light or chemical energy.
- The other includes all the **organisms that need preformed organic compounds**.
- All pathogenic microbes fall in the second group, but within it, they have many gradations of nutritional needs. Some, like E. coli, are satisfied with glucose and some inorganic material. Other pathogenic bacteria, like their human host, are unable to make one or more essential metabolites—vitamins, amino acids, purines, pyrimidines, and so on—which must be supplied as growth factors

NUTRITION AND ENERGY METABOLISM

- Bacteria also have a wide range of responses to oxygen.
- At the extremes are the **strict aerobes**, which must have oxygen to grow. An example is the tubercle bacillus, which thrives in the portions of the body that are well oxygenated, such as the lungs.
- At the other extreme are the **strict or obligate anaerobes**, bacteria that cannot grow in the presence of oxygen, such as the organisms that cause botulism and tetanus.
- The largest number of bacteria that are medically important can grow whether or not oxygen is present. They are called **facultative anaerobes** and include E. coli and other intestinal bacteria

NUTRITION AND ENERGY METABOLISM

- Strict aerobes perform **respiration** only. In a series of coupled oxidation–reductions, the final electron acceptor is molecular oxygen.

- Strict anaerobes usually carry out **fermentation**, where the final electron acceptor is an organic molecule. Examples of organic electron acceptors are pyruvate, which is reduced to lactate in the lactic acid fermentation, and acetyl coenzyme A, which is reduced to alcohol in ethanol fermentation.

- Facultative anaerobes are capable of either form of metabolism, depending on whether oxygen is present or absent. Thus, they will respire in the presence of oxygen and ferment in its absence.

- Per molecule of substrate oxidized, respiration yields more energy than fermentation. Therefore, fermentative organisms must turn over more substrate to obtain the same amount of energy.

GROWING AND RESTING STATES

- When bacteria find themselves in a suitable environment, they grow and eventually divide. The time it takes for a bacterium to become two is called the **generation time** or **doubling time**. For example, E. coli requires about 20 minutes to double

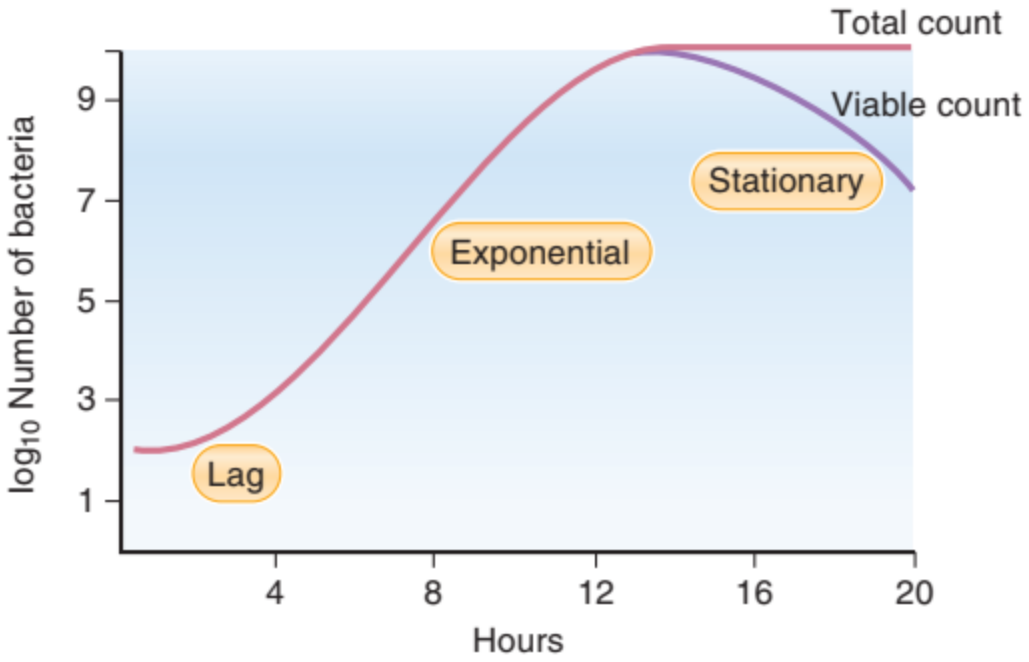


FIGURE 3-15. The growth of a bacterial culture. Bacteria in the inoculum sometimes resume growth slowly (lag phase, hours 0 to 5). They then enter the exponential phase of growth (hours 5 to 10). When foodstuff is exhausted or toxic material accumulates, they enter the stationary phase (hours 10 onward). During the stationary phase, bacterial cultures may lose their viability, as reflected in the viable count, often without losing cell integrity (maintaining a constant total count).

GROWING AND RESTING STATES

- When bacteria grow to a certain density, they either exhaust required nutrients or they accumulate toxic levels of metabolites.
- They may run out of the carbon source, a required inorganic compound, or essential amino acids or vitamins.
- For aerobic bacteria, crowding leads to the exhaustion of oxygen, which is poorly soluble in water. Toxic metabolites may be hydrogen peroxide, which is the case for some anaerobes that lack catalase, or acids formed by fermentation, which results in a pH too low to be compatible with growth.
- The stage of the culture where growth stops is known as the **stationary phase**.
- The explosiveness of exponential growth means that even a small number of bacteria can rapidly initiate an infection. An example of unhampered growth that leads to dangerous illness is acute bacterial meningitis in a child.

GROWING AND RESTING STATES

- In the tissues of the body bacteria are often stressed by nutritional limitations or by the damaging action of the defense mechanisms.

- To permit them to adapt to such conditions, bacteria do not cease all metabolic activities when they stop growing.
- Instead, although they cease net growth, they continue some synthetic activities that permit them to make specific constituents needed for adaptation.

sporulation

- Cessation of growth of some bacterial species initiates **sporulation**.
- This results in the production of metabolically inert **spores** extraordinarily resistant to chemical and physical insults.
- During sporulation, the “mother cell” eventually lyses. The cytoplasmic contents that are released sometimes contain large amounts of toxins.
- This happens in tetanus, gas gangrene, and other diseases caused by sporulating bacteria.

Bacterial structure

Spore

dehydrated metabolically inactive forms that allow bacteria to survive in poor conditions

Extremely resistant to high temperatures (calcium dipicolinate)

The resistance of the spores contributes to a special envelope consisting of:

1. a **spore membrane** equivalent to a cell membrane;
2. thick **cortex** consisting of special forms of peptidoglycans;
3. a **coating** containing an insoluble protein with plenty of cysteine and keratin;
4. outer shell of lipoproteins and carbohydrates called **exosporium**

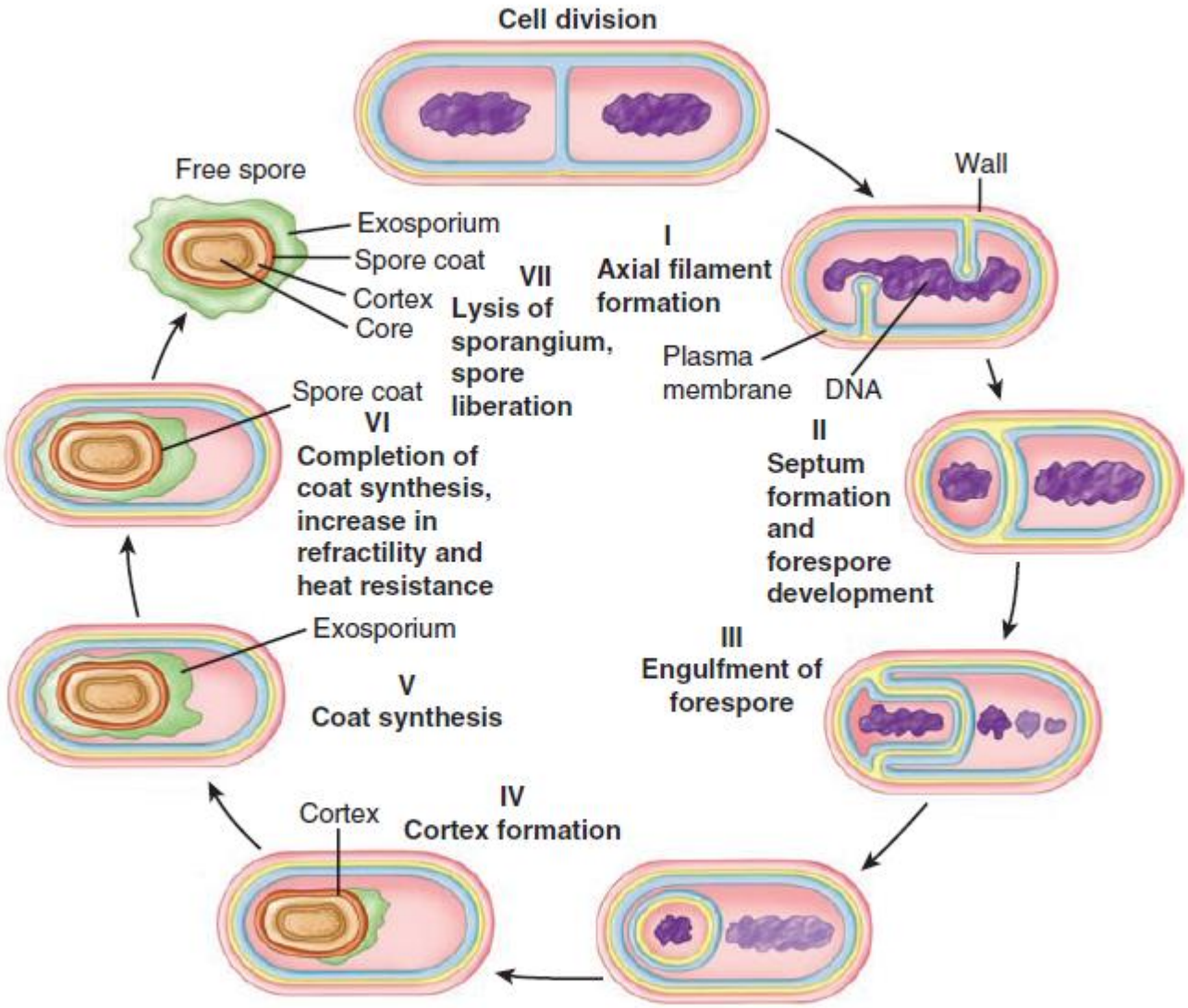
Spores are not reproductive structures

Bacterial structure

Spores

Sporulation: the process by which the vegetative form of a bacteria in poor environmental conditions turns into a spores.

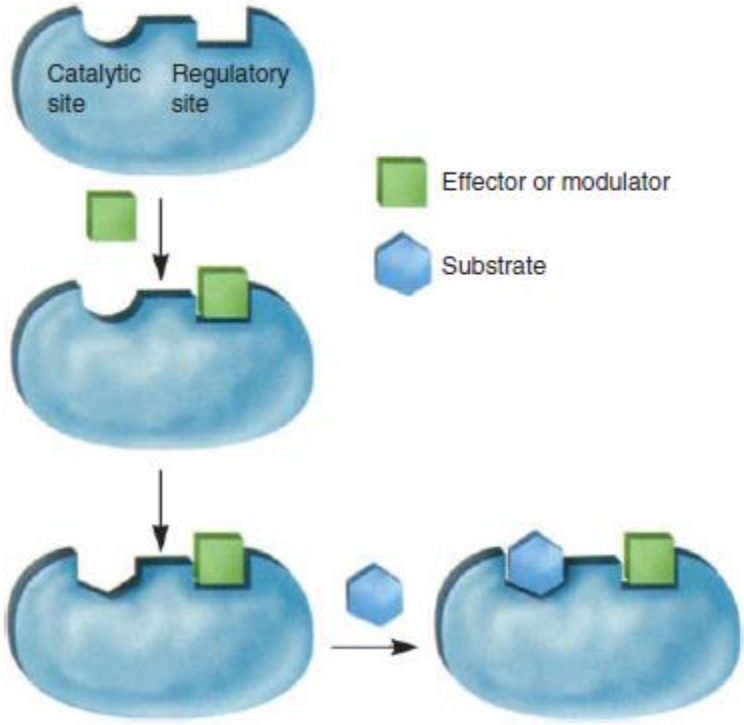
Germination: the process by which a spore in favorable conditions turns into a vegetative form.



Bacterial adaptation

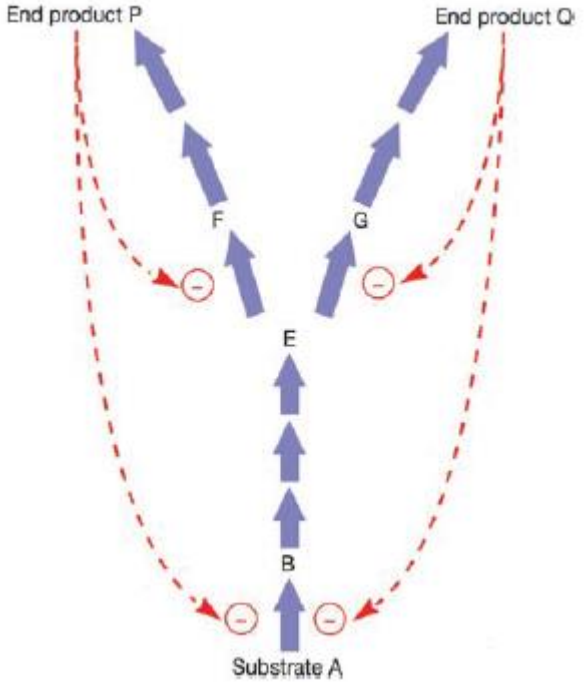
- **Control of enzyme activity**

allosteric regulation



AMP, ADP, ATP induce the conformational changes of allosteric enzymes and control their activity

the end product regulation



The end product affects the first enzyme in the pathway and controls the speed of the biochemical pathway

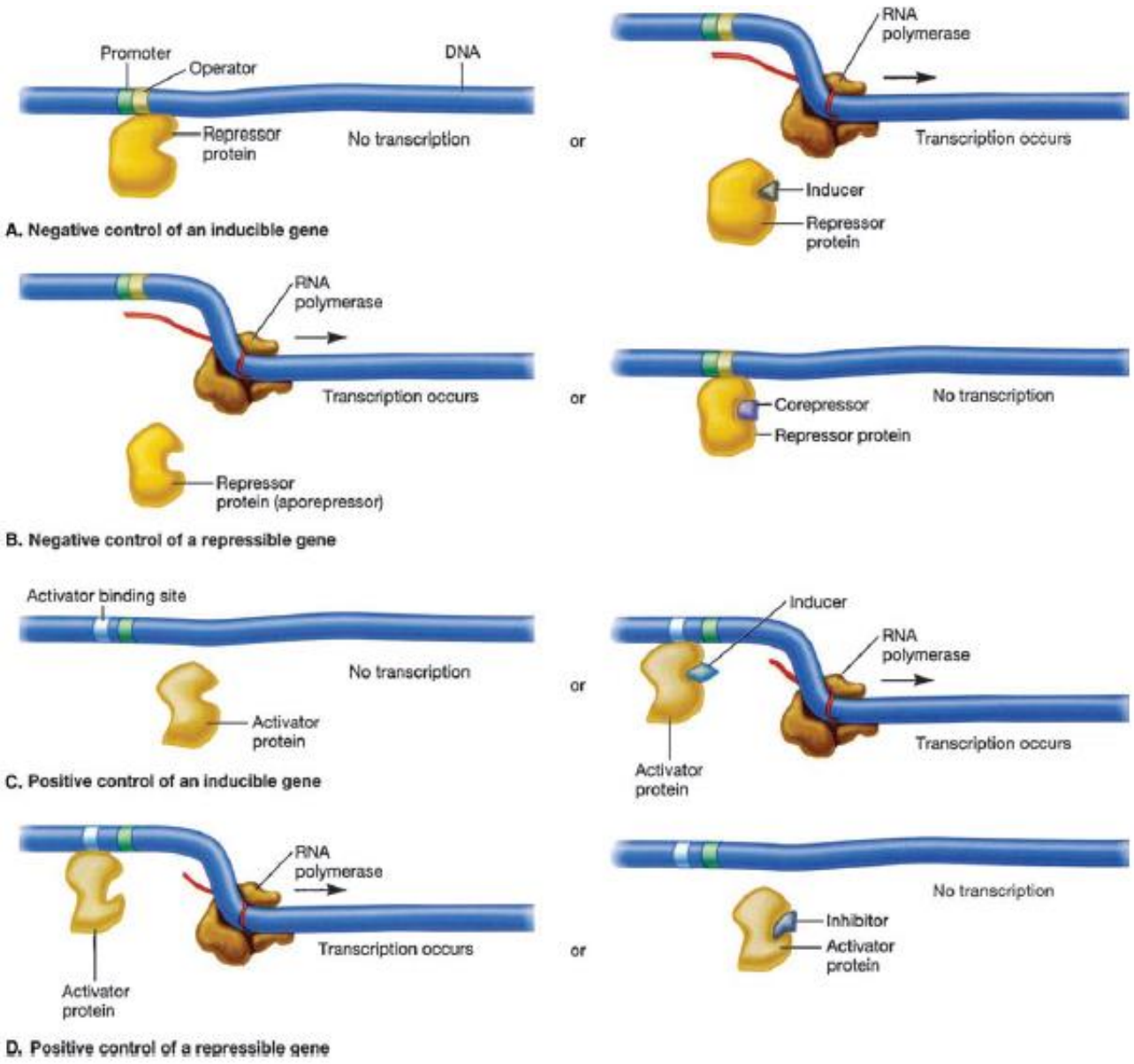
Bacterial adaptation

▪Control of gene expression

The rate of protein (enzyme) synthesis is regulated by gene transcription (by controlling the start of transcription) because mRNA is degraded very quickly so that the synthesis of a particular enzyme is rapidly ended but also rapidly activated.

Most genes in bacteria are organized as multicistron operons. The DNA segment encoding a particular polypeptide is a **cistron**.
Operon is a unit of transcription, ie. **cistron** and the corresponding single-stranded mRNA.
The structure of a typical operon includes: regions of **promoter, operator, cistron** and **terminator.**
Repressors / activators bind to the region of the operator and thus regulate transcription.
Groups of genes controlled by independent operons must work together for a cell to respond to a change in the environment called **regulon**.

Control of gene expression



Bacterial survival

Mechanisms of bacterial survival

- Cellular stress regulators
- Endospore
- Stationary growth phase
- Mobility and chemotaxis

Bacterial survival

Mechanisms of bacterial survival

- **Cellular stress regulators**

❖in response to nutritional stress, for example if there is not enough glucose, the cell can redirect the expression of its genes to alternative sources of carbon present in the environment

❖if a cell survives DNA damage, it activates the genes involved in DNA repair. During this process, cell division is blocked

Bacterial survival

Mechanisms of bacterial survival

- **Endospores**

❖ Bacteria cell can survive for a long period of time without growing in the form of spores; in several types of G+ bacteria, **sporulation** takes place, by activating several regulons whose coordinated activity creates **endospores**.

Bacterial survival

Mechanisms of bacterial survival

- **Stationary growth phase**

❖All bacteria adapt to external conditions by transitioning to a stationary growth phase.Bacterial cells in the stationary phase of growth differ morphologically from endospores, they are resistant and metabolically inactive, have a firmer shell and tightly packed chromosome.

❖This condition is important in diseases such as tuberculosis, which have a long latency period after infection.

Bacterial survival

Mechanisms of bacterial survival

- **Mobility and chemotaxis**

❖Chemotaxis is a directed movement towards the chemical attractant as far as possible from the chemical repellents, and is enabled by a molecular sensory system that has memory and adaptation.

❖Chemotaxis also has a role in survival because it enables the avoidance of toxic substances, but also in the promotion of growth.

❖It can also be a virulence factor because it facilitates the colonization of the host.

Bacterial genetics

For acquiring the diversity and specificity of the bacterias use:
- mutation and recombination processes as well as eukaryotic cells
  -very powerful mechanisms for exchanging genetic material with each other

TRANSPOSITION

transfer of transposable elements (**insertion sequences and transposons**) from **one chromosome to another**, from **one place to another within the same chromosome**, or **between chromosomes and plasmids**

Insertion sequences: genes whose products are involved in transposition and in the regulation of their own frequency, cause of mutations.

Transposons: transposable segments of DNA that also contain additional genes necessary for transposition; contain genes that can encode molecules that give certain characteristics to bacteria (Antimicrobial resistance, metabolism)

Bacterial genetics

For acquiring the diversity and specificity of the bacterias use:
- mutation and recombination processes as well as eukaryotic cells
  -very powerful mechanisms for exchanging genetic material with each other

GENETIC MATERIAL EXCHANGE

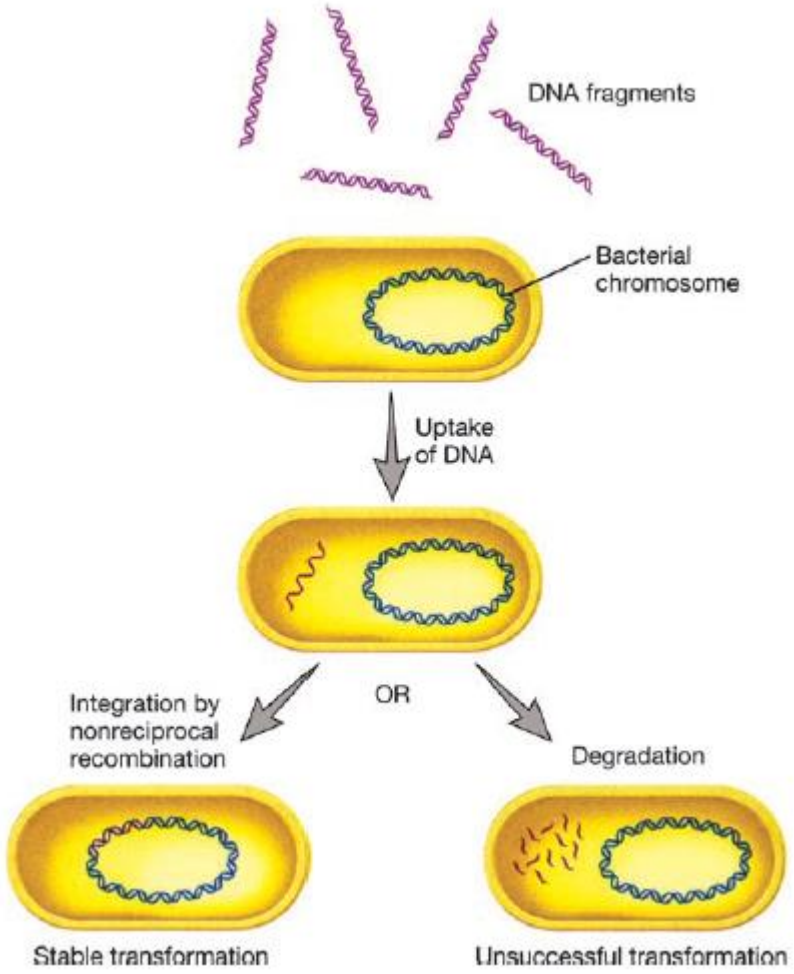
- Transformation (free DNA)
- Transduction (bacteriophage)
- Conjugation (direct contact of two cells)

Bacterial genetics

Acquiring the diversity, specificity of the bacterial cell

Transformation

Competence
DNA degradation / recombination

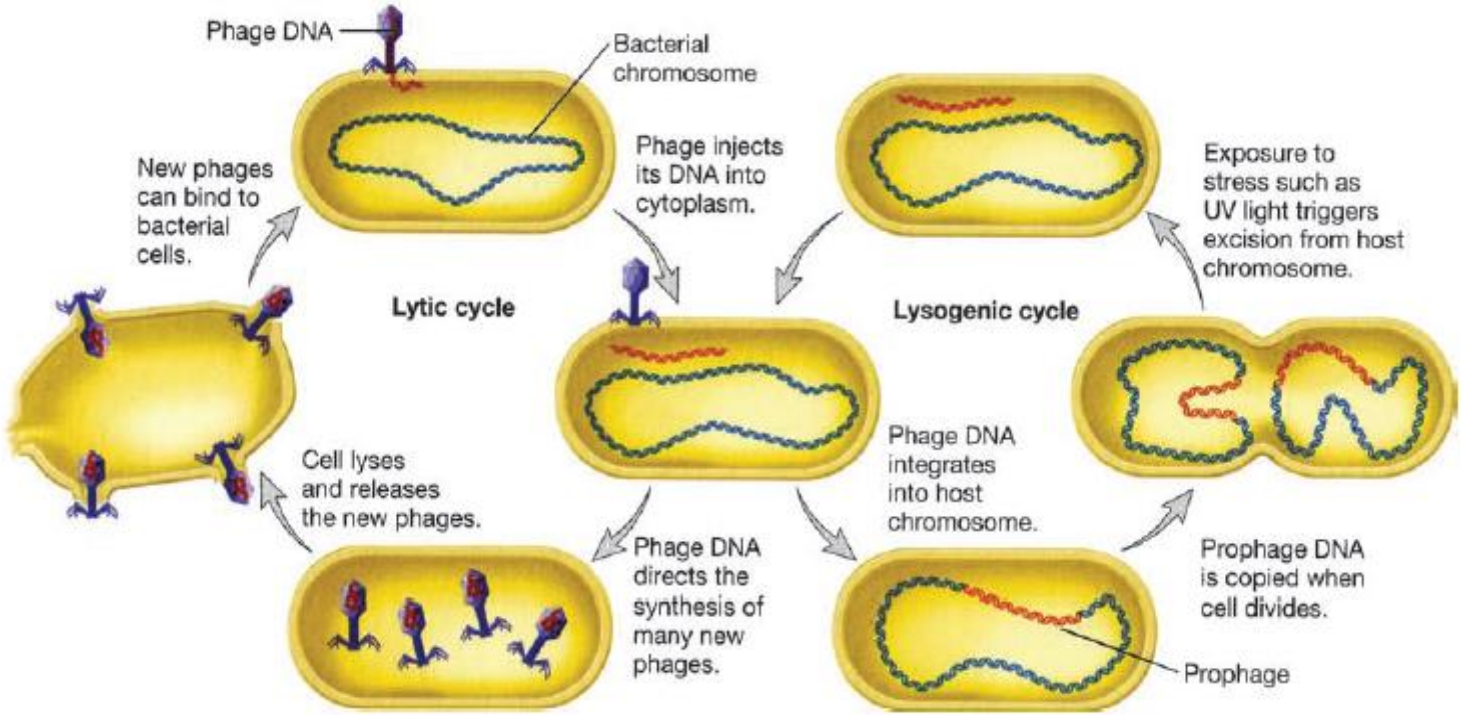


https://www.youtube.com/watch?v=MRBdbKFisgI

Bacterial genetics

Acquiring the diversity, specificity of the bacterial cell

Transduction



Temperate phage / prophage; lysogeny
Generalized / specialized transduction

https://www.youtube.com/watch?v=3DP-MAhr0YY https://www.youtube.com/watch?v=hFwA0aBX5bE

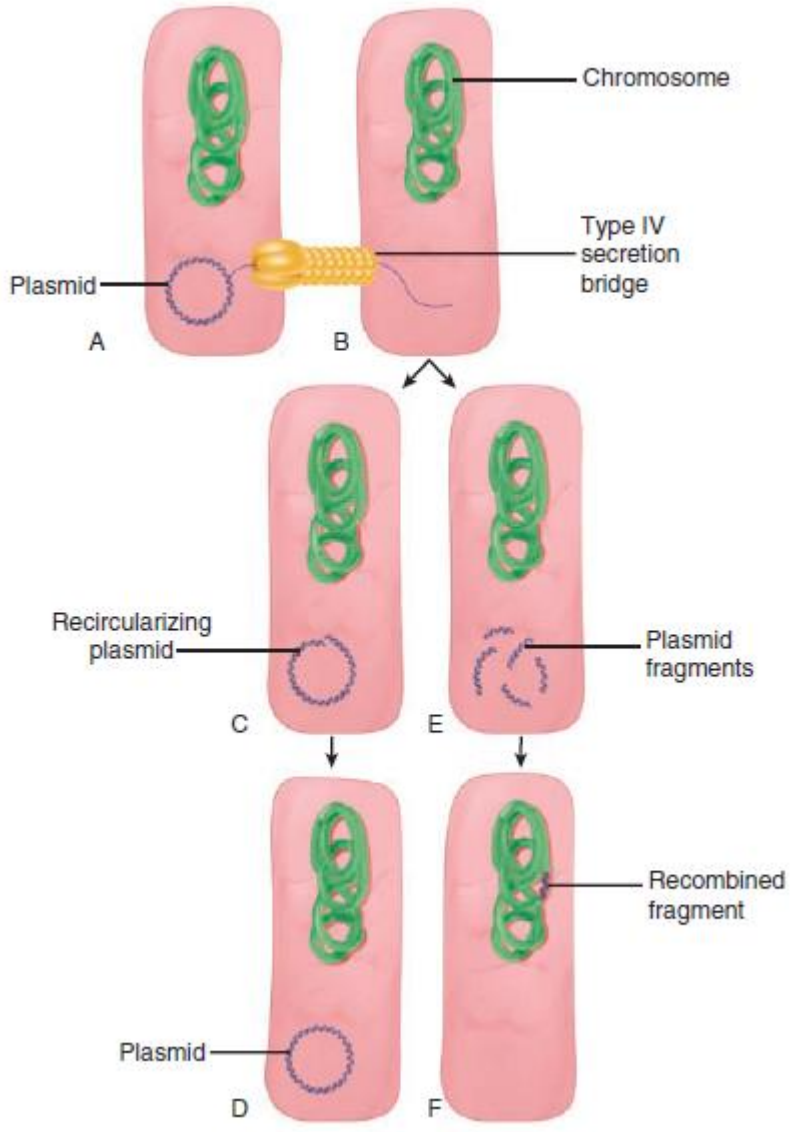
Bacterial genetics

Acquiring the diversity, specificity of the bacterial cell

Conjugation

- Conjugative plasmids
- Formation of conjugative bridges
- Separation of plasmid DNA, 1 strand goes to a new cell
- Synthesis complementary chain
- Circular plasmid or integration in the chromosome

https://www.youtube.com/watch?v=Gfm2LNqTD



Establishment of Infectious Diseases

- Most of us first encounter microorganisms at birth. Microbiologically speaking, we lead a sterile existence while in our mother's womb.
- The placenta is a formidable barrier to the transmission of microorganisms to the fetus. Such transmission is possible, however, and some diseases are transmitted to the fetus through the placenta. Examples of these so-called congenital infections are rubella and syphilis or those caused by HIV or cytomegalovirus (CMV).

- The first encounter with environmental microorganisms usually takes place at birth. During parturition, the newborn comes in contact with microorganisms present in the mother's vaginal canal and on her skin.

- The mother, however, does not send the newborn into the world totally unprotected. Through her circulation, she endows the fetus with a vast repertoire of specific antibodies. Some immunological protection is also provided by the mother's milk (colostrum), which also contains maternal antibodies. However, these acquired defenses soon wane and the child must cope on its own.

Exogenously Acquired Diseases

- Exogenously acquired diseases result from encounters with agents in the environment.
- Thus, we “catch” a cold from others, or we get typhoid fever from eating or drinking contaminated food or water.
- There are various ways in which disease-causing agents can be acquired from the environment: food, water, air, objects, insect bites, or humans or animals with whom we share our environment.
- Many agents are readily transmitted among humans through the exchange of bodily fluids—for instance, by sneezing, touching, or sexual intercourse. The way we encounter a disease agent often suggests a mode of prevention.
- Infection by an agent spread through the fecal– oral route can be dramatically reduced by ensuring that wastewater and drinking water are maintained separately. Prevention has been successful for many serious epidemics

TABLE 1-1 Examples of Encounters and Disease Prevention

Type of Contact	Example	Type of Agent	Source	Strategy for Prevention	Preventive Aim
Inhalation	Common cold	Virus	Aerosol from infected persons	None	—
	Coccidioidomycosis	Fungus	Soil	None	—
Ingestion	Typhoid fever	Bacterium	Water, food	Sanitation	Lower infecting dose
Sexual contact	Gonorrhea	Bacterium	Person	Social behavior	Avoid contact
Wound	Surgical infections	Bacteria	Normal flora surroundings	Aseptic techniques	Avoid contact
Insect bite	Malaria	Protozoan	Mosquito	Insect control	Eliminate vector

Endogenously Acquired Diseases

- Endogenously acquired diseases are caused by agents present in or on the body.
- Members of the normal collection of microbes that are normally found on our skin or mucous membranes (our microbiota) may cause disease, usually when they penetrate into deeper tissues.
- Thus, a cut can lead to the production of pus caused by the staphylococci that inhabit healthy skin.
- A distinction must be made between colonization and infectious disease.
- Colonization simply denotes the presence of microorganisms in a site of the body that may or may not lead to tissue damage and signs and symptoms of disease.

Normal Microbiota

- Some people harbor certain strains of virulent streptococci in their throat for a considerable period but only rarely come down with strep throat.
- Are these streptococci members of the normal microbiota?
- The answer is yes if normal microbiota refers to organisms in or on the body that are not in the process of causing disease.
- The answer is no if this kind of streptococcus is considered not found in the throats of approximately 95% of all healthy people.

ENTRY

- Most of the tissues we normally think of as being inside the body are topologically connected with the outside the surface of the lumen of the intestine, the alveoli of the lung, the bile canaliculi, and the tubules of the kidney are in direct contact with the exterior environment.

- In fact, this is true of almost all the organs contained within the thorax and abdomen.

- The term entry then can be used in two senses: it means either the ingress of microorganisms into body cavities that are contiguous with the outside environment or the penetration of microorganisms into deeper tissue after crossing an epithelial barrier.

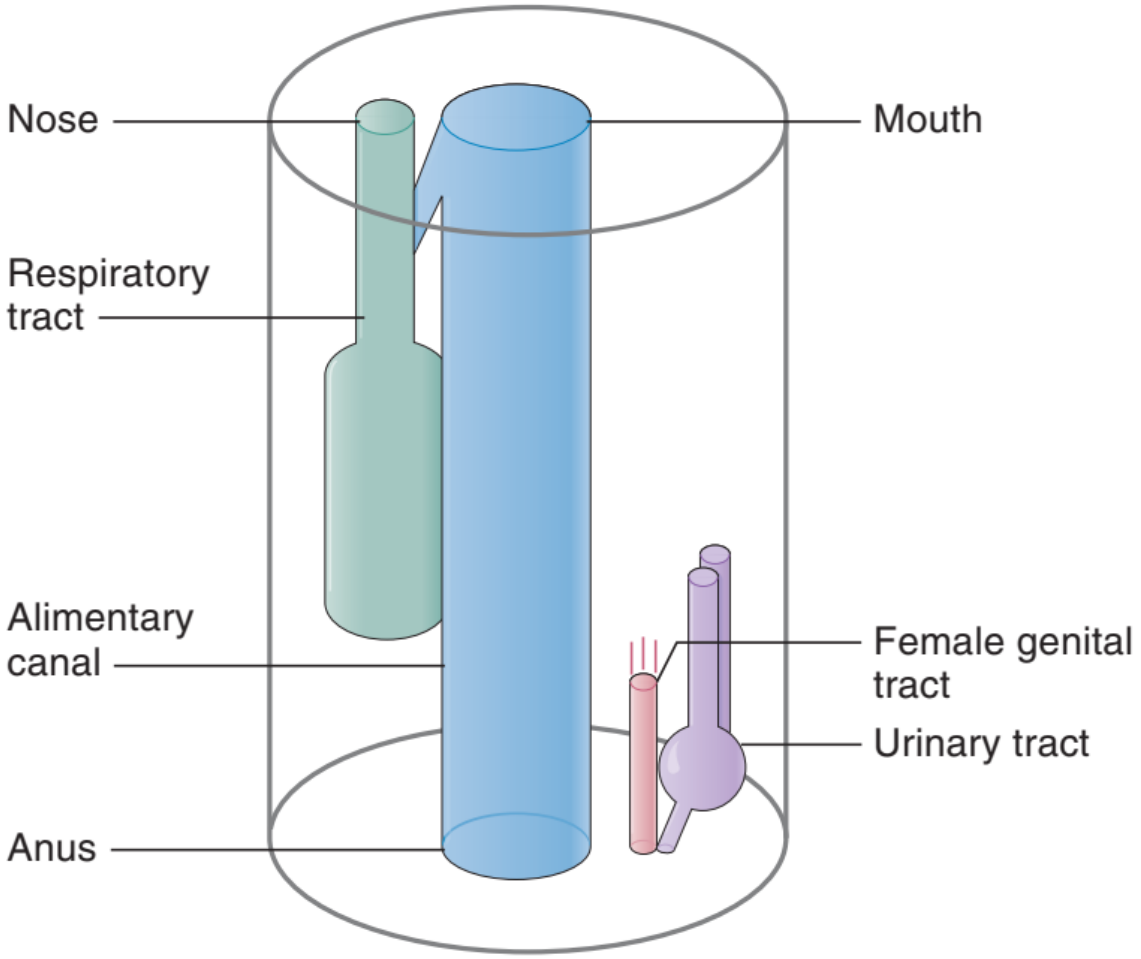


FIGURE 1-1. A schematic diagram showing the regions of the body in direct contact with the exterior. These include the outer aspects of the digestive, respiratory, and urogenital systems. These systems account for most of the organs of the thorax and abdomen. The main systems that do not have such direct connections are the musculoskeletal, nervous, circulatory, and endocrine systems. In women, the genital tract is connected to the peritoneal cavity via the fallopian tubes.

Ingress: Entry without Crossing Epithelial Barriers

- Microorganisms enter the intestine by being swallowed and enter the lung by being inhaled.
- External microorganisms can also enter the urinary tract or the genital system.
- To cause disease, microorganisms do not have to enter deep into tissues but can stay only on mucosal surfaces.
- Examples of serious infectious diseases that occur without bacterial penetration through epithelial surfaces are cholera and whooping cough

Inhalation

- To enter the respiratory system, microorganisms face a series of aerodynamic and hydrodynamic obstacles.
- Microorganisms are inhaled in aerosol droplets or dust particles contained in the air we breathe.
- They take a circuitous path through the respiratory tract because they must navigate through complex anatomic structures such as nasal turbinates, the oropharynx, and the larynx.
- Microorganisms that arrive in the lower reaches of the respiratory tree face the powerful upward-sweeping action of the ciliary epithelium.
- Colonization of these sites requires that the microorganisms be able to stick to the epithelial surface.

Ingestion

- When contaminated food or water is ingested, microorganisms face a powerful host defense, the acid in the stomach.
- The stomach is a chemical disinfection chamber where many microorganisms are destroyed. Its effectiveness in killing microbes, however, is determined by the length of time the microorganisms spend in the stomach, which in turn depends on the kind and amount of the food eaten.
- Even after great destruction, some bacteria and yeasts escape the stomach alive, although their original number may have been reduced a millionfold or more.
- The way that bacteria are ingested can influence their level of acid sensitivity because certain foods can protect the microbes from acid killing.
- Some species have inherently greater resistance to acid and are therefore infectious at lower doses.

Ingestion

- Bacteria, fungi, parasites, and viruses that escape the acid barrier of the stomach enter the duodenum.

- There they meet the enzymes of the pancreatic juice, the bile salts, and the strong sweeping force of peristalsis.
- Very few microbes can colonize the duodenum or anywhere else in the upper reaches of the small intestine.
- Toward the ileum, the environment is more favorable to bacterial life, but even there, the few organisms that gain a foothold must avoid being washed away.
- Bacteria found in this region have special mechanisms that allow them to adhere to the epithelial cells of the intestinal mucosa.
- Several surface components of these bacteria serve as adhesins.

- Bacteria at this site may cause disease without penetrating the mucosal epithelium. Cholera and its milder relative traveler's diarrhea are the manifestations of the local production of powerful toxins in the intestine that affect the epithelial cells.

Penetration: Entry into Tissues after Crossing Epithelial Barriers

- Some microorganisms pass directly through epithelia, especially mucous membranes that consist of a single cell layer.

- To penetrate the skin, which is tough and multilayered, most infecting agents must be carried across by insect bites or await breaks in the skin surface.
- Certain worms can burrow unaided through the skin and invade the host.
- To penetrate mucosal epithelial cells, many agents first interact with specific receptors on the surface of the host cell.

- Microorganisms can also be actively carried into tissues by white cells or macrophages that lie outside the body.
- Macrophages that reside in the alveoli of the lungs—alveolar macrophages, also known as dust cells—can pick up inhaled infectious agents by phagocytosis. Most of the time, microorganism-containing macrophages are carried upward on the ciliary epithelium, but occasionally, infected macrophages can reenter the body and carry their load of microorganisms into deeper locations.

Insect Bites

- Insect bites can lead to the penetration of viruses (viral encephalitis, yellow fever), bacteria (plague, typhus), protozoa (malaria, sleeping sickness), or worms (river blindness, elephantiasis).

- Insects also spread diseases by carrying microorganisms on their surfaces and by contaminating skin or food.

Cuts and Wounds

- Penetration from cuts and wounds is a common occurrence that often goes unnoticed because it does not usually lead to symptoms of disease.

Organ Transplants and Blood Transfusions

- Yet another way for organisms to penetrate into deeper tissue is through organ transplants or blood transfusions.

- Kidney transplants sometimes result in infections by CMV, perhaps because of virus residing in the transplanted kidney.

Inoculum Size

- Whether organisms from the microbiota of the skin or mucous membranes will cause disease depends on many factors.
- Among them is the size of the inoculum, the number of invading organisms.
- An encounter with a small number of organisms is unlikely to result in an infection.
- Medical professionals are well aware of the importance of inoculum size in infection. Before making an incision in the skin, a surgeon prepares the area to reduce the number of bacteria that could invade a surgical wound.

SPREAD

- The term spread has two shades of meaning.
- It suggests direct, lateral propagation of organisms from the original site of entry to contiguous tissues, but it can also refer to dissemination to distant sites.
- Microorganisms spread and multiply only if they overcome host defenses.
- Spread can precede or follow microbial multiplication in the body.

Active Participation by Microbes

- Infectious agents are not always passive participants in the process of spread; some contribute to it by actively moving.

- Streptococci manufacture a variety of extracellular hydrolases that allow them to break out of the walled defenses erected by the inflammatory response.
- These organisms make a protease that breaks up fibrin, a hyaluronidase that hydrolyzes the hyaluronic acid of connective tissue and may allow the organism to spread, and a deoxyribonuclease that reduces the viscosity of pus caused by release of DNA from lysed white cells.

- Some bacteria make elastases, collagenases, or other powerful proteases.

MULTIPLICATION

- Rarely do infectious agents cause disease without first multiplying within the body.
- The number of microorganisms we inhale or ingest or the number that survives initial host barriers is usually too small to produce symptoms directly.
- Infectious agents must reproduce before their presence is noted by symptoms.
- Exceptions to the rule are agents that cause disease through production of a toxin, such as Clostridium botulinum, which produces the botulinum toxin that leads to botulism. This condition is an intoxication, not an infection.
- Incubation period reflects the time needed for the infectious agents to overcome early defenses and grow to a certain population size.

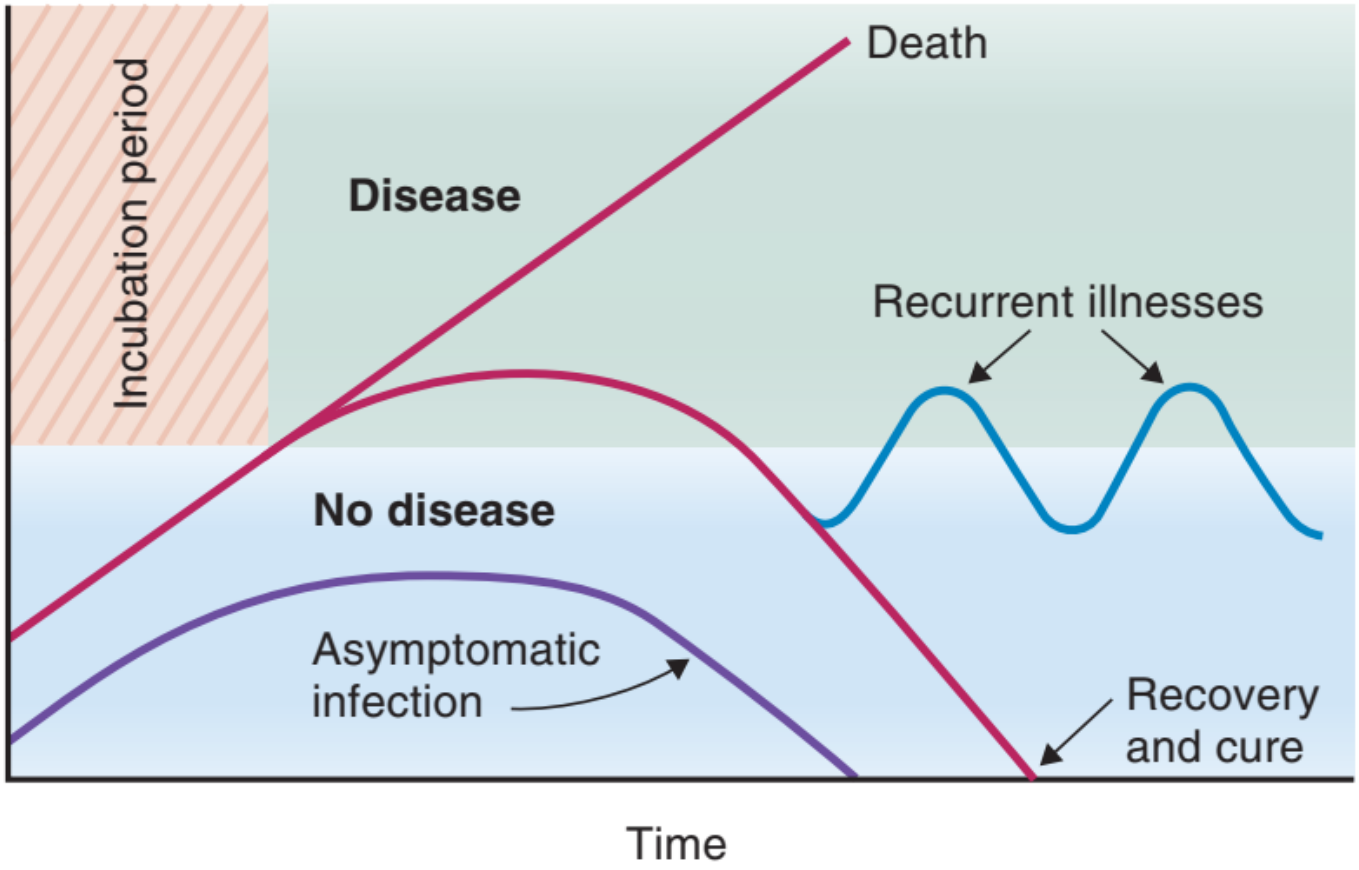


FIGURE 1-2. Microbial multiplication and clinical manifestations of disease. The number of microorganisms present in a patient must exceed a given threshold to cause disease. If the number is below that threshold, no signs and symptoms of disease will be apparent. In some cases, the numbers oscillate above and below the threshold, resulting in recurrent bouts of disease. Note that this drawing is idealized; in reality, the threshold of overt disease is not fixed but varies with the physiological state of the host.

Environmental Factors That Influence Multiplication

- The physical environment of the body selects for microbes that grow within certain ranges of temperature, osmotic pressure, and pH.

- Fever may be a defense mechanism that helps restrict the disease.

Subversion of Host Defenses

- Microbial countermeasures do not contribute directly to tissue damage, yet they can be thought of as virulence factors because they are essential for the microbe to be pathogenic.
- Each species of infectious agents develops a unique spectrum of survival strategies.
- The strategies that microbes use to subvert host defence are: complement and phagocytosis, and humoral and cellular immunity.
- One type of microbial defensive strategy is protective covering.
- Some microbes produce extracellular capsules that block recognition and binding by both complement and antibodies and prevent phagocytosis.
- This is the strategy of such important mucosal pathogens as the pneumococcus or the meningococcus.
- These capsules are themselves immunogenic and antigenic and thus have become the basis of vaccines used to prevent pneumonia and meningitis.
- Another way that bacteria avoid immune recognition is by altering their surface antigens in a genetically programmed way. Certain pathogens, such as the gonococci or the salmonellae, can modify surface structures that are recognized by the immune system.

Subversion of Host Defenses

- Many microbes survive in the body because they do not cause too much damage to the host. The best adapted of them cause no disease at all, elicit no inflammatory response, and actually are a help to the host by providing nutrients in the gastrointestinal tract (e.g., vitamin B12) or crowding out potential pathogens (on the skin and mucosal surfaces).

- They may even stimulate the immune system to raise useful cross-protective antibodies (e.g., protection against meningococci may be the result of the colonization by related but nonpathogenic commensal species).

- Among the most successful pathogens are those that cause sexually transmitted diseases. In some cases, these bacteria cause minimal disease (e.g., chlamydial infection, gonorrhea), whereas in other cases, disease comes on slowly or as part of a chronic process (e.g., syphilis).

- When nonpathogenic strains cause trouble, it is a result of their getting into places they do not belong

Subversion of Host Defenses

- The occasional highly pathogenic invaders with their well-developed set of toxins and other virulence mechanisms cause significant acute damage and disease but may end up killing the host.

DAMAGE

- The type and intensity of the damage depend on the tissues and organs affected.
- Damage is not always caused by activities of the invading agent alone but is often the consequence of a vehement host response.

Mechanisms of Direct Damage

- Some bacteria produce extracellular factors, broadly called toxins, that are directly responsible for tissue damage. Some of these, such as the toxins of botulism and tetanus, are among the strongest poisons known.
- These factors cause damage in various ways: some just help bacteria spread in tissues, others lyse host cells, yet others stop cell growth, and still others exaggerate normal physiological mechanisms.
- By depressing or augmenting particular functions, a toxin can kill a person without directly damaging any cells.

- One of the most dramatic manifestations of infection is cell death. This comes about in a variety of ways: direct action of cytolytic toxins, activation of cell-killing white blood cells, or induction of programmed cell death.
- The damage caused by the death of tissue cells is most serious when it occurs in essential organs, such as the brain or the heart.

- With many of the common Gram-negative bacteria, the host response is elicited by a major component of their surface, a lipopolysaccharide known as endotoxin. In small amounts, endotoxin elicits fever and mobilizes certain defense mechanisms. In large amounts, it results in shock and intravascular coagulation.

Immune Response

- Innate immunity refers to immune mechanisms that are always present and available for action.

- These responses tend to be less specific than acquired, adaptive immunity, but since they require no prior exposure to a pathogen to be active, they represent the first line against microbial invasion.

- Alternatively, adaptive immune responses are usually classified as humoral when they lead to the production of circulating antibodies or as cellular when special immune system cells seek out and destroy infected cells.

- Both responses can cause damage.

Humoral Immunity

- Infecting agents elicit the formation of specific antibodies.
- In the circulation and tissues, antibodies combine with the infecting agents or with some of their soluble products.
- These antigen–antibody complexes evoke an inflammatory response by facilitating the activation of a complex set of serum proteins, the complement system.
- In the presence of antigen–antibody complexes, these proteins are activated by a series of proteolytic reactions, called the classical pathway of activation.
- The complement system can also be activated by the presence of microorganisms alone, through a process called the alternative pathway.
- Antigen–antibody complexes are sometimes deposited on the membrane of the glomeruli of the kidneys, resulting in impairment of kidney function, a condition called glomerulonephritis.

Cellular Immunity

- A different type of response is expressed via special cells of the immune system and is called cell-mediated immunity (CMI).

- This complex phenomenon leads to the activation and mobilization of macrophages, the powerful phagocytic cells that participate in the later stages of inflammation.

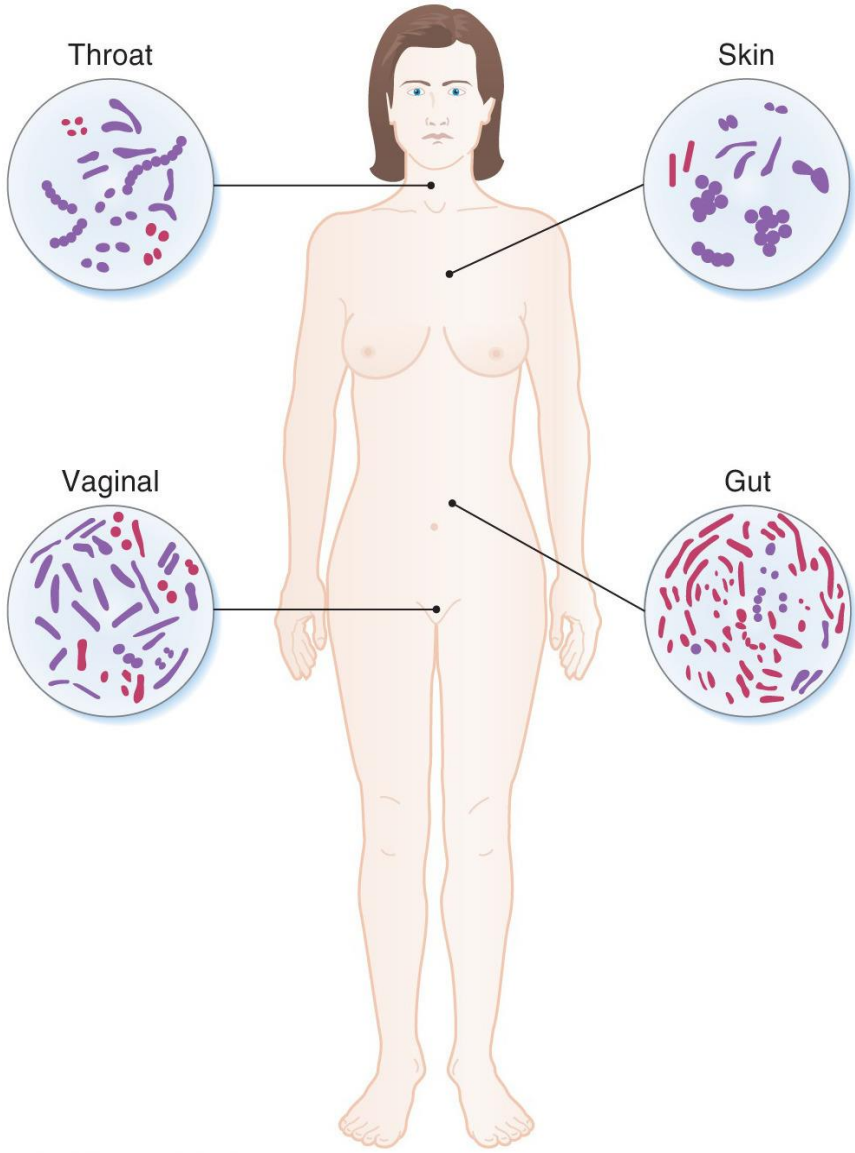
- Although the immune responses can cause tissue damage, in most instances, the price is well worth it.

Normal microflora

Normal microflora

Members of the normal microflora are defined as **microorganisms that are found on, or in the body of the healthy individual**

Majority of members of normal microflora coexist with people, without causing harm, and in some cases provide great benefit (**mutualism**)



Normal microflora

Resident microflora (strains that form niches in certain areas of the organism and remain there permanently)

Transient microflora (acquired from the environment, microorganisms quickly take their places, but are in competition with members of the resident flora or are in constant struggle with the host's immune mechanisms)

Properties of microorganisms that enable successful colonization

Bacterial adhesins	
Anti-colonizing characteristics of the host	**How bacterias overcome these problems**
Flushing of microorganisms by fluid flow	Adeherence for epithelial cells (gonococcus attaches to the epithelium of the urethra)
Killing of microorganisms by host phagocytes	Avoidance of phagocytosis (pneumococcus has a mucous capsule)
	Killing phagocytes (streptococcus produces a toxin that makes pores in the neutrophil membrane)
Insufficient nutrients	Intake of nutrients from host cells (some staphylococci lyse erythrocytes and use hemoglobin as a source of iron)
Growth inhibition by production of antimicrobial cationic peptides	Modification of surface molecules (lipid A) so that cationic protein cannot bind

- Affinity for certain regions of some infectious agents

The role of normal microflora

- Opportunistic infections
- Stimulation of the immune system
- Elimination (disabling colonization by pathogens)
- Role in human nutrition and metabolism?

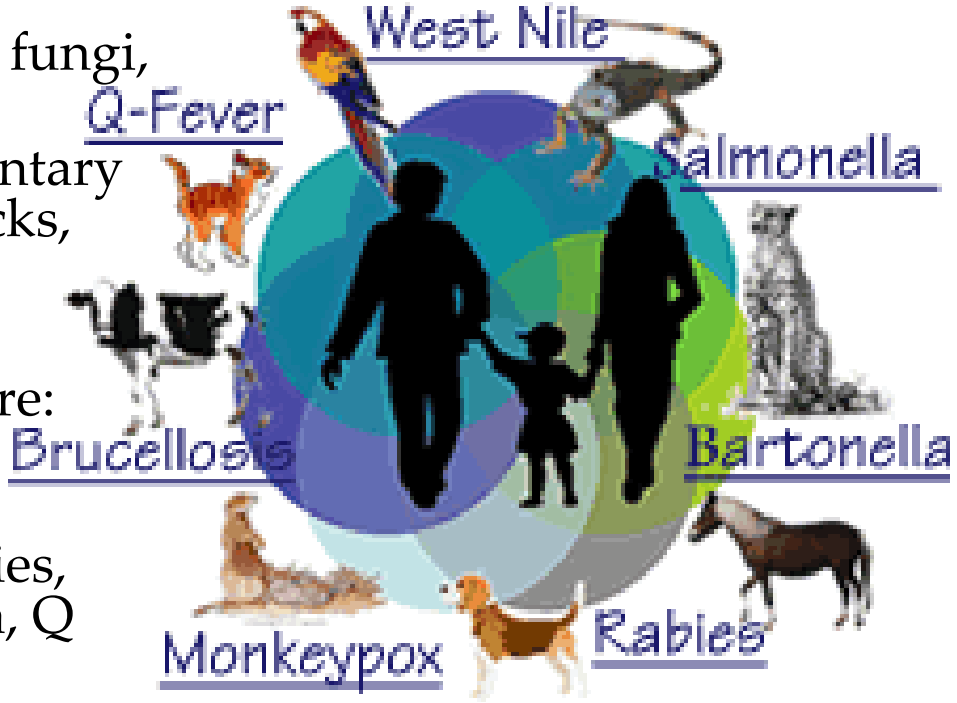
Good and bad conversion of ingested compounds

Cyclamate cyclohexamine (carcingen for bladder) ⟶

Toxic nitrosamine is converted by the activity of bacteria into harmless compounds.

Zoonoses

- Infections and diseases of animals whose causative agents can be transmitted to humans in natural conditions.
- Reservoirs: forest animals, rodents, mice, etc.
- Causes: bacteria, viruses, parasites, fungi, rickettsiae, etc.
- Transmission routes: contact, alimentary and aerogenic and over vectors - ticks, mosquitoes
- Polymorphic clinical picture
- In our country, the most common are: salmonellosis, Lyme disease and trichinosis.
- Zoonoses also include anthrax, rabies, brucellosis, leptospirosis, tularemia, Q fever, psittacosis, trichinosis, etc.
- Prevention and treatment of zoonoses have a multidisciplinary approach and require joint cooperation of doctors and veterinarians.



Antibiotics

Definitions

Antibiotics: Antimicrobial agents that originate from microorganisms, mostly produced by fungi or bacteria themselves.

Antimicrobial agents: substances used to treat infectious diseases.

Bactericidal action: antimicrobial activity that not only inhibits growth but is also lethal to bacteria.

Bacteriostatic action: antimicrobial activity that inhibits growth but does not kill the microorganism.

Minimum inhibitory concentration or MIC: the lowest concentration capable of inhibiting the growth of microorganisms *in vitro*

Resistance: the microorganism cannot be inhibited by clinical concentrations of antimicrobial drug.

Sensitivity: the microorganism may be inhibited by clinical, therapeutic concentrations of antimicrobial drug.

Spectrum denotes all categories of microorganisms against which a certain antimicrobial drug is active.

Antibiotics

bacteriostatic vs bactericidal drugs

Bactericidal and bacteriostatic drugs are not equally effective in all situations.

Bacteriostatic therapy requires the activation of the host's defense mechanisms in order to eliminate the bacteria.

If the host is immunodeficient or if the site of infection is inaccessible to the adequate functioning of the immune system (endocarditis or meningitis), then bactericidal antibiotics are mandatory.

Most agents that are bactericidal show bacteriostatic effect at lower concentrations

Antibiotics

BIOLOGICAL BASIS FOR ANTIBACTERIAL ACTIVITY

There are three types of restrictions on the effective action of antibiotics that are directly related to the way they work:

1. rate of action (bactericidal / bacteriostatic drugs),
2. susceptibility of microorganisms (type of microorganisms and conditions of infection) i
3. side effects on the host (allergy, toxicity).

Antibiotics

Antimicrobial drugs that act on cell wall synthesis
β-lactams and glycopeptides

Cross-links between tetrapeptides in peptidoglycan are the target site of action of two very important groups of antimicrobial agents

Antibiotics

β-lactam antimicrobial agents

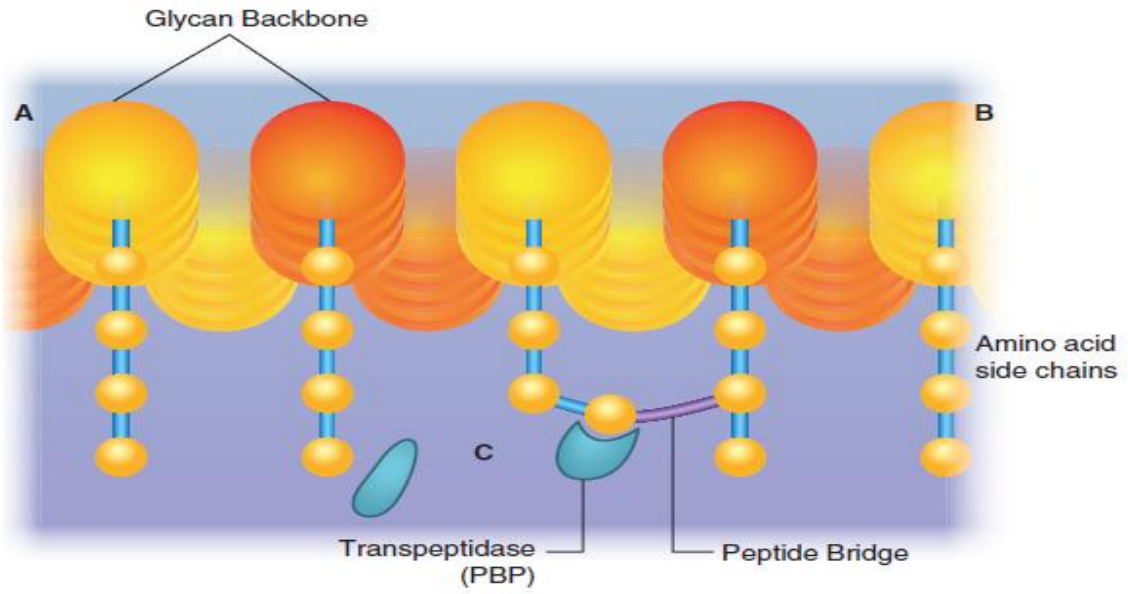
penicillins, cephalosporins, carbapenems and monobactams

β-lactam ring in their structure - crucial for their antibacterial activity

Antibiotics

transpeptidation reaction - peptide cross-links between glycan chains

β-lactams block the transpeptidase activity – no cross-linking



Antibiotics

β-lactam antimicrobial drugs are in generally highly **bactericidal**, but only for bacteria that **grow and actively synthesize the cell wall**

Bactericidal method:

1. interrupting the growth of the peptidoglycan chain

2. releasing or activating autolytic enzymes that later cleave cell wall

3. osmotic lysis of the bacteria

Microorganisms that **do not have a cell wall** such as mycoplasmas are **not sensitive** to β-lactam antimicrobial drugs.

Antibiotics

Penicillin

derived from a fungus Penicilium, and bacteria Streptomyces

semisynthetic process - chemical modifications of fermentation products

against G+, G-cocci, some spirochetes including treponema palidum

Antibiotics

Penicillin

Three important drug development strategies have allowed penicillins to remain the most important group of antibiotics:

1. resistanse to **staphylococcal penicillinase**

Penicillinase is an enzyme (family of β-lactamases) that inactivate β-lactam antibiotics

Penicillinase-resistant penicillins - methicillin, nafcillin, oxacillin

meticillin is no longer in use - meticillin-resistant Staphylococcus

Antibiotics

Penicillin

2. Efective against G + and G-bacteria
aminopenicillins, ampicillin, and amoxicillin are efective against G-pathogens but not against Pseudomonas aeruginosa.
ureidopenicillins, piperacillin, and ticarcillin are efective against Pseudomonas aeruginosa

3. β-lactams + β-lactamase inhibitors – eficciant against bacterias with β-lactamases

Antibiotics

Cephalosporins

resistance to staphylococcal penicillinase, and to the activity of β-lactamases by G-bacteria

5 generations

expansion of the spectrum by modifying the side chains **-** higher-generation - broader spectrum - stronger action - lower minimum inhibitory concentration against G-bacteria

Antibiotics

Cephalosporins

1.generation — cefazolin and **cephalexin**

- similar spectrum of action as penicillinase-resistant penicillin
- active against some Enterobacteriaceae

2. generation cefoxithim and **cefaclor**

- resistant to β-lactamase of G-microorganisms that inactivate the first generation of cephalosporins
- wider effect on Enterobacteriaceae

Antibiotics

Cephalosporins

3.generation-ceftriaxone,cefotaxime and ceftazidime

- wider spectrum of action
- active against G-microorganisms, in concentrations that are 10 to 100 times lower compared to the first generation

Ceftazidine - active against Pseudomonas aeruginosa

wide range of effects, potency and less toxicity make third-generation the drugs most commonly used to treat life-threatening infections

Antibiotics

Cephalosporins

4.fourth generation – cefepime

•penetrate the outer membrane of G-bacteria

•resistance to most β-lactamases

•active against a large number of Enterobacteriaceae and Pseudomonas aeruginosa

•high activity against Neisseria meningitidis and Haemophilus influenzae

Antibiotics

Cephalosporins

5.generation – ceftaroline

• binding with high affinity the protein that binds penicillin 2A

Protein penicillin binding protein 2A allows resistance to other β-lactam antibiotics in meticillin-resistant strains of Staphylococcus aureus

Antibiotics

Carbapenems

Imipenem, merapenem and doripenem

•have the widest spectrum among all beta lactam antibiotics

•Penetration in G- and G + bacteria

•resistance to β-lactamases

• They are very effective against obligate anaerobes such as Basteroides fragilis

Antibiotics

Monobactams

Aztreonam

- Against aerobic and facultative anaerobic G-bacteria (Enterobacteriaceae, Pseudomonas aeruginosa, Neisseria meningitidis, Haemophilus influenzae)

- resistant to β-lactamases produced by G-bacillus

Antibiotics

β-lactamase inhibitors

•bind to the enzyme β-lactamase and thus inactivate it

•These compounds are clavulanic acid, sulbactam and tazobactam

•suicidal inhibitors - they are first hydrolyzed by β-lactamase before becoming effective inactivators of these enzymes

•They are very effective against staphylococcal penicillinases and a wider range of β-lactamases

Antibiotics

β-lactamase inhibitors

- They have a weaker ability to inactivate cephalosporinases

- The combination of these inhibitors with the appropriate β-lactam - powerful therapeutic agents (amoxicillin + clavulanic acid, or ampilin + sulbactam)

Antibiotics

Glycopeptide antimicrobial drugs

vancomycin and teicoplanin

- Inhibition of the association of linear peptidoglycan molecules
- binding directly to the terminal amino groups in the tetrapeptides
- inhibition of cross-linking of peptidoglycan chains

- bactericidal effect

- effective against G + bacterias

- used for infections with multidrug-resistant G + bacteria

Antibiotics

Inhibitors of protein synthesis

Aminoglycosides

aminocyclitol ring with attached carbohydrates

differ according to the exact structure of that ring and the number and nature of bound carbohydrates

effective against the bacteria that can transport them within the cell by mechanisms that involve oxidative phosphorylation

They are weakly active against strict anaerobes and facultative microorganisms that use fermentation, such as streptococci.

Antibiotics

Inhibitors of protein synthesis

Aminoglycosides

inhibit protein synthesis by binding to bacterial ribosomes

Destabilization of ribosomes and thus prevents the elongation of the polypeptide chain

Eukaryotic ribosomes are resistant to aminoglycosides - **selective toxicity**

do not act on intracellular microorganisms such as rickettsiae and chlamydia

Antibiotics

Inhibitors of protein synthesis

Aminoglycosides

Gentamicin and tobramycin – effective against Enterobacteriaceae and Pseudomonas aeruginosa – often in combination with other drugs

Streptomycin and amikacin are mainly used in combination with other antimicrobial agents in the treatment of tuberculosis and other diseases caused by mycobacteria

Neomycin is the most toxic aminoglycoside, it is used only for topical application, and *per os* only before some operations because of the poor absorption

Antibiotics

Inhibitors of protein synthesis

Tetracyclines

four fused benzene rings

Tetracycline and doxacycline are obtained by substitution of benzene rings

Inhibition of protein synthesis by binding to the 30S ribosomal subunit - blocking the binding of aminoacyl transport RNA to the acceptor site on the mRNA-ribosome complex

the effect of tetracycline is reversible- more **bacteriostatic** effect

Antibiotics

Inhibitors of protein synthesis

Tetracyclines

Tigecillin – against anaerobic bacteria – for treatment polymicrobial intra-abdominal infections and other complicated soft tissue infections

Effective against:

- G +, G-bacilli, cocci
- aerobes and anaerobes microorganisms that do not have a cell wall such as mycoplasma
- intracellular bacteria such as rickettsiae and chlamydia

Antibiotics

Inhibitors of protein synthesis

Chloramphenicol

simple nitrobenzene ring

Inhibition of protein synthesis - binding to the 50S subunit of the ribosome and blocking the action **of peptidyl transferase –** blocking the formation of peptide bond

The action is reversible - it acts **bacteriostatically**

.

Antibiotics

Inhibitors of protein synthesis

Chloramphenicol

effective against aerobic and anaerobic species, and obligatorily intracellular pathogens such as rickettsiae and chlamydia

cheap drug with a broad spectrum of action, **but** it is rarely used due to its **high toxicity**

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Antibiotics

Inhibitors of protein synthesis

Macrolides

erythromycin, azithromycin, and clarithromycin

bind to the 50S subunit of the ribosome and block translocation reactions

bacteriostatic effect

Macrolides can be concentrated in phagocytes and other cells - effective against intracellular pathogens

Antibiotics

Inhibitors of protein synthesis

Clindamycin

similar mode of action as as macrolides

effective against some G-anaerobes including Bacteroides fragilis

most often used against **anaerobic** bacteria

Antibiotics

Inhibitors of protein synthesis

Linezolid

binds to the bacterial 50S subunit of the ribosome

Effective against G+, anaerobes G- bacterias, mycobacteria

useful in the treatment of **pneumonia** and other **soft tissue infections**, especially those caused by **resistant strains of staphylococci** and **enterococci**

Antibiotics

Inhibitors of protein synthesis

Streptogramins

quinupristin and **dalfopristin**

They block preotein synthesis by binding to **50S ribosomes** of certain G+ bacteria including **methicillin-resistant staphylococci** and **vancomycin-resistant enterococci**

Quinupristin inhibits peptide chain elongation and dalfopristin interferes with peptidyl transferase

Antibiotics

Inhibitors of nucleic acid synthesis

Quinolones

two linked chains which when substituted with fluorine - fluorinated quinolones

ciprofloxacin, levofloxacin, gemifloxacin, moxifloxacin

target site - **DNA gyrase and topoisomerase 4** - enzymes involved in making breaks in the DNA chain and super threads

effective against aerobes and facultative microorganisms, but relatively ineffective against anaerobes

Antibiotics

Inhibitors of nucleic acid synthesis
Folate inhibitors

Agents that interfere with the synthesis of folic acid

selective toxicity- mammalian cells do not synthesize folate but ingest it through diet

Folate is an essential coenzyme in the reactions of synthesis of purines, thymidines and some amino acids

sulfonamides, trimethoprim, paraaminosalicylic acid

Antibiotics

Inhibitors of nucleic acid synthesis
Folate inhibitors

Sulfonamides
structural analogues of paraaminobenzoic acid

It binds to the enzyme **dihydropteorate synthetase** important for the initial stages of folate synthesis

Bacteriostatic effect

Effective in tretment the uncomplicated urinary tract infections mainly caused by Enterobacteriaceae and Escherichia coli

Antibiotics

Inhibitors of nucleic acid synthesis
Folate inhibitors

Trimethoprim sulfamethoxazole

competitively inhibiting the activity of bacterial **dihydrofolate reductase** that catalyzes the conversion of folate to a reduced form

combination with sulfonamide - - **synergistic bacteriostatic or bactericidal effect**

Effective against G + or G-cocci or bacilli, but it does not act on anaerobes and Pseudomonas aeruginosa.

Antibiotics

Metronidazole

effective against bacteria, fungi and parasites

induction of breaks in the DNA chain

clinically effective for any infection caused by anaerobic microorganisms (Bacteroides fragilis)

Antibiotics

Rifamycins

binds to the **DNA-dependent RNA polymerase**-prevens the initiation of RNA synthesis

the most significant clinical effect - effect on **Mycobacterium tuberculosis**

because resistance often occurs due to a mutation in the polymerase gene, rifampin is combined with other agents

active against most G+ bacteria and certain G-microorganisms monotherapy for the prophylaxis of Neisseria meningitidis and Haemophilus influenzae

Antibiotics

Antimicrobial agents that act on the outer membrane and on the cytoplasmic membrane

polymyxin V and **colistin** - cationic detergents

They bind and change permeability of G- cell wall - loss of essential cytoplasmic components

increasing permeability - bacterial death

Effective against G-bacteria, Pseudomonas aeruginosa

high toxicity - topical application

advantage - rarely developed resistance

Sterilization and disinfection

Elimination of microorganisms is the most important strategy for disease prevention

The death or killing of a microorganism means the loss of the ability of microorganisms to multiply in culture.

Sterilization is the complete killing or removal of all living microorganisms from a particular area or object (exposure to heat, certain gases, ionizing radiation, liquid chemicals and filtration)

Pasteurization is the use of heat of sufficient temperature (but lower than those used for sterilization) to inactivate important pathogenic microorganisms in liquids(water, milk). Spores are not killed at these temperatures, only vegetative forms are killed.

Disinfection is the destruction of pathogenic microorganisms by processes that do not meet the sterilization criteria.

Sterilization and disinfection

Antiseptics are disinfectants that can be used on body surfaces (skin, oral mucosa ...) in order to reduce the number of microorganisms that are members of the normal microflora, as well as pathogenic microorganisms that are placed on these surfaces

Disinfectants are used to remove microorganisms from non living objects.

Asepsis, a procedure that prevents access of microorganisms to a certain, protected area, is applied in many procedures in operating rooms, in the processes of preparation of various drugs as well as in technical manipulations in microbiological laboratories. Asepsis is achieved by sterilizing the materials and equipment.

Sterilization and disinfection

Sterilization

- **Heat**

Open flame: for sterilization of instruments in microbiological laboratories or for emergency sterilization of a knife or needle.

Dry heat: rapid sterilization of the material being disposed of. Dry heat: 160 degrees Celsius for 2 hours (charring of organic matter and destruction of microorganisms including spores) is used for glass and metal.

Wet heat: (much faster and more efficient sterilization method than dry heat because reactive water molecules, at relatively lower temperatures, irreversibly denature proteins by cleaving hydrogen bonds between peptide groups) in an autoclave at 121 degrees Celsius for 10 to 15 minutes depending on the material being sterilized.

Sterilization and disinfection

Sterilization

- **Gas**

Ethylene oxide: an alkylating agent that inactivates (kills) microorganisms by removing labile hydrogen atoms on the hydroxyl, carboxyl groups of guanine and adenine in DNA.

The material (not resistant to high heat, artificial valves ...) is sterilized by exposure to 10% ethylene oxide in carbon dioxide at 50-60oC in controlled humidity conditions, 4 to 6 hours, followed by prolonged exposure to air so the gas, that is the absorbed material, could diffuse (exit from the gas-absorbing material).

Aeration is crucial because the absorbed gas in sterilized materials can cause damage to tissues and skin.

Sterilization and disinfection

Sterilization

- **Ultraviolet and ionizing radiation**

Ultraviolet rays

wavelength of 240-270 nm are absorbed by nucleic acids, which causes gene damage (thymidine dimers are formed). The main application of UV rays is in the radiation of air in hospitals as well as in the decontamination of certain rooms where potentially dangerous microorganisms are placed.

Ionizing radiation has much higher energy than UV radiation and also causes direct DNA damage but also the formation of toxic free radicals and hydrogen peroxide from water. Sterilization of many surgical materials such as gloves, plastic syringes, certain containers...

Sterilization and disinfection

Disinfection

- **Physical methods**

Filtration

Removal of living and dead microorganisms from the liquid by positive / negative filtration pressure. Cellulose ester membrane filters with different pore sizes are used to remove bacteria.

Pasteurization

Pasteurization involves exposing liquids to temperatures of 55-75 degrees to remove vegetative bacteria (not spores). Pasteurization of water at 70 degrees for 30 minutes is a very efficient method for the preparation of plastics used for inhalation therapies.

Microwave

Microwaves in the form of microwave ovens do not use pressure but can reach temperatures close to boiling if moisture is present and are used as an alternative to dry heat to disinfect hospital waste.

Sterilization and disinfection

Disinfection

- **Chemical methods**

Chemical disinfectants are classified based on their ability to sterilize material**:**
high levels of disinfection: they kill all agents except the most resistant bacterial spores
intermediate level: kill all agents except spores
low levels: they kill only most vegetative bacteria and viruses that have a lipid envelope

Most of the substances used for disinfection are protoplasmic poisons, they are not used to treat infections (except superficial ones).The presence of organic matter significantly interferes with the disinfection process

Sterilization and disinfection

Disinfection

Alcohol

in a liquid state in a concentration of 70-95%, it denatures proteins, so it kills vegetative forms of bacteria very quickly, it does not act on spores and most viruses.

100% alcohol dehydrates microorganisms quickly but does not kill them because **the killing process requires water molecules.**

Alcohol (70-90%) and isopropyl alcohol (90-95%) are very often used to decontaminate the skin before some simple invasive procedures such as venipuncture.

* however, **their effect is not immediate, it is very slow** and the traditional wiping of the skin with alcohol before drawing blood is more symbolic than effective because it does not have enough time to kill microorganisms.

Sterilization and disinfection

Disinfection

Halogens

Iodine oxidizes key components of the cell wall of microorganisms. It was originally used as a tincture of 2% iodine in 55% alcohol and thus kills microorganisms much faster and more efficiently than alcohol. Preparations in which iodine is combined with carriers (povidone) or with non-ionic detergents (iodophores) gradually release small amounts of iodine, color the skin less and are mainly used to prepare the skin before surgery.

Chlorine oxidizes cell wall compounds and is lethal within seconds to most vegetative bacteria and inactivates most viruses in extremely low concentrations.

It is used to maintain safe drinking water as well as chlorination of pool water

Sterilization and disinfection

Disinfection

Hydrogen peroxide

A powerful oxidizing agent that attacks membrane lipids, it acts very quickly on most bacteria and viruses, and kills catalase-producing bacteria more slowly as well as spores.

Surfactants

Compounds with hydrophobic and hydrophilic groups that bind to various compounds and dissolve them, or change their characteristics. Cationic detergents, namely **quaternary ammonium compounds** (benzalkonium chloride) are very bactericidal, but in the absence of contaminating organic matter.

They have low toxicity to the skin and mucous membranes, so they are used as antibacterial agents in a concentration of 0.1%. They do not affect spores and most viruses.

Sterilization and disinfection

Disinfection

Phenols

Phenol denatures proteins and has a bactericidal effect. They are very toxic to the skin and tissues and cannot be used as antiseptics. Short exposure to phenols can be tolerated, so active phenols are present in most mouthwashes.

Chlorine hexidine alters the membrane permeability of both G + and G- bacteria and is routinely used as a disinfectant for hands and skin. It binds to the skin and gives a persistent antibacterial effect.

Sterilization and disinfection

Disinfection

Glutaraldehyde and formaldehyde

Alkylating agents that are lethal to all medically important microorganisms.

Formaldehyde is a gas that is an irritant, an allergen and is very unpleasant, which limits its use in the form of a solution or gas.

Glutaraldehyde is a high-level disinfectant for devices that cannot be exposed to high temperatures (lenses, equipment for respiratory devices).

Formaldehyde gases are very effective in decontaminating the environment in conditions of high humidity, so they are sometimes used for decontamination of laboratory rooms that have been accidentally and extensively contaminated with bacteria.