KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2018 onwards)

DEPARTMENT OF MICROBIOLOGY

SEMESTER : I
SUBJECT CODE: 17MBP102
CLASS : I MSc Microbiology
SUBJECT: Microbial Physiology and metabolism

Unit I

S.No	Duration	Topic	Reference
1	1	Pokaryotic cell structures	T1-18-20
2	1	Cell organization of cell wall; G+ve& G-ve	T2-25-28
3	1	Plasma membrane-structure & functions	R1-15-29
4	1	Organelles inside cytoplasmic matrix-ribosome	R1-41-44
5	1	nucleoid, inclusion bodies	T2-32-38
6	1	Capsule, slime layers & layers, pili	R2-48-49
7	1	Fimbriae, flagella	R2-53-63
8	1	Motility	R2 64
9	1	Eukaryotic cell structure	W1-12-19
10	1	Lichens & microalgae	R2-70-74
11	1	Mycoplasma	R2-75-76
12	1	Basic structure of virus	R2-80-91
13	1	Unit Revision	
		Total hours 13 hours	

Text Book: T1 : Doelle, 2005 Bacterial metabolism
T2 : Moat and Foster, 2003- Microbial physiology
Reference books R1 Caldwell, 2008, microbial physiology & metabolism
R2 Microbiology, Prescott, Harley, Ulein fifth edition 2009.
Website W1 http://www.science.smith.edu/department/biology

Unit II

Sl.No	Duration	Topic	Reference
1	1	Bacterial endospores & structure	R2-66-68
2	1	Endospore formation in *Bacillus*	R2-66-68
3	1	Exospores formation in *Streptomyces*	W2
4	1	Sporulation in fungi-	R2-100-110

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		Aspergillussp&Penicillium sp	
5	1	Spore cycle -Stages	R2-68-69
6	1	factors affecting spore formation	R2-120-140
7	1	Growth and nutritional requirements	W2
8	1	Control of microorganisms	R1-16-18
9	1	Physical method & Chemical method to control microorganism	R1-25-30
10	1	Biofilm	W2
11	1	Biosurfactant production & industrial application	W2
12	1	Unit Revision	
		Total hours:12 Hours	

R1 : Caldwell, 2008/ Microbial physiology.
R2 : Prescott, Harley-2009/ Microbiology.
W2: www.science.smith.edu/biology.

Unit III

Sl.No	Duration	Topic	Reference
1	1	Embden-Meyerhof –Parnas pathway	R1 89-90
2	1	Hexose monophosphate pathway	
3	1	EntnerDuodoroff pathway	R1 91-92
4	1	Tricarboxylic acid cycle	R1 97-98
5	1	Glyoxylate cycle/ aerobic respiration & a Anaerobic respiration	R1 200-210
6	1	Electron transport chain	R1 157-159

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7	1	Substrate level phosphorylation	W3, W4
8	1	Oxidation phosphorylation	T3 125-127
9	1	ATP generation	T3 126-129
10	1	Aerobic respiration	T3 – 130-132
11	1	Anaerobic respiration & Energy utilization.	T3 -133 -134 & W4
12	1	Unit discussion	
		Total hours : 12 hours	

Text Book: T3 : Text Book of Microbiology by Prescott and Pelczar
Website W3 : www.khanaacademy.org/scince/biology
W4 : www.ncbi.Wm.nih.gov/books

Unit IV

Sl.No	Duration	Topic	Reference
1	1	Biosynthesis of fatty acids	T3 201-202
2	1	Biosynthesis of nucleotides	T3 203-207
3	1	Biosynthesis of aminoacids	T3-12-15
4	1	Biosynthesis of protein	T3-17-22
5	1	Biosynthesis of phospholipids	T3-23-40
6	1	Archaeal lipids & cell wall	T3208-210
7	1	Biosynthesis of G+ve bacteria	T3-45-55
8	1	Gram –ve bacteria cell wall synthesis	T3-57-60
9	1	Synthesis of secondary metabolites	T3-70-85
10	1	Toxins	T3-90-95
11	1	Characterization Toxins	T3-100-105 & R1-66-68
12	1	Mechanism of action	R1- 67-71

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13	1	Unit revision	
	Total Hrs: 13		

Text Book: T3 : Text Book of Microbiology by Michael Pelczar
R1 : Caldwell, 2008/ Microbial physiology.

Unit 5

Sl.No	Duration	Topic	Reference
1	1	Aerobic and anaerobic fermentation, types	T3-94-95
2	1	Photosynthesis; bacteria & cyanobacteria	T3-11-20
3	1	Oxygenic and Anoxygenic photosynthesis	T3-25-30
4	1	Purple/ green sulfur and non sulfur bacteria	T3-30-35
5	1	Bacterial pigments (photosynthetic)	T3-40-45 & W3
6	1	Methanogenesis- assimilation of CO_2	T3-56-60
7	1	Bioluminescence	T3-67-80 & W3
8	1	Quorum sensing mechanism	T3-90-98 &W3
9	1	Application of Bioluminescence	W3
10	1	Application of of quorum sensing	T3-110-120
11	1	Model and old QP discussion	T3-125-130
12	1	Old QP discussion	
13	1	Old QP discussion	
14	1	Old QP discussion	
		TOTAL HOURS : 14	

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Unit I:

An Overview of Procaryotic Cell Structure

Size, Shape, and Arrangement

One might expect that small, relatively simple organisms like procaryotes would be uniform in shape and size. Although it is true that many procaryotes are similar in morphology, there is a remarkable amount of variation due to differences in genetics and ecology. Most commonly encountered bacteria have one of two shapes. **Cocci** (**coccus**) are roughly spherical cells. They can exist as individual cells, but also are associated in characteristic arrangements that are frequently useful in bacterial identification. **Diplococci** (**diplococcus**) arise when cocci divide and remain together to form pairs. Long chains of cocci result when cells adhere after repeated divisions in one plane; this pattern is seen in the genera *Streptococcus, Enterococcus,* and *Lactococcus* . *Staphylococcus* divides in random planes to generate irregular grapelike clumps. Divisions in two or three planes can produce symmetrical clusters of cocci. Members of the genus *Micrococcus* often divide in two planes to form square groups of four cells called tetrads. In the genus *Sarcina,* cocci divide in three planes producing cubical packets of eight cells. The other common bacterial shape is that of a **rod,** often called a **bacillus**. *Bacillus megaterium* is a typical example of a bacterium with a rod shape. Bacilli differ considerably in their length-towidth ratio, the coccobacilli being so short and wide that they resemble cocci. The shape of the rod's end often varies between species and may be flat, rounded, cigar-shaped, or bifurcated. Although many rods do occur singly, they may remain together after division to form pairs or chains (e.g., *Bacillus megaterium* is found in long chains).

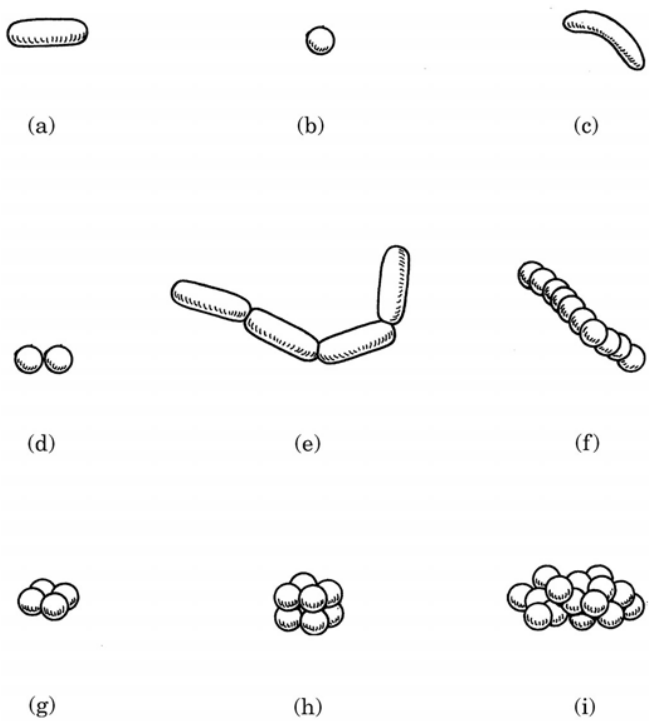


Figure Bacterial shapes. Most bacteria are (a) rod shaped, (b) spherical or (c) curved. These basic shapes may join to form (d) pairs, (e and f) chains, (g) sheets, (h) packets or (i) irregular aggregates

A few rod-shaped bacteria, the **vibrios,** are curved to form distinctive commas or incomplete spirals. Bacteria can assume a great variety of shapes, although they often are simple spheres or rods. Actinomycetes characteristically form long multinucleate filaments or hyphae that may branch to produce a network called a **mycelium**. Many bacteria are shaped like long rods twisted into spirals or helices; they are called **spirilla** if rigid and **spirochetes** when flexible. The oval- to pear-shaped *Hyphomicrobium* produces a bud at the end of a long hypha. Other bacteria such as *Gallionella* produce nonliving stalks. A few bacteria actually are flat. For example,

Anthony E. Walsby has discovered square bacteria living in salt ponds. These bacteria are shaped like flat, square-to rectangular boxes about 2 µm by 2 to 4 µm, and only 0.25µm thick. Finally, some bacteria are variable in shape and lack a single, characteristic form. These are called **pleomorphic** even though they may, like *Corynebacterium,* have a generally rodlike form. Bacteria vary in size as much as in shape. The smallest (e.g., some members of the genus *Mycoplasma*) are about 0.3 µm in diameter, approximately the size of the largest viruses (the poxviruses). Recently there have been reports of even smaller cells. Nanobacteria or ultramicrobacteria appear to range from around 0.2 µm to less than 0.05 µm in diameter. A few strains have been cultured, but most are simply very small bacteria-like objects only observed microscopically. It has been thought that the smallest possible cell is about 0.14 to 0.2 µm in diameter, but many nanobacteria are reported to be smaller. Some microbiologists think nanobacteria are artifacts, and more research will be required before the significance of these forms becomes clear. *Escherichia coli,* a bacillus of about average size, is 1.1 to 1.5 µm wide by 2.0 to 6.0 µm long. A few bacteria become fairly large; some spirochetes occasionally reach 500 µm in length, and the cyanobacterium *Oscillatoria* is about 7µm in diameter (the same diameter as a red blood cell). A huge bacterium lives in the intestine of the brown surgeonfish, *Acanthurus nigrofuscus. Epulopiscium fishelsoni* grows as large as 600 by 80 µm, a little smaller than a printed hyphen. More recently an even larger bacterium, *Thiomargarita namibiensis,* has been discovered in ocean sediment. Thus a few bacteria are much larger than the average eucaryotic cell (typical plant and animal cells are around 10–50µm in diameter).

Procaryotic Cell Organization

A variety of structures is found in procaryotic cells. Not all structures are found in every genus. Furthermore, gram negative and gram-positive cells differ, particularly with respect to their cell walls. Despite these variations procaryotes are consistent in

their fundamental structure and most important components. Procaryotic cells almost always are bounded by a chemically complex cell wall. Inside this wall, and separated from it by a periplasmic space, lies the plasma membrane. This membrane can be invaginated to form simple internal membranous structures. Since the procaryotic cell does not contain internal membrane-bound organelles, its interior appears morphologically simple. The genetic material is localized in a discrete region, the nucleoid, and is not separated from the surrounding cytoplasm by membranes. Ribosomes and larger masses called inclusion bodies are scattered about in the cytoplasmic matrix. Both gram-positive and gram-negative cells can use flagella for locomotion. In addition, many cells are surrounded by a capsule or slime layer external to the cell wall.

Table: Functions of Procaryotic Structures

Plasma membrane	Selectively permeable barrier, mechanical boundary of cell, nutrient and waste transport, location of many metabolic processes (respiration, photosynthesis), detection of environmental cues for chemotaxis
Gas vacuole	Buoyancy for floating in aquatic environments
Ribosomes	Protein synthesis
Inclusion bodies	Storage of carbon, phosphate, and other substances
Nucleoid	Localization of genetic material (DNA)

Periplasmic space	Contains hydrolytic enzymes and binding proteins for nutrient processing and uptake
Cell wall	Gives bacteria shape and protection from lysis in dilute solutions
Capsules and slime layers	Resistance to phagocytosis, adherence to surfaces
Fimbriae and pili	Attachment to surfaces, bacterial mating
Flagella	Movement
Endospore	Survival under harsh environmental conditions

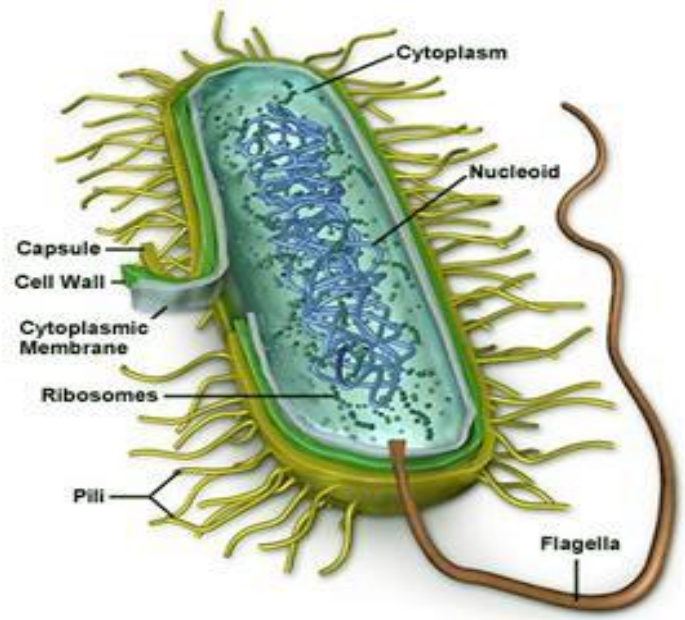


Figure Structure of a typical bacterial cell.

Procaryotic Cell Membranes

Membranes are an absolute requirement for all living organisms. Cells must interact in a selective fashion with their environment, whether it is the internal environment of a multicellular organism or a less protected and more variable external environment. Cells must not only be able to acquire nutrients and eliminate wastes, but they also have to maintain their interior in a constant, highly organized state in the face of external changes. The **plasma membrane** encompasses the cytoplasm of both procaryotic and eucaryotic cells. This membrane is the chief point of contact with the cell's environment and thus is responsible for much of its relationship with the outside world. To understand membrane function, it is necessary to become familiar with membrane structure, and particularly with plasma membrane structure. **The Plasma Membrane** Membranes contain both proteins and lipids, although the exact proportions of protein and lipid vary widely. Bacterial plasma membranes usually have a higher proportion of protein than do eucaryotic membranes, presumably because they fulfill so

many different functions that are carried out by other organelle membranes in eucaryotes. Most membrane-associated lipids are structurally asymmetric with polar and nonpolar ends and are called amphipathic. The polar ends interact with water and are **hydrophilic;** the nonpolar **hydrophobic** ends are insoluble in water and tend to associate with one another. This property of lipids enables them to form a bilayer in membranes. The outer surfaces are hydrophilic, whereas hydrophobic ends are buried in the interior away from the surrounding water. Many of these amphipathic lipids are phospholipids. Bacterial membranes usually differ from eucaryotic membranes in lacking sterols such as cholesterol. However, many bacterial membranes do contain pentacyclic sterol-like molecules called hopanoids , and huge quantities of hopanoids are present in our ecosystem. Hopanoids are synthesized from the same precursors as steroids. Like steroids in eucaryotes, they probably stabilize the bacterial membrane. The membrane lipid is organized in two layers, or sheets, of molecules arranged end-to-end.

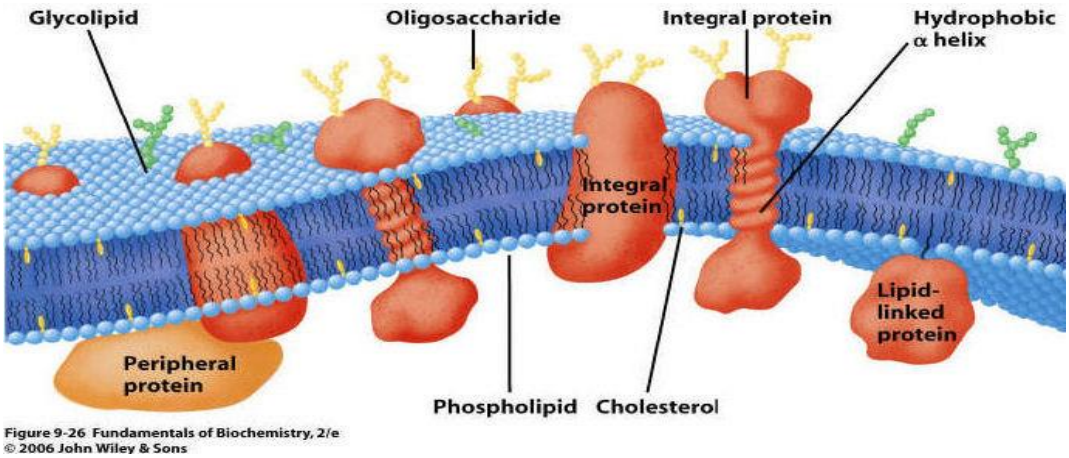


Figure Plasma Membrane Structure. This diagram of the fluid mosaic model of bacterial membrane structure shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and

wiggly tails, the hydrophobic fatty acid chains. Other membrane lipids such as hopanoids (pink) may be present. For the sake of clarity, phospholipids are shown in proportionately much larger size than in real membranes.

Many archaeal membranes differ from other bacterial membranes in having a monolayer with lipid molecules spanning the whole membrane. Cell membranes are very thin structures, about 5 to 10 nm thick, and can only be seen with the electron microscope. The freeze-etching technique has been used to cleave membranes down the center of the lipid bilayer, splitting them in half and exposing the interior. In this way it has been discovered that many membranes, including the plasma membrane, have a complex internal structure. The small globular particles seen in these membranes are thought to be membrane proteins that lie within the membrane lipid bilayer .

The most widely accepted current model for membrane structure is the **fluid mosaic model** of S. Jonathan Singer and Garth Nicholson. They distinguish between two types of membrane proteins. **Peripheral proteins** are loosely connected to the membrane and can be easily removed. They are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. About 70 to 80% of membrane proteins are **integral proteins.** These are not easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Integral proteins, like membrane lipids, are amphipathic; their hydrophobic regions are buried in the lipid while the hydrophilic portions project from the membrane surface. Some of these proteins even extend all the way through the lipid layer. Integral proteins can diffuse laterally around the surface to new locations, but do not flip-flop or rotate through the lipid layer. Often carbohydrates are attached to the outer surface of plasma membrane proteins and seem to have important functions.

The emerging picture of the cell membrane is one of a highly organized and asymmetric system, which also is flexible and dynamic. Although membranes apparently have a common basic design, there are wide variations in both their structure

and functional capacities. The differences are so large and characteristic that membrane chemistry can be used in bacterial identification. The plasma membranes of procaryotic cells must fill an incredible variety of roles successfully. Many major plasma membrane functions are noted here even though they are discussed individually at later points in the text. The plasma membrane retains the cytoplasm, particularly in cells without cell walls, and separates it from the surroundings.

The plasma membrane also serves as a selectively permeable barrier: it allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others. Thus the membrane prevents the loss of essential components through leakage while allowing the movement of other molecules. Because many substances cannot cross the plasma membrane without assistance, it must aid such movement when necessary. Transport systems can be used for such tasks as nutrient uptake, waste excretion, and protein secretion. The prokaryotic plasma membrane also is the location of a variety of crucial metabolic processes: respiration, photosynthesis, the synthesis of lipids and cell wall constituents, and probably chromosome segregation. Finally, the membrane contains special receptor molecules that help procaryotes detect and respond to chemicals in their surroundings. Clearly the plasma membrane is essential to the survival of microorganisms.

Internal Membrane Systems

Although procaryotic cytoplasm does not contain complex membranous organelles like mitochondria or chloroplasts, membranous structures of several kinds can be observed. A commonly observed structure is the mesosome. Mesosomes are invaginations of the plasma membrane in the shape of vesicles, tubules, or lamellae .

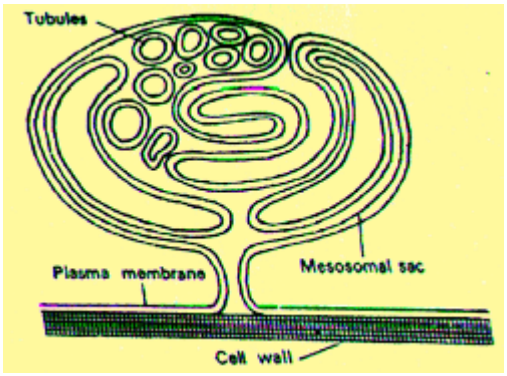


Figure Mesosome Structure

They are seen in both grampositive and gram-negative bacteria, although they are generally more prominent in the former. Mesosomes often are found next to septa or cross-walls in dividing bacteria and sometimes seem attached to the bacterial chromosome. Thus they may be involved in cell wall formation during division or play a role in chromosome replication and distribution to daughter cells.

Currently many bacteriologists believe that mesosomes are artifacts generated during the chemical fixation of bacteria for elec tron microscopy. Possibly they represent parts of the plasma membrane that are chemically different and more disrupted by fixatives. Many bacteria have internal membrane systems quite different from the mesosome. Plasma membrane infoldings can become extensive and complex in photosynthetic bacteria such as the cyanobacteria and purple bacteria or in bacteria with very high respiratory activity like the nitrifying bacteria. They may be aggregates of spherical vesicles, flattened vesicles, or tubular membranes. Their function may be to provide a larger membrane surface for greater metabolic activity.

The Cytoplasmic Matrix

Procaryotic cytoplasm, unlike that of eucaryotes, lacks unit membrane-bound organelles. The **cytoplasmic matrix** is the substance lying between the plasma membrane and the nucleoid. The matrix is largely water (about 70% of bacterial mass is

water). It is featureless in electron micrographs but often is packed with ribosomes and highly organized. Specific proteins are positioned at particular sites such as the cell pole and the place where the bacterial cell will divide. Thus although bacteria may lack a true cytoskeleton, they do have a cytoskeletonlike system of proteins in their cytoplasmic matrix. The plasma membrane and everything within is called the **protoplast;** thus the cytoplasmic matrix is a major part of the protoplast.

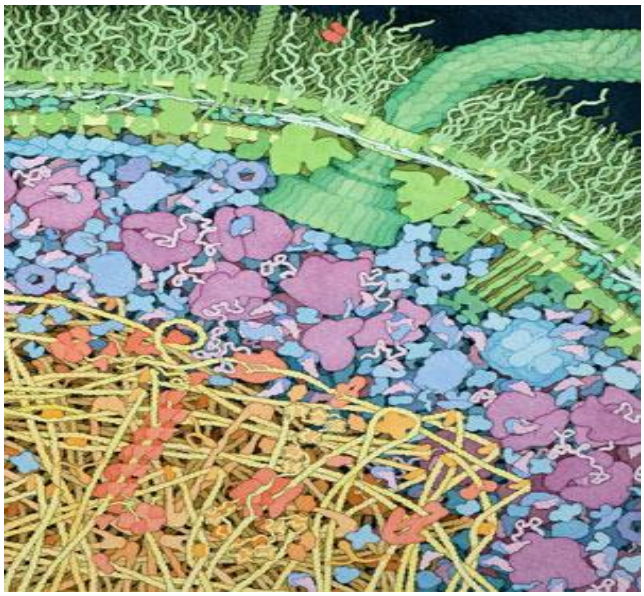


Figure A Cross Section of the Bacterium *Escherichia coli* Drawn at a Magnification of a Million Times. The glycocalyx, flagellum, gram-negative cell wall, and plasma membrane are at the top. Ribosomes synthesizing proteins fill the underlying cytoplasmic matrix. At the bottom is the nucleoid with its dense tangle of DNA and associated proteins.

Inclusion Bodies

A variety of **inclusion bodies,** granules of organic or inorganic material that often are clearly visible in a light microscope, is present in the cytoplasmic matrix.

These bodies usually are used for storage (e.g., carbon compounds, inorganic substances, and

energy), and also reduce osmotic pressure by tying up molecules in particulate form. Some inclusion bodies are not bounded by a membrane and lie free in the cytoplasm—for example, polyphosphate granules, cyanophycin granules, and some glycogen granules. Other inclusion bodies are enclosed by a membrane about 2.0 to 4.0 nm thick, which is single-layered and not a typical bilayer membrane. Examples of membrane-enclosed inclusion bodies are poly-_-hydroxybutyrate granules, some glycogen and sulfur granules, carboxysomes, and gas vacuoles. Inclusion body membranes vary in composition. Some are protein in nature, whereas others contain lipid. Because inclusion bodies are used for storage, their quantity will vary with the nutritional status of the cell. For example, polyphosphate granules will be depleted in freshwater habitats that are phosphate limited.

Organic inclusion bodies usually contain either glycogen or poly hydroxybutyrate. **Glycogen** is a polymer of glucose units composed of long chains formed by (1 4) glycosidic bonds and branching chains connected to them by (1 6) glycosidic bonds. **Poly-hydroxybutyrate (PHB)** contains hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. Usually only one of these polymers is found in a species, but purple photosynthetic bacteria have both. Polyhydroxybutyrate accumulates in distinct bodies, around 0.2 to 0.7 µm in diameter, that are readily stained with Sudan black for light microscopy and are clearly visible in the electron microscope. Glycogen is dispersed more evenly throughout the matrix as small granules (about 20 to 100 nm in diameter) and often can be seen only with the electron microscope. If cells contain a large amount of glycogen, staining with an iodine solution will turn them reddish-brown. Glycogen and PHB inclusion bodies are carbon storage reservoirs providing material for energy and biosynthesis. Many bacteria also store carbon as lipid droplets.

Cyanobacteria have two distinctive organic inclusion bodies. **Cyanophycin granules** are composed of large polypeptides containing approximately equal amounts of the amino acids arginine and aspartic acid. The granules often are large enough to be visible in the light microscope and store extra nitrogen for the bacteria. **Carboxysomes** are present in many cyanobacteria, nitrifying bacteria, and thiobacilli. They are polyhedral, about 100 nm in diameter, and contain the enzyme ribulose- 1,5-bisphosphate carboxylase in a paracrystalline arrangement. They serve as a reserve of this enzyme and may be a site of CO2 fixation. A most remarkable organic inclusion body, the **gas vacuole,** is present in many cyanobacteria, purple and green photosynthetic bacteria, and a few other aquatic forms such as *Halobacterium* and *Thiothrix*. These bacteria float at or near the surface, because gas vacuoles give them buoyancy. This is vividly demonstrated by a simple but dramatic experiment. Cyanobacteria held in a full, tightly stoppered bottle will float, but if the stopper is struck with a hammer, the bacteria sink to the bottom. Examination of the bacteria at the beginning and end of the experiment shows that the sudden pressure increase has collapsed the gas vacuoles and destroyed the microorganisms' buoyancy. Gas vacuoles are aggregates of enormous numbers of small, hollow, cylindrical structures called **gas vesicles**

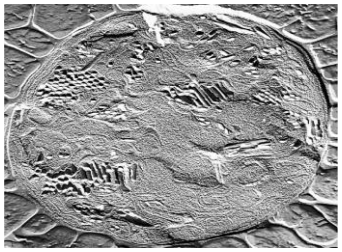
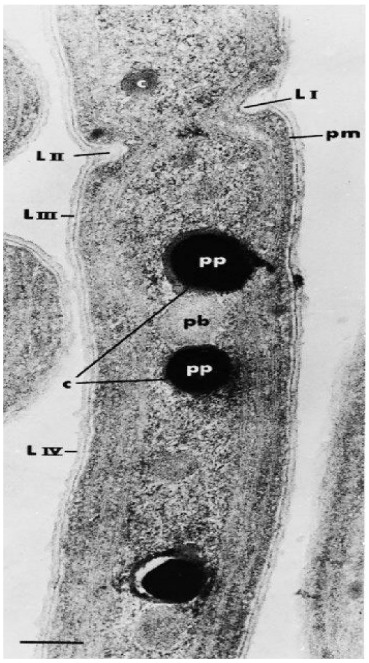
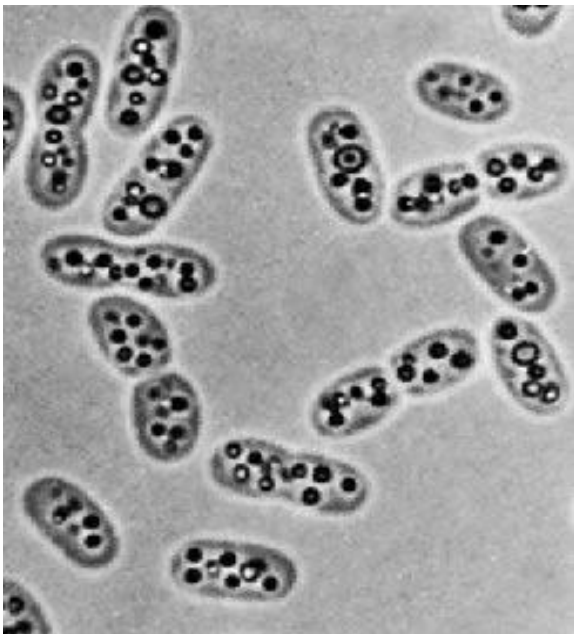
. 

Figure Gas Vesicles and Vacuoles. A freeze-fracture preparation of *Anabaena flos-aquae* (_89,000). Clusters of the cigar shaped vesicles form gas vacuoles. Both longitudinal and cross-sectional views of gas vesicles can be seen.

Gas vesicle walls do not contain lipid and are composed entirely of a single small protein. These protein subunits assemble to form a rigid enclosed cylinder that is hollow and impermeable to water but freely permeable to atmospheric gases. Bacteria with gas vacuoles can regulate their buoyancy to float at the depth necessary for proper light intensity, oxygen concentration, and nutrient levels. They descend by simply collapsing vesicles and float upward when new ones are constructed. Two major types of inorganic inclusion bodies are seen. Many bacteria store phosphate as **polyphosphate granules** or

volutin granules





(a) (b)

Figure Inclusion Bodies in Bacteria. (**a**) Ultrastructure of the cyanobacterium *Anacystis nidulans.* The bacterium is dividing, and a septum is partially formed, LI and

LII. Several structural features can be seen, including cell wall layers, LIII and LIV; the plasma membrane, pm; polyphosphate granules, pp; a polyhedral body, pb; and cyanophycin material, c. Thylakoids run along the length of the cell. Bar 0.1 µm.

(**b**) *Chromatium vinosum*, a purple sulfur bacterium, with intracellular sulfur granules, light field.

Polyphosphate is a linear polymer of orthophosphates joined by ester bonds. Thus volutin granules function as storage reservoirs for phosphate, an important component of cell constituents such as nucleic acids. In some cells they act as an energy reserve, and polyphosphate can serve as an energy source in reactions. These granules are sometimes called **metachromatic granules** because they show the metachromatic effect; that is, they appear red or a different shade of blue when stained with the blue dyes methylene blue or toluidine blue. Some bacteria also store sulfur temporarily as sulfur granules, a second type of inorganic inclusion body. For example, purple photosynthetic bacteria can use hydrogen sulfide as a photosynthetic electron donor and accumulate the resulting sulfur in either the periplasmic space or in special cytoplasmic globules. Inorganic inclusion bodies can be used for purposes other than storage. An excellent example is the **magnetosome,** which is used by some bacteria to orient in the earth's magnetic field. These inclusion bodies contain iron in the form of magnetite

Ribosomes

The cytoplasmic matrix often is packed with **ribosomes;** they also may be loosely attached to the plasma membrane. Ribosomes look like small, featureless particles at low magnification in electron micrographs but are actually very complex objects made of both protein and ribonucleic acid (RNA).

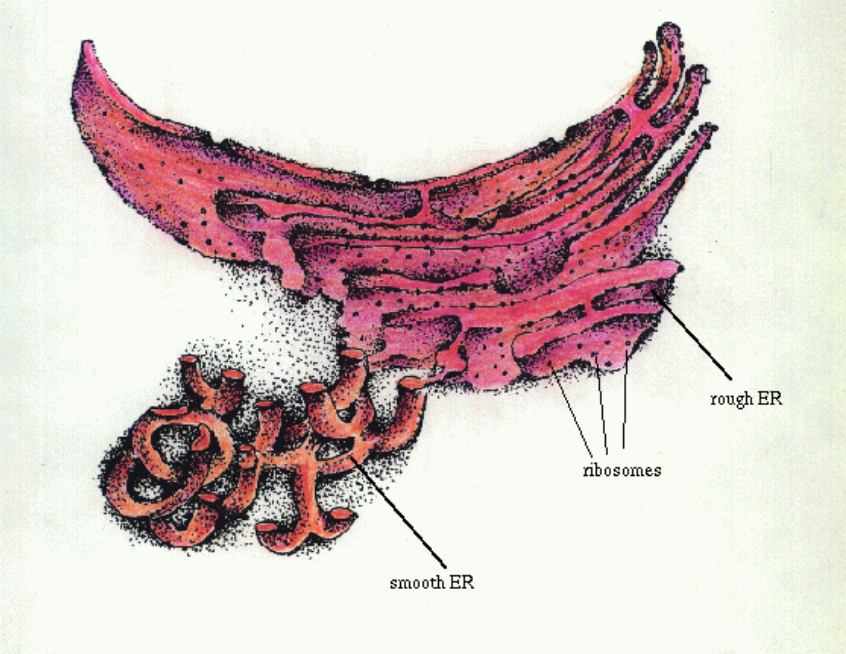


Fig: Structure of ribosome

They are the site of protein synthesis; matrix ribosomes synthesize proteins destined to remain within the cell, whereas the plasma membrane ribosomes make proteins for transport to the outside. The newly formed polypeptide folds into its final shape either as it is synthesized by the ribosome or shortly after completion of protein synthesis. The shape of each protein is determined by its amino acid sequence. Special proteins called molecular chaperones, or chaperones, aid the polypeptide in folding to its proper shape. Note that procaryotic ribosomes are smaller than eukaryotic ribosomes. They commonly are called 70S ribosomes, have dimensions of about 14 to 15 nm by 20 nm, a molecular weight of approximately 2.7 million, and are constructed of a 50S and a

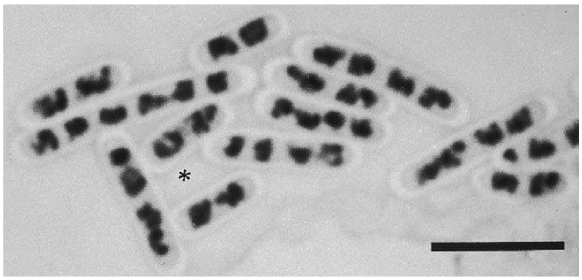
30S subunit. The S in 70S and similar values stands for **Svedberg unit.** This is the unit of the sedimentation coefficient, a measure of the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater its Svedberg value

or sedimentation coefficient. The sedimentation coefficient is a function of a particle's molecular weight, volume, and shape. Heavier and more compact particles normally have larger Svedberg numbers or sediment faster. Ribosomes in the cytoplasmic matrix of eucaryotic cells are 80S ribosomes and about 22 nm in diameter. Despite their overall difference in size, both types of ribosomes are similarly composed of a large and a small subunit.

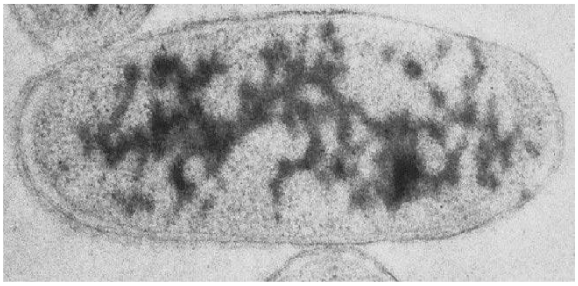
The Nucleoid

Probably the most striking difference between procaryotes and eucaryotes is the way in which their genetic material is packaged. Eucaryotic cells have two or more chromosomes contained within a membrane-delimited organelle, the nucleus. In contrast,

procaryotes lack a membrane-delimited nucleus. The prokaryotic chromosome is located in an irregularly shaped region called the **nucleoid** (other names are also used: the nuclear body, chromatin body, nuclear region). Usually procaryotes contain a single circleof double-stranded **deoxyribonucleic acid (DNA),** but some have a linear DNA chromosome. Recently it has been discovered that some bacteria such as *Vibrio cholerae* have more than one chromosome. Although nucleoid appearance varies with the method of fixation and staining, fibers often are seen in electron micrographs and are probably DNA.



(a)



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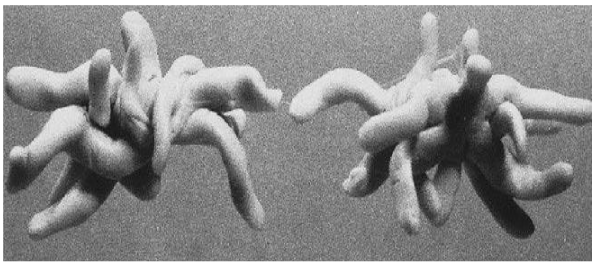


(c)

Figure The Bacterial Nucleoid. (**a**) Nucleoids in growing *Bacillus* cells stained using HCl-Giemsa stain and viewed with a light microscope. (**b**) A section of actively growing *E. coli* immunostained specifically for DNA and examined in the transmission electron microscope. Coupled transcription and translation occur in parts of the nucleoid that extend out into the cytoplasm. (**c**) A model of two nucleoids in an actively growing *E. coli* cell. Note that a metabolically active nucleoid is not compact and spherical but has projections that extend into the cytoplasmic matrix.

The nucleoid also is visible in the light microscope after staining with the Feulgen stain, which specifically reacts with DNA. A cell can have more than one nucleoid when cell division occurs after the genetic material has been duplicated. In actively growing bacteria, the nucleoid has projections that extend into the cytoplasmic matrix. Presumably

these projections contain DNA that is being actively transcribed to produce mRNA. Careful electron microscopic studies often have shown the nucleoid in contact with either the mesosome or the plasma membrane. Membranes also are found attached to isolated nucleoids. Thus there is evidence that bacterial DNA is attached to cell membranes, and membranes may be involved in the separation of DNA into daughter cells during division. Nucleoids have been isolated intact and free from membranes. Chemical analysis reveals that they are composed of about 60% DNA, 30% RNA, and 10% protein by weight. In *Escherichia coli*, a rod-shaped cell about 2 to 6 µm long, the closed DNA circle measures approximately 1,400 µm. Obviously it must be very efficiently packaged to fit within the nucleoid. The DNA is looped and coiled extensively, probably with the aid of RNA and nucleoid proteins (these proteins differ from the histone proteins present in eukaryotic nuclei).

Many bacteria possess **plasmids** in addition to their chromosome. These are double-stranded DNA molecules, usually circular, that can exist and replicate independently of the chromosome or may be integrated with it; in either case they normally are inherited or passed on to the progeny. However, plasmids are not usually attached to the plasma membrane and sometimes are lost Many bacteria possess **plasmids** in addition to their chromosome. These are double-stranded DNA molecules, usually circular, that can exist and replicate independently of the chromosome or may be integrated with it; in either case they normally are inherited or passed on to the progeny. However, plasmids are not usually attached to the plasma membrane and sometimes are lost to one of the progeny cells during division. Plasmids are not required for host growth and reproduction, although they may carry genes that give their bacterial host a selective advantage. Plasmid genes can render bacteria drug-resistant, give them new metabolic abilities, make them pathogenic, or endow them with a number of other properties. Because plasmids often move between bacteria, properties such as drug resistance can spread throughout a population.

The Procaryotic Cell Wall

The cell wall is the layer, usually fairly rigid, that lies just outside the plasma membrane. It is one of the most important parts of a procaryotic cell for several reasons. Except for the mycoplasmas and some Archaea, most bacteria have strong walls that give them shape and protect them from osmotic lysis ; wall shape and strength is primarily due to peptidoglycan, as we will see shortly. The cell walls of many pathogens have components that contribute to their pathogenicity. The wall can protect a cell from toxic substances and is the site of action of several antibiotics.

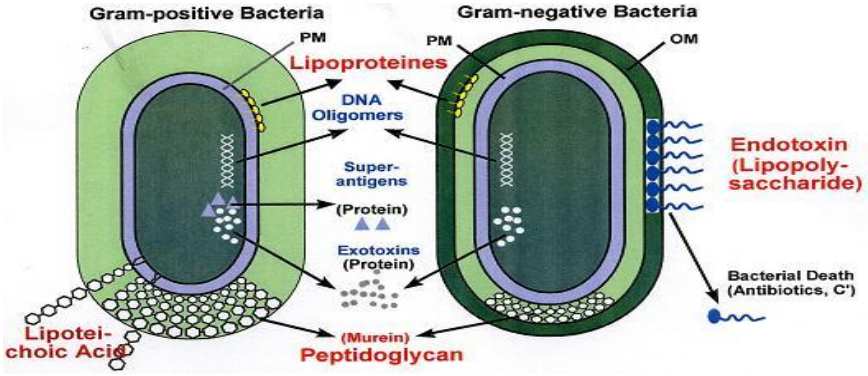


Figure Gram-Positive and Gram-Negative Cell Walls.

After Christian Gram developed the Gram stain in 1884, it soon became evident that bacteria could be divided into two major groups based on their response to the Gram-stain procedure. Gram-positive bacteria stained purple, whereas gram-negative bacteria were colored pink or red by the technique. The true structural difference between these two groups became clear with the advent of the transmission electron microscope. The gram-positive cell wall consists of a single 20 to 80 nm thick homogeneous **peptidoglycan** or **murein** layer lying outside the plasma membrane. In contrast, the gram-negative cell wall is quite complex. It has a 2 to 7 nm peptidoglycan layer surrounded by a 7 to 8 nm thick **outer membrane.** Because of the thicker peptidoglycan layer, the walls of gram-positive cells are stronger than those of gram-negative bacteria. Microbiologists often call all the structures from the plasma

membrane outward the **envelope** or cell envelope. This includes the wall and structures like capsules.

Frequently a space is seen between the plasma membrane and the outer membrane in electron micrographs of gramnegative bacteria, and sometimes a similar but smaller gap may be observed between the plasma membrane and wall in grampositive bacteria. This space is called the **periplasmic space.** Recent evidence indicates that the periplasmic space may be filled with a loose network of peptidoglycan. Possibly it is more a gel than a fluid-filled space. The substance that occupies the periplasmic space is the **periplasm.** Gram-positive cells may have periplasm even if they lack a discrete, obvious periplasmic space. Size estimates of the periplasmic space in gram-negative bacteria range from 1 nm to as great as 71 nm.

When cell walls are disrupted carefully or removed without disturbing the underlying plasma membrane, periplasmic enzymes and other proteins are released and may be easily studied. The periplasmic space of gram-negative bacteria contains many proteins that participate in nutrient acquisition. The periplasmic space also contains enzymes involved in peptidoglycan synthesis and the modification of toxic compounds that could harm the cell. Gram positive bacteria may not have a visible periplasmic space and do not appear to have as many periplasmic proteins; rather, they secrete several enzymes that ordinarily would be periplasmic in gram-negative bacteria. Such secreted enzymes are often called **exoenzymes.** Some enzymes remain in the periplasm and are attached to the plasma membrane.

Peptidoglycan Structure

Peptidoglycan or murein is an enormous polymer composed of many identical subunits. The polymer contains two sugar derivatives, *N*-acetylglucosamine and *N*-acetylmuramic acid (the lactyl ether of *N*-acetylglucosamine), and several different

amino acids, three of which—D-glutamic acid, D-alanine, and *meso*-diaminopimelic acid—are not found in proteins. The presence of D-amino acids protects against attack by most peptidases. The peptidoglycan subunitpresent in most gram-negative bacteria and many gram-positive ones**.**

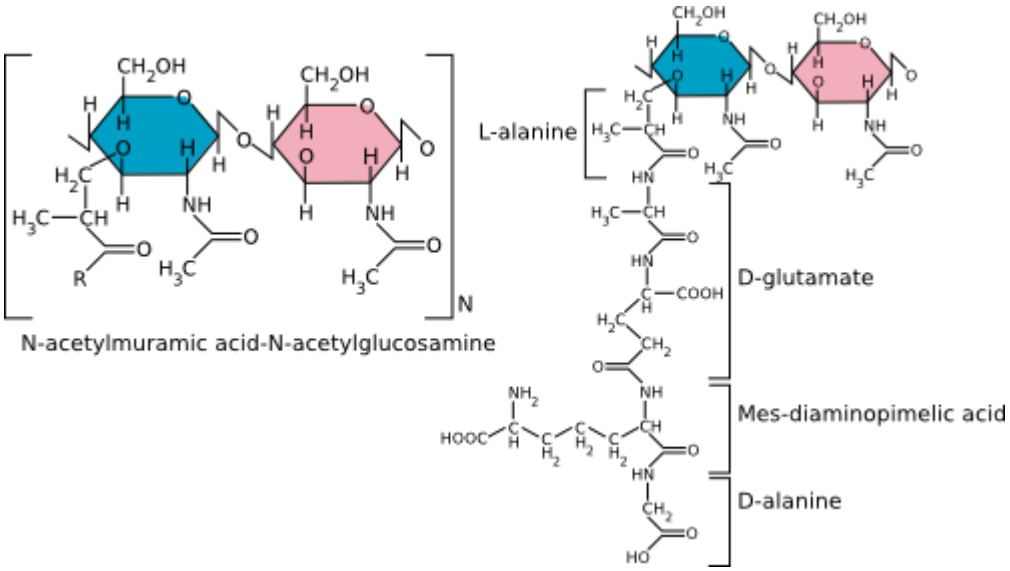


Figure The chemical structure of peptidoglycan. The generalized peptidoglycan monomer showing the two sugars that make up the backbone. The R group consists of four amino acids, with the best-studied cell walls containing L-alanine, D-alanine, D-glutamic acid and diaminopimelic acid.

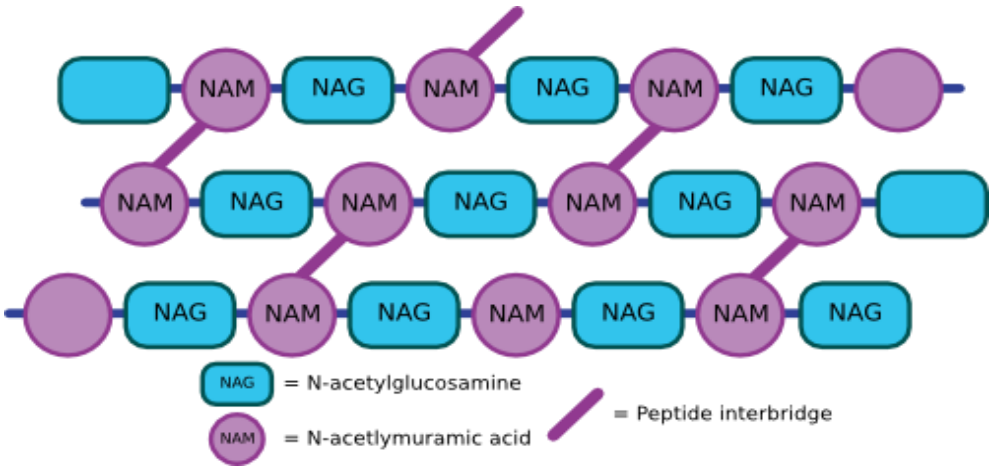


Figure A cartoon of the peptiodglycan mesh. The peptidoglycan polymers then crosslink with other peptidoglycan chains to form a complex mesh that wraps the cell in a structure a kin to chicken wire.

The backbone of this polymer is composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues. A peptide chain of four alternating D- and L-amino acids is connected to the carboxyl group of *N*-acetylmuramic acid. Many bacteria substitute another diaminoacid, usually L-lysine, in the third position for *meso*-diaminopimelic acid. Chains of linked peptidoglycan subunits are joined by crosslinks between the peptides. Often the carboxyl group of the terminal D-alanine is connected directly to the amino group of diaminopimelic acid, but a **peptide interbridge** may be used instead. Most gram-negative cell wall peptidoglycan lacks the peptide interbridge. This cross-linking results in an enormous peptidoglycan sac that is actually one dense, interconnected network.

These sacs have been isolated from gram-positive bacteria and are strong enough to retain their shape and integrity , yet they are elastic and somewhat stretchable, unlike cellulose. They also must be porous, as molecules can penetrate them.

Gram-Positive Cell Walls

Normally the thick, homogeneous cell wall of gram-positive bacteria is composed primarily of peptidoglycan, which often contains a peptide interbridge. However gram-positive cell walls usually also contain large amounts of **teichoic acids,** polymers of glycerol or ribitol joined by phosphate groups. Amino acids such as D-alanine or sugars like glucose are attached to the glycerol and ribitol groups. The teichoic acids are connected to either the peptidoglycan itself by a covalent bond with the six hydroxyl of *N*-acetylmuramic acid or to plasma membrane lipids; in the latter case they are called lipoteichoic acids. Teichoic acids appear to extend to the surface of the peptidoglycan, and, because they are negatively charged, help give the gram-

positive cell wall its negative charge. The functions of these molecules are still unclear, but they may be important in maintaining the structure of the wall. Teichoic acids are not present in gram-negative bacteria.

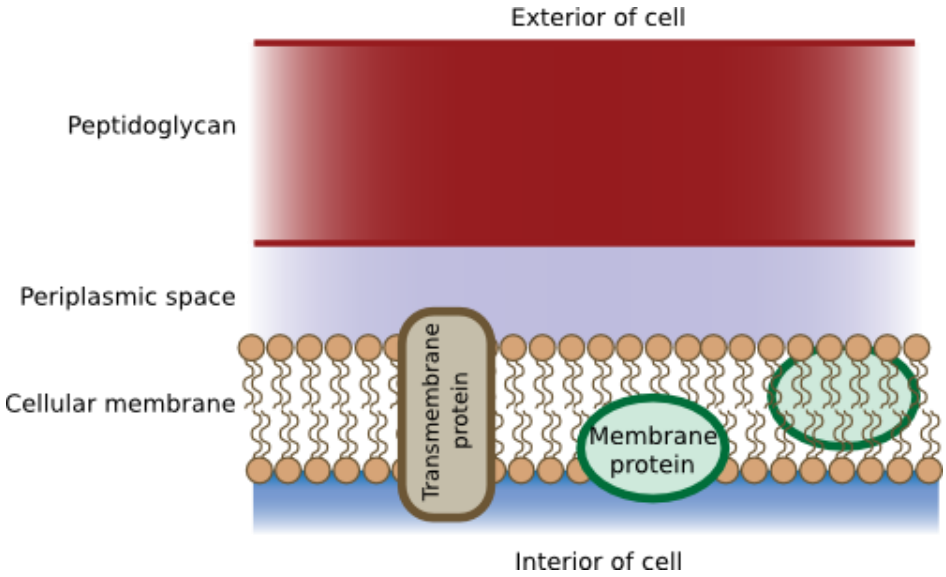


Figure The gram-positive cell wall. The cell wall is made mostly of peptidoglycan, interspersed with teichoic acid which knits the different layers together. The amount of crosslinking is higher and the wall is thicker than in gram-negative cell walls.

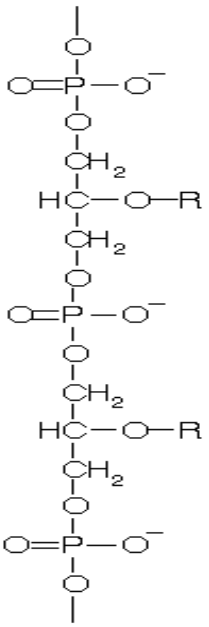


Figure Teichoic acid. Teichoic acid is a long, thin molecule that weaves through the peptidoglycan.

Gram-Negative Cell Walls

The thin peptidoglycan layer next to the plasma membrane may constitute not more than 5 to 10% of the wall weight. In *E. coli* it is about 2 nm thick and contains only one or two layers or sheets of peptidoglycan. The outer membrane lies outside the thin peptidoglycan layer.

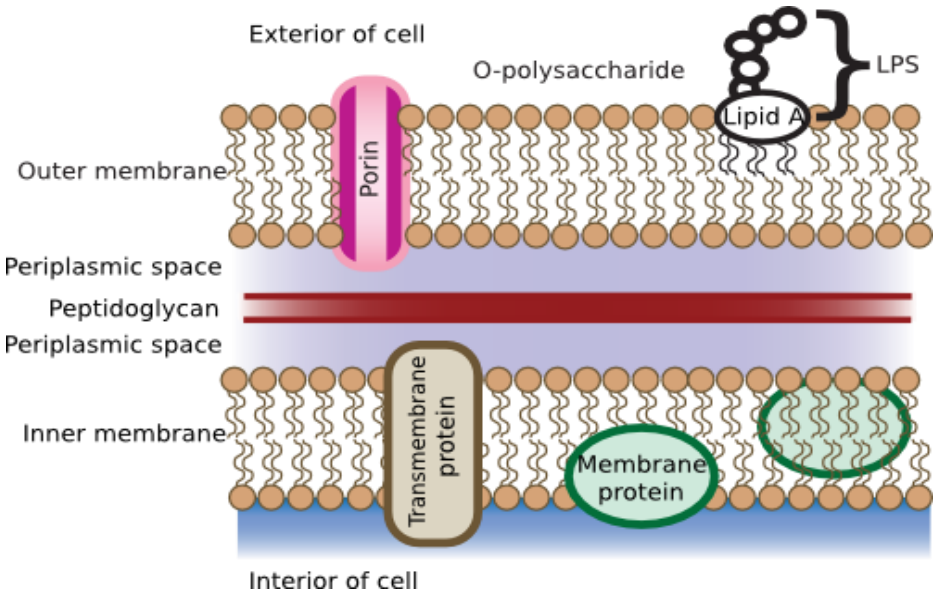


Figure The gram-negative cell wall. The cell wall in gram-negative bacteria contains much less peptidoglycan and is surrounded by an outer membrane. There is much less crosslinking between the peptidoglycan. LPS is also present in the outer membrane and penetrates into the surrounding environment.

The most abundant membrane protein is Braun's lipoprotein, a small lipoprotein covalently joined to the underlying peptidoglycan and embedded in the outer membrane by its hydrophobic end. The outer membrane and peptidoglycan are so firmly linked by this lipoprotein that they can be isolated as one unit. Another structure that may strengthen the gram-negative wall and hold the outer membrane in place is the adhesion site. The outer membrane and plasma membrane appear to be in direct contact at many locations in the gram-negative wall. In *E. coli* 20 to 100 nm areas of contact between the two membranes are seen in plasmolyzed cells. Adhesion sites may be regions of direct contact or possibly true membrane fusions. It has been proposed that substances can move into the cell through these adhesion sites rather than traveling through the periplasm. Possibly the most unusual constituents of the outer membrane

are its **lipopolysaccharides (LPSs).** These large, complex molecules contain both lipid and carbohydrate, and consist of three parts: (1) lipid A, (2) the core polysaccharide, and (3) the O side chain.

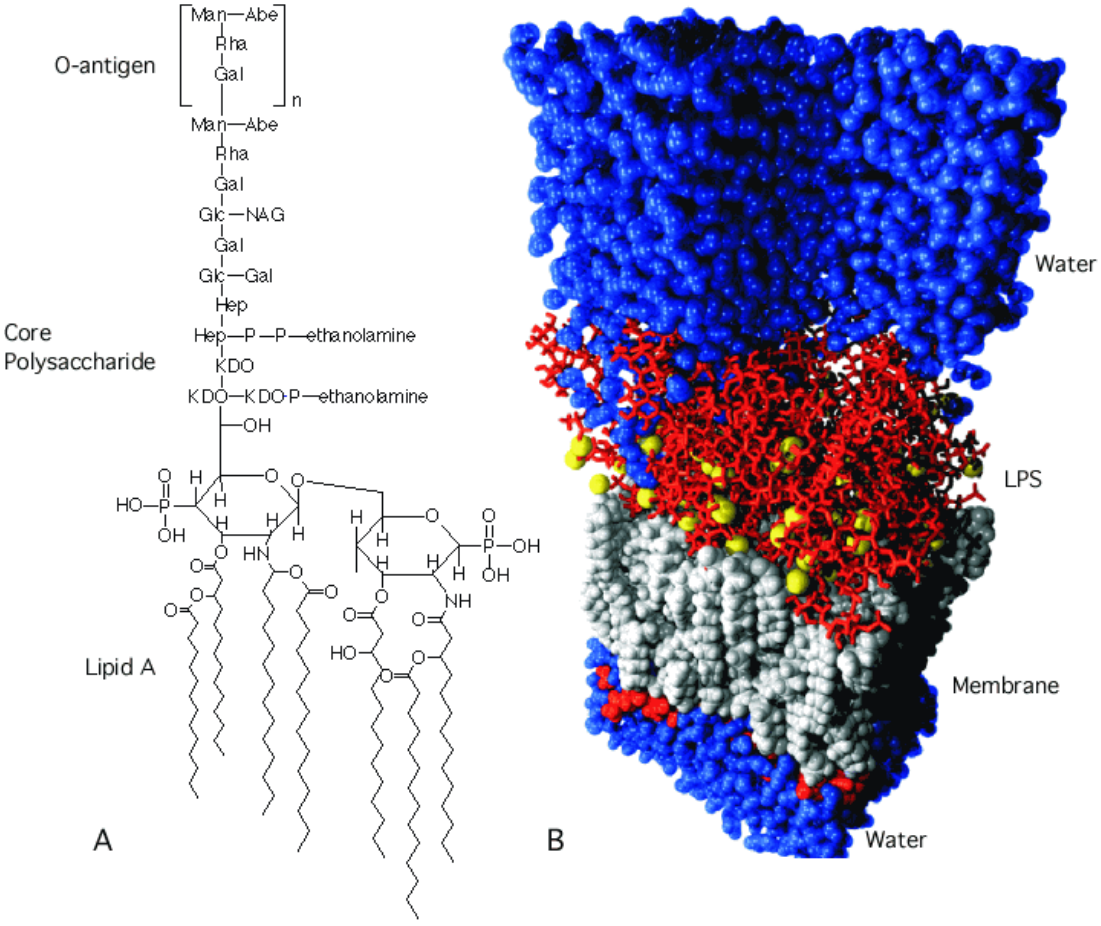


Figure The structure of LPS. LPS is composed of three sections: the lipid A region, a conserved core polysaccharide, and a highly variable O-polysaccharide. (A) The chemical structure of LPS. (B) A molecular model of the membrane from *Pseudomonas aeruginosa*. Source (T. P. Straatsma, Pacific Northwest National Laboratory)

The **lipid A** region contains two glucosamine sugar derivatives, each with three fatty acids and phosphate or pyrophosphate attached. It is buried in the outer membrane and the remainder of the LPS molecule projects from the surface. The **core polysaccharide** is joined to lipid A. In *Salmonella* it is constructed of 10 sugars, many of them unusual in structure. The **O side chain** or **O antigen** is a polysaccharide chain extending outward from the core. It has several peculiar sugars and varies in composition between bacterial strains. Although O side chains are readily recognized by host antibodies, gramnegative bacteria may thwart host defenses by rapidly changing the nature of their O side chains to avoid detection.

Antibody interaction with the LPS before reaching the outer membrane proper may also protect the cell wall from direct attack. The LPS is important for several reasons other than the avoidance of host defenses. Since the core polysaccharide usually contains charged sugars and phosphate, LPS contributes to the negative charge on the bacterial surface. Lipid A is a major constituent of the outer membrane, and the LPS helps stabilize membrane structure. Furthermore, lipid A often is toxic; as a result the LPS can act as an endotoxin and cause some of the symptoms that arise in gram-negative bacterial infections.

A most important outer membrane function is to serve as a protective barrier. It prevents or slows the entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium. Even so, the outer membrane is more permeable than the plasma membrane and permits the passage of small molecules like glucose and other monosaccharides. This is due to the presence of special **porin protein**. Three porin molecules cluster together and span the outer membrane to form a narrow channel through which molecules smaller than about 600 to 700 daltons can pass. Larger molecules such as vitamin B12 must be transported across the outer membrane by specific carriers. The outer membrane also prevents the loss of constituents like periplasmic enzymes.

The Mechanism of Gram Staining

Although several explanations have been given for the Gramstain reaction results, it seems likely that the difference between gram-positive and gram-negative bacteria is due to the physical nature of their cell walls. If the cell wall is removed from grampositive bacteria, they become gram negative. The peptidoglycan itself is not stained; instead it seems to act as a permeability barrier preventing loss of crystal violet. During the procedure the bacteria are first stained with crystal violet and next treated with

iodine to promote dye retention. When gram-positive bacteria then are decolorized with ethanol, the alcohol is thought to shrink the pores of the thick peptidoglycan. Thus the dye-iodine complex is retained during the short decolorization step and the bacteria remain purple. In contrast, gram-negative peptidoglycan is very thin, not as highly cross-linked, and has larger pores. Alcohol treatment also may extract enough lipid from the gramnegative wall to increase its porosity further. For these reasons, alcohol more readily removes the purple crystal violet-iodine complex from gram-negative bacteria.

Components External to the Cell Wall

Bacteria have a variety of structures outside the cell wall that can function in protection, attachment to objects, and cell movement.

Capsules, Slime Layers, and S-Layers

Some bacteria have a layer of material lying outside the cell wall. When the layer is well organized and not easily washed off, it is called a **capsule.** A **slime layer** is a zone of diffuse, unorganized material that is removed easily. A **glycocalyx** is a network of polysaccharides extending from the surface of bacteria and other cells (in

this sense it could encompass both capsules and slime layers). Capsules and slime layers usually are composed of polysaccharides, but they may be constructed of other materials. For example, *Klebsiella pneumonia* has a capsule of poly- D-glutamic acid. Capsules are clearly visible in the light microscope when negative stains or special capsule stains are employed

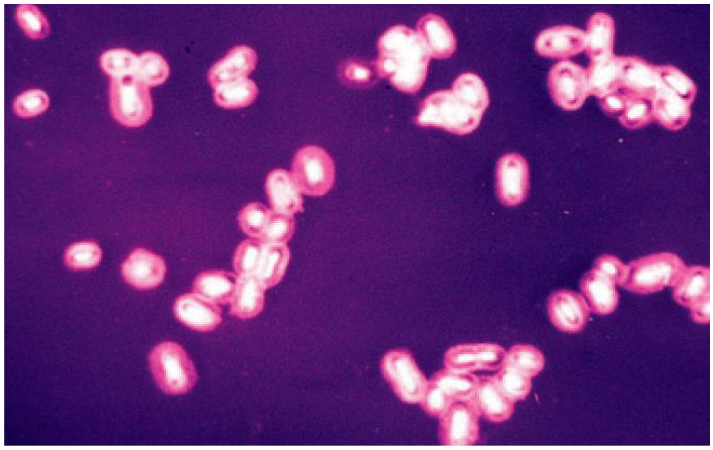


Figure Bacterial Capsules. (**a**) *Klebsiella pneumoniae* with its capsule stained for observation in the light microscope

Although capsules are not required for bacterial growth and reproduction in laboratory cultures, they do confer several advantages when bacteria grow in their normal habitats. They help bacteria resist phagocytosis by host phagocytic cells. *Streptococcus pneumoniae* provides a classic example. When it lacks a capsule, it is destroyed easily and does not cause disease, whereas the capsulated variant quickly kills mice. Capsules contain a great deal of water and can protect bacteria against desiccation. They exclude bacterial viruses and most hydrophobic toxic materials such as detergents. The glycocalyx also aids bacterial attachment to surfaces of solid objects in aquatic environments or to tissue surfaces in plant and animal hosts.

Gliding bacteria often produce slime, which presumably aids in their motility Many gram-positive and gram-negative bacteria have a regularly structured layer called

an **S-layer** on their surface. Slayers also are very common among Archaea, where they may be the only wall structure outside the plasma membrane. The Slayer has a pattern something like floor tiles and is composed of protein or glycoprotein. In gram-negative bacteria the S-layer adheres directly to the outer membrane; it is associated with the peptidoglycan surface in gram-positive bacteria. It may protect the cell against ion and pH fluctuations, osmotic stress, enzymes, or the predacious bacterium *Bdellovibrio*. The S-layer also helps maintain the shape and envelope rigidity of at least some bacterial cells. It can promote cell adhesion to surfaces. Finally, the layer seems to protect some pathogens against complement attack and phagocytosis, thus contributing to their virulence.

Pili and Fimbriae

Many gram-negative bacteria have short, fine, hairlike appendages that are thinner than flagella and not involved in motility. These are usually called **fimbriae** (s., **fimbria**).

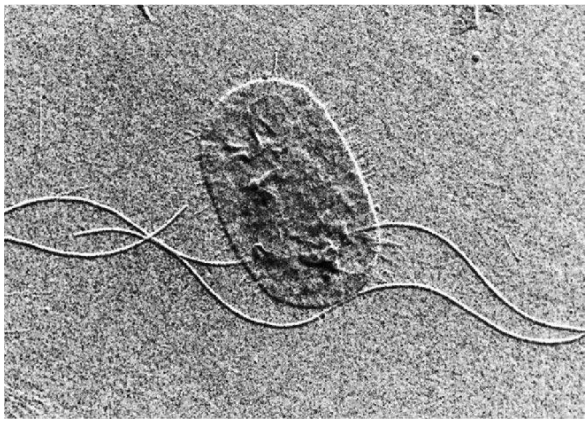


Figure Flagella and Fimbriae. The long flagella and the numerous shorter fimbriae are very evident in this electron micrograph of *Proteus vulgaris*.

Although a cell may be covered with up to 1,000 fimbriae, they are only visible in an electron microscope due to their small size. They seem to be slender tubes composed of helically arranged protein subunits and are about 3 to 10 nm in diameter and up to several micrometers long. At least some types of fimbriae attach bacteria to solid surfaces

such as rocks in streams and host tissues. **Sex pili** (s., **pilus**) are similar appendages, about 1 to 10 per cell, that differ from fimbriae in the following ways. Pili often are larger than fimbriae (around 9 to 10 nm in diameter). They are genetically determined by sex factors or conjugative plasmids and are required for bacterial mating. Some bacterial viruses attach specifically to receptors on sex pili at the start of their reproductive cycle.

Flagella and Motility

Most motile bacteria move by use of **flagella** (s., **flagellum**), threadlike locomotor appendages extending outward from the plasma membrane and cell wall. They are slender, rigid structures, about 20 nm across and up to 15 or 20 _m long. Flagella are so thin they cannot be observed directly with a bright-field microscope, but must be stained with special techniques designed to increase their thickness. The detailed structure of a flagellum can only be seen in the electron microscope. Bacterial species often differ distinctively in their patterns of flagella distribution. **Polar** or **Monotrichous** bacteria (*trichous* means hair) have one flagellum; if it is located at an end, it is said to be a **polar flagellum**. **Amphitrichous** bacteria (*amphi* means "on both sides" have a single flagellum at each pole. In contrast, **lophotrichous** bacteria (*lopho* means tuft) have a cluster of flagella at one or both ends. Flagella are spread fairly evenly over the whole surface of **peritrichous** (*peri* means "around") bacteria. Flagellation patterns are very useful in identifying bacteria.

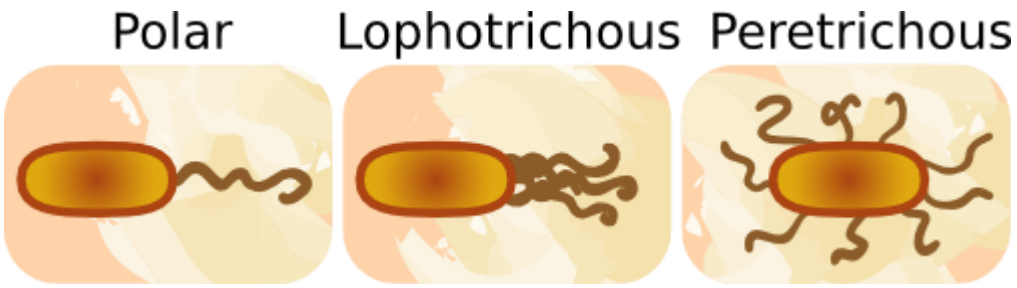


Figure Flagellar arrangements. A cartoon of several common flagellar arrangements.

Flagellar Ultrastructure

Transmission electron microscope studies have shown that the bacterial flagellum is composed of three parts. (1) The longest and most obvious portion is the **filament,** which extends from the cell surface to the tip. (2) A **basal body** is embedded in the cell;

and (3) a short, curved segment, the **hook,** links the filament to its basal body and acts as a flexible coupling. The filament is a hollow, rigid cylinder constructed of a single protein called **flagellin,** which ranges in molecular weight from 30,000 to 60,000. The filament ends with a capping protein. Some bacteria have sheaths surrounding their flagella. For example *Bdellovibrio* has a membranous structure surrounding the filament. *Vibrio cholerae* has a lipopolysaccharide sheath. The hook and basal body are quite different from the filament. Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex part of a flagellum.

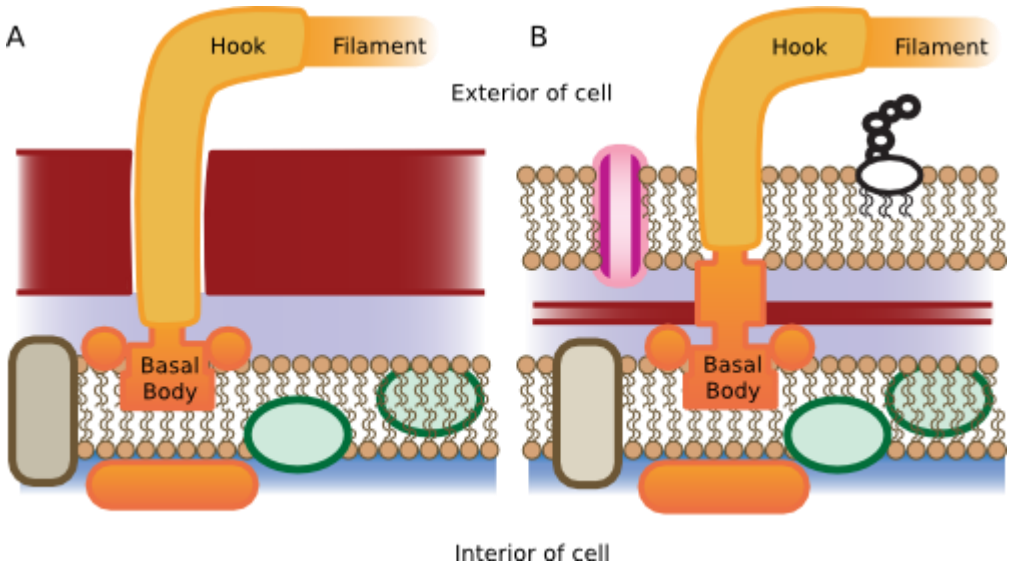


Figure Flagella attachment in bacteria. Flagella are attached by a hook and rings that anchor it to the cell wall of the microorganism. In gram-positive bacteria (A) the rings are located in the cytoplasmic membrane and the flagella passes through the peptidoglycan to the outside environment. In gram-negative bacteria (B) there are additional protein rings in the outer membrane.

In *E.coli* and most gram-negative bacteria, the body has four rings connected to a central rod. The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers, respectively. The inner M ring contacts the plasma membrane. Grampositive bacteria have only two basal body rings, an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan.

The Mechanism of Flagellar Movement

Procaryotic flagella operate differently from eucaryotic flagella. The filament is in the shape of a rigid helix, and the bacterium moves when this helix rotates. Considerable evidence shows that flagella act just like propellers on a boat. Bacterial mutants with straight flagella or abnormally long hook regions (polyhook mutants) cannot swim. When bacteria are tethered to a glass slide using antibodies to filament or

hook proteins, the cell body rotates rapidly about the stationary flagellum. If polystyrene-latex beads are attached to flagella, the beads spin about the flagellar axis due to flagellar rotation. The flagellar motor can rotate very rapidly. The *E. coli* motor rotates 270 revolutions per second; *Vibrio alginolyticus* averages 1,100 rps.

The direction of flagellar rotation determines the nature of bacterial movement. Monotrichous, polar flagella rotate counterclockwise (when viewed from outside the cell) during normal forward movement, whereas the cell itself rotates slowly clockwise. The rotating helical flagellar filament thrusts the cell forward in a run with the flagellum trailing behind. Monotrichous bacteria stop and tumble randomly by reversing the direction of flagellar rotation. Peritrichously flagellated bacteria operate in a somewhat similar way. To move forward, the flagella rotate counterclockwise. As they do so, they bend at their hooks to form a rotating bundle that propels them forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles. Because bacteria swim through rotation of their rigid flagella, there must be some sort of motor at the base. A rod or shaft extends from the hook and ends in the M ring, which can rotate freely in the plasma membrane.

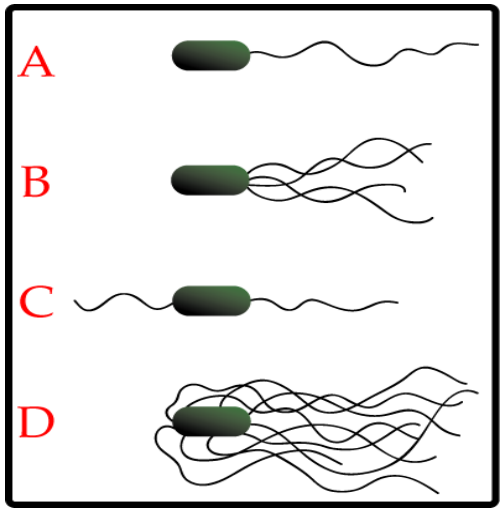


Figure Flagellar Motility. The relationship of flagellar rotation to bacterial movement. Parts (**a)** and (**b)**, describe the motion of monotrichous, polar bacteria. Parts (**c**) and (**d**) illustrate the movements of peritrichous organisms.

It is believed that the S ring is attached to the cell wall in gram-positive cells and does not rotate. The P and L rings of gram-negative bacteria would act as bearings for the rotating rod. There is some evidence that the basal body is a passive structure and rotates within a membrane-embedded protein complex much like the rotor of an electrical motor turns in the center of a ring of electromagnets (the stator). The rotor portion of the motor seems to be made primarily of a rod, the M ring, and a C ring joined to it on the cytoplasmic side of the basal body. These two rings are made of several proteins; Fli G is particularly important in generating flagellar rotation. The two most important proteins in the stator part of the motor are Mot A and Mot B. These form a proton channel through the plasma membrane, and Mot B also anchors the Mot complex to cell wall peptidoglycan. There is some evidence that Mot A and Fli G directly interact during flagellar rotation. This rotation is driven by proton or sodium gradients in procaryotes, not directly by ATP as is the case with eucaryotic flagella. The flagellum is a very effective swimming device.

From the bacterium's point of view, swimming is quite a task because the surrounding water seems as thick and viscous as molasses. The cell must bore through the water with its helical or corkscrewshaped flagella, and if flagellar activity ceases, it stops almost instantly. Despite such environmental resistance to movement, bacteria can swim from 20 to almost 90 _m/second. This is equivalent to traveling from 2 to over 100 cell lengths per second. In contrast, an exceptionally fast 6 ft human might be able to run around 5 body lengths per second. Bacteria can move by mechanisms other than flagellar rotation. Spirochetes are helical bacteria that travel through viscous substances such as mucus or mud by flexing and spinning movements caused by a special **axial filament** composed of periplasmic flagella. A very different type of motility, **gliding**

motility, is employed by many bacteria: cyanobacteria , myxobacteria and cytophagas, and some mycoplasmas.

Chemotaxis

Bacteria do not always swim aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products. Movement toward chemical attractants and away from repellents is known as **chemotaxis.** Such behavior is of obvious advantage to bacteria.

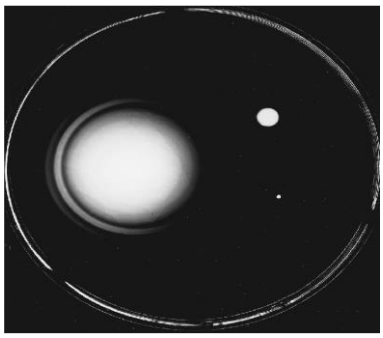


Figure Positive Bacterial Chemotaxis. Chemotaxis can be demonstrated on an agar plate that contains various nutrients. Positive chemotaxis by *Escherichia coli* on the left. The outer ring is composed of bacteria consuming serine. The second ring was formed by *E. coli* consuming aspartate, a less powerful attractant. The upper right colony is composed of motile, but nonchemotactic mutants. The bottom right colony is formed by nonmotile bacteria.

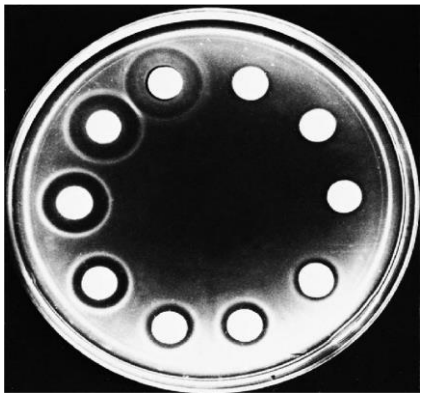


Figure Negative Bacterial Chemotaxis. Negative chemotaxis by *E. coli* in response to the repellent acetate. The bright disks are plugs of concentrated agar containing acetate that have been placed in dilute agar inoculated with *E. coli.* Acetate concentration increases from zero at the top right to 3 M at top left. Note the increasing size of bacteria-free zones with increasing acetate. The bacteria have migrated for 30 minutes.

Chemotaxis may be demonstrated by observing bacteria in the chemical gradient produced when a thin capillary tube is filled with an attractant and lowered into a bacterial suspension. As the attractant diffuses from the end of the capillary, bacteria collect and swim up the tube. The number of bacteria within the capillary after a short length of time reflects the strength of attraction and rate of chemotaxis. Positive and negative chemotaxis also can be studied with petri dish cultures. If bacteria are placed in the center of a dish of agar containing an attractant, the bacteria will exhaust the local supply and then swim outward following the attractant gradient they have created. The result is an expanding ring of bacteria. When a disk of repellent is placed in a petri dish of semisolid agar and bacteria, the bacteria will swim away from the repellent, creating a clear zone around the disk.

Bacteria can respond to very low levels of attractants (about 10-8 M for some sugars), the magnitude of their response increasing with attractant concentration. Usually they sense repellents only at higher concentrations. If an attractant and a repellent are present together, the bacterium will compare both signals and respond to

the chemical with the most effective concentration. Attractants and repellents are detected by **chemoreceptors,** special proteins that bind chemicals and transmit signals to the other components of the chemosensing system. About 20 attractant chemoreceptors and 10 chemoreceptors for repellents have been discovered thus far. These chemoreceptor proteins may be located in the periplasmic space or the plasma membrane. Some receptors participate in the initial stages of sugar transport into the cell. The chemotactic behavior of bacteria has been studied using the tracking microscope, a microscope with a moving stage that automatically keeps an individual bacterium in view. In the absence of a chemical gradient, *E. coli* and other bacteria move randomly. A bacterium travels in a straight or slightly curved line, a **run,** for a few seconds; then it will stop and **tumble** or **twiddle** about. The tumble is followed by a run in a different direction.

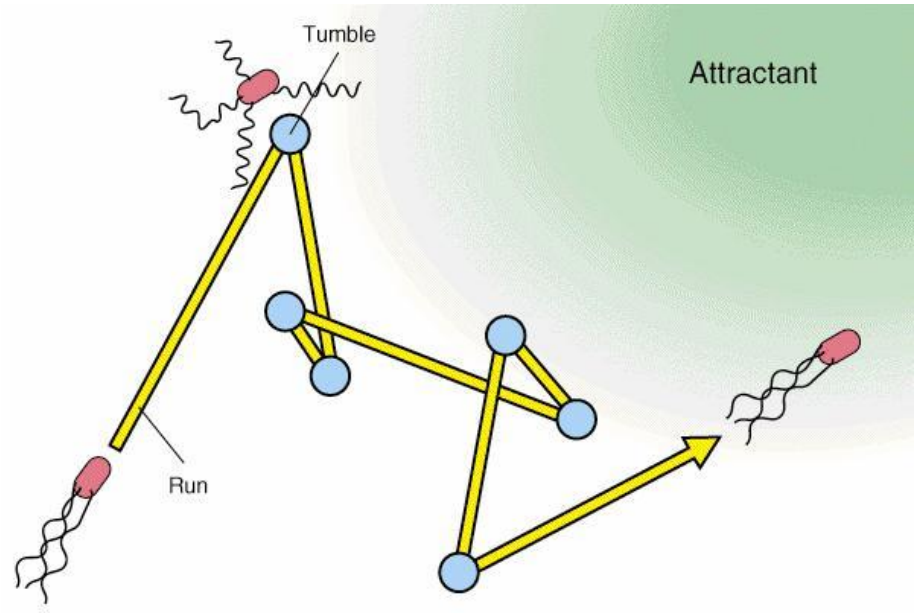


Figure Directed Movement in Bacteria. Random movement of a bacterium in the absence of a concentration gradient. Tumbling frequency is fairly constant. Movement in an attractant gradient. Tumbling frequency is reduced when the bacterium

is moving up the gradient. Therefore runs in the direction of increasing attractant are longer.

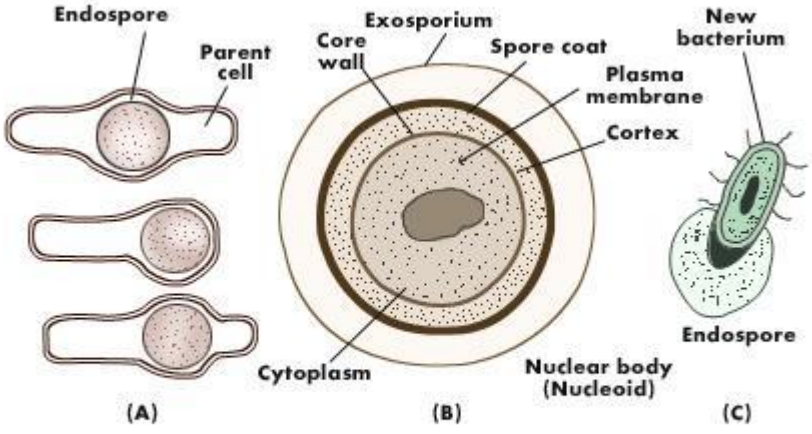
When the bacterium is exposed to an attractant gradient, it tumbles less frequently (or has longer runs) when traveling up the gradient, but tumbles at normal frequency if moving down the gradient. Consequently the bacterium moves up the gradient. Behavior is shaped by temporal changes in chemical concentration: the bacterium compares its current environment with that experienced a few moments previously; if the attractant concentration is higher, tumbling is suppressed and the run is longer. The opposite response occurs with a repellent gradient. Tumbling frequency decreases (the run lengthens) when the bacterium moves down the gradient away from the repellent. When the environment is constant, bacteria tend to move in a random walk. That is, there is a random sequence of runs followed by tumbles. If a run is in the direction of improving conditions, tumbles are suppressed so that the cell tends to move in the preferred direction. This is said to be a biased random walk toward attractants and away from repellants. Individual cells do not choose a particular direction. Instead, they determine whether or not to continue in the same direction.

The Bacterial Endospore

A number of gram-positive bacteria can form a special resistant, dormant structure called an **endospore.** Endospores develop within vegetative bacterial cells of several genera: *Bacillus* and *Clostridium* (rods), *Sporosarcina* (cocci), and others. These structures are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants, and desiccation. In fact, some endospores have remained viable for around 100,000 years, and actinomycete spores (which are not true endospores) have been recovered alive after burial in the mud for 7,500 years. Because of their resistance and the fact that several species of endospore-forming bacteria are dangerous pathogens, endospores are of great practical importance

in food, industrial, and medical microbiology. This is because it is essential to be able to sterilize solutions and solid objects.

Endospores often survive boiling for an hour or more; therefore autoclaves must be used to sterilize many materials. Endospores are also of considerable theoretical interest. Because bacteria manufacture these intricate entities in a very organized fashion over a period of a few hours, spore formation is well suited for research on the construction of complex biological structures. In the environment, endospores aid in survival when moisture or nutrients are scarce. Endospores can be examined with both light and electron microscopes. Because spores are impermeable to most stains, they often are seen as colorless areas in bacteria treated with methylene blue and other simple stains; special spore stains are used to make them clearly visible. Spore position in the mother cell or **sporangium** frequently differs among species, making it of considerable value in identification. Spores may be centrally located, close to one end (subterminal), or definitely terminal. Sometimes a spore is so large that it swells the sporangium.



Endospore formation. A, Endospores according to their position in parent cells. B, An edospore in cross-section. C, Germination of endospore

The spore often is surrounded by a thin, delicate covering called the **exosporium.**A **spore coat** lies beneath the exosporium, is composed of several protein layers, and may be fairly thick. It is impermeable and responsible for the spore's resistance to chemicals. The **cortex,** which may occupy as much as half the spore volume, rests beneath the spore coat. It is made of a peptidoglycan that is less cross-linked than that in vegetative cells. The **spore cell wall** (or core wall) is inside the cortex and surrounds the protoplast or **core.** The core has the normal cell structures such as ribosomes and a nucleoid, but is metabolically inactive. It is still not known precisely why the endospore is so resistant to heat and other lethal agents. As much as 15% of the spore's dry weight consists of **dipicolinic acid** complexed with calcium ions, which is located in the core. It has long been thought that dipicolinic acid was directly involved in

spore heat resistance, but heat-resistant mutants lacking dipicolinic acid now have been isolated. Calcium does aid in resistance to wet heat, oxidizing agents, and sometimes dry heat. It may be that calcium-dipicolinate often stabilizes spore nucleic acids.

Recently specialized small, acid-soluble DNA-binding proteins have been discovered in the endospore. They saturate spore DNA and protect it from heat, radiation, dessication, and chemicals. Dehydration of the protoplast appears to be very important in heat resistance. The cortex may osmotically remove water from the protoplast, thereby protecting it from both heat and radiation damage. The spore coat also seems to protect against enzymes and chemicals such as hydrogen peroxide. Finally, spores contain some DNA repair enzymes. DNA is repaired during germination and outgrowth after the core has become active once again. In summary, endospore heat resistance probably is due to several factors: calcium-dipicolinate and acid-soluble protein stabilization of DNA, protoplast dehydration, the spore coat, DNA repair, the greater stability of cell proteins in bacteria adapted to growth at high temperatures, and

others. Spore formation, **sporogenesis** or **sporulation,** normally commences when growth ceases due to lack of nutrients. It is a complex process and may be divided into seven stages.

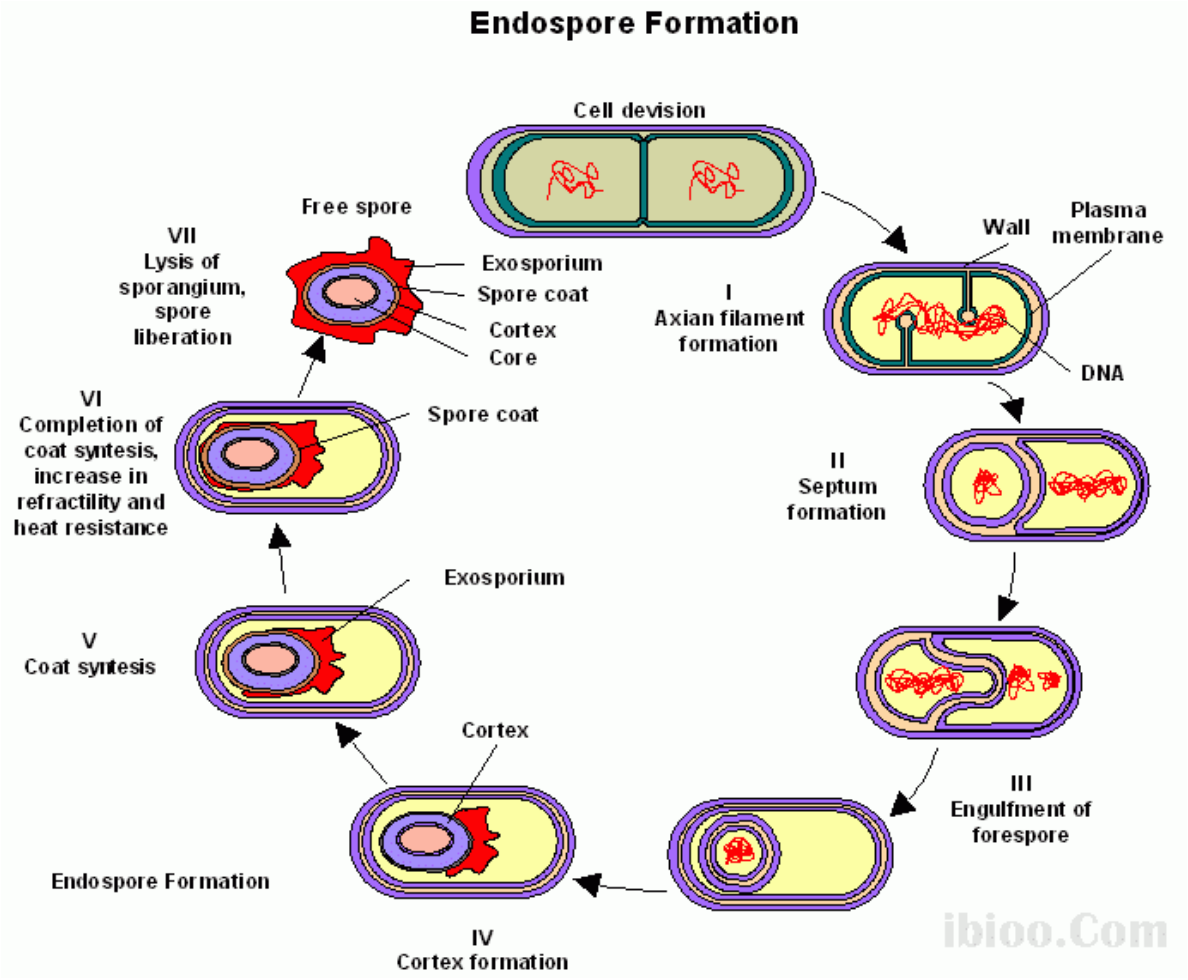


Figure A typical sporulation cycle in *Bacillus* species from the active vegetative cell to release and germination.

An axial filament of nuclear material forms (stage I), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum (stage II). The membrane continues to grow and engulfs the immature spore in a second membrane (stage III). Next, cortex is laid down in the space between the two

membranes, and both calcium and dipicolinic acid are accumulated (stage IV). Protein coats then are formed around the cortex (stage V), and maturation of the spore occurs (stage VI). Finally, lytic enzymes destroy the sporangium releasing the spore (stage VII). Sporulation requires only about 10 hours in *Bacillus megaterium.* The transformation of dormant spores into active vegetative cells seems almost as complex a process as sporogenesis. It occurs in three stages: (1) activation, (2) germination, and (3) outgrowth. Often an endospore will not germinate successfully, even in a nutrient-rich medium, unless it has been activated.

Activation is a reversible process that prepares spores for germination and usually results from treatments like heating. It is followed by **germination,** the breaking of the spore's dormant state. This process is characterized by spore swelling, rupture or absorption of the spore coat, loss of resistance to heat and other stresses, loss of refractility, release of spore components, and increase in metabolic activity. Many normal

metabolites or nutrients (e.g., amino acids and sugars) can trigger germination after activation. Germination is followed by the third stage, outgrowth. The spore protoplast makes new components, emerges from the remains of the spore coat, and develops again into an active bacterium.

An Overview of Eucaryotic Cell Structure

The most obvious difference between eucaryotic and prokaryotic cells is in their use of membranes. Eucaryotic cells have membranedelimited nuclei, and membranes also play a prominent part in the structure of many other organelles (**figures**). **Organelles** are intracellular structures that perform specific functions in cells analogous to the functions of organs in the body. The name organelle (little organ) was coined because biologists saw a parallel between the relationship of organelles to a cell and that of organs to the whole body. It is not satisfactory to define organelles as membrane-bound structures because this would exclude such components as ribosomes and

bacterial flagella. The partitioning of the eucaryotic cell interior by membranes makes possible the placement of different biochemical and physiological functions in separate compartments so that they can more easily take place simultaneously under independent control and proper coordination. Large membrane surfaces make possible greater respiratory and photosynthetic activity because these processes are located exclusively in membranes. The intracytoplasmic membrane complex also serves as a transport system to move materials between different cell locations. Thus abundant membrane systems probably are necessary in eucaryotic cells because of their large volume and the need for adequate regulation, metabolic activity, and transport. **Table** briefly summarizes the functions of the major eucaryotic organelles.

SUMMARY TABLE 7.2 **Eukaryotic Cell Components**

Icons not to scale		Structure		Function
		Membrane	Components	
	**Nucleus**	Double ("envelope"); openings called nuclear pores	Chromosomes Nucleolus Nuclear lamina	Genetic information Assembly of ribosome subur Structural support
	**Ribosomes**	None	Complex of RNA and proteins	Protein synthesis
	**Endomembrane system** ***Rough ER***	Single; contains receptors for entry of selected proteins	Network of branching sacs Ribosomes associated	Protein synthesis and processing
	***Golgi apparatus***	Single; contains receptors for products of rough ER	Stack of flattened cisternae	Protein processing (e.g., glycosylation)
	***Smooth ER***	Single; contains enzymes for synthesizing phospholipids	Network of branching sacs Enzymes for synthesizing lipids	Lipid synthesis
	***Lysosomes***	Single; contains proton pumps	Acid hydrolases (catalyze hydrolysis reactions)	Digestion and recycling
	**Peroxisomes**	Single; contains transporters for selected macromolecules	Enzymes that catalyze oxidation reactions Catalase (processes peroxide)	Oxidation of fatty acids, ethanol, or other compounds
	**Vacuoles**	Single; contains transporters for selected molecules	Varies—pigments, oils, carbohydrates, water, or toxins	Varies—coloration, storage of oils, carbohydrates, water, or toxins
	**Mitochondria**	Double; inner contains enzymes for ATP production	Enzymes that catalyze oxidation-reduction reactions, ATP synthesis	ATP production
	**Chloroplasts**	Double; plus membrane-bound sacs in interior	Pigments Enzymes that catalyze oxidation-reduction reactions	Production of ATP and sugars via photosynthesis
	**Cytoskeleton**	None	Actin filaments Intermediate filaments Microtubules	Structural support; movement of materials; in some species, movement of whole cell
	**Plasma membrane**	Single; contains transport and receptor proteins	Phospholipid bilayer with transport and receptor proteins	Selective permeability—maintains intracellular environment
	**Cell wall**	None	Carbohydrate fibers running through carbohydrate or protein matrix	Protection, structural support

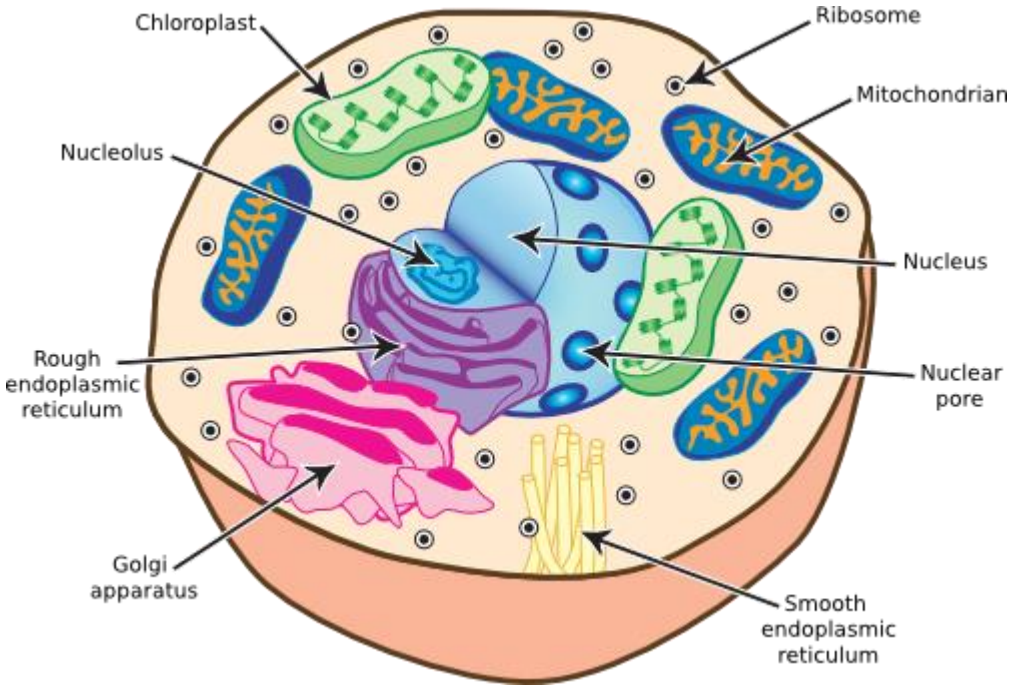


Figure Eukaryotic cell structure. A diagram of the common structures found in eukaryotic cells.

The Cytoplasmic Matrix, Microfilaments, Intermediate Filaments, and Microtubules

When a eucaryotic cell is examined at low power with the electron microscope, its larger organelles are seen to lie in an apparently featureless, homogeneous substance called the **cytoplasmic matrix.** The matrix, although superficially uninteresting, is actually one of the most important and complex parts of the cell. It is the "environment" of the organelles and the location of many important biochemical processes. Several physical changes seen in cells viscosity changes, cytoplasmic streaming, and others also are due to matrix activity. Water constitutes about 70 to 85% by weight of a eukaryotic cell. Thus a large part of the cytoplasmic matrix is water. Cellular water can exist in two different forms. Some of it is bulk or free water; this is normal, osmotically active water. Water also can exist as bound water or water of hydration. This water is bound to

the surface of proteins and other macromolecules and is osmotically inactive and more ordered than bulk water. There is some evidence that bound water is the site of many metabolic processes. The protein content of cells is so high that the cytoplasmic matrix often may be semicrystalline. Usually matrix pH is around neutrality, about pH 6.8 to 7.1, but can vary widely. For example, protozoan digestive vacuoles may reach pHs as low as 3 to 4.

Probably all eucaryotic cells have **microfilaments,** minute protein filaments, 4 to 7 nm in diameter, which may be either scattered within the cytoplasmic matrix or organized into networks and parallel arrays. Microfilaments are involved in cell motion and shape changes. Some examples of cellular movements associated with microfilament activity are the motion of pigment granules, amoeboid movement, and protoplasmic streaming in slime molds. The participation of microfilaments in cell movement is suggested by electron microscopic studies showing that they frequently are found at locations appropriate for such a role. For example, they are concentrated at the interface between stationary and flowing cytoplasm in plant cells and slime molds. Experiments using the drug cytochalasin B have provided additional evidence. Cytochalasin B disrupts microfilament structure and often simultaneously inhibits cell movements. However, because the drug has additional effects in cells, a direct cause-and-effect interpretation of these experiments is sometimes difficult. Microfilament protein has been isolated and analyzed chemically. It is an actin, very similar to the actin contractile protein of muscle tissue. This is further indirect evidence for microfilament involvement in cell movement.

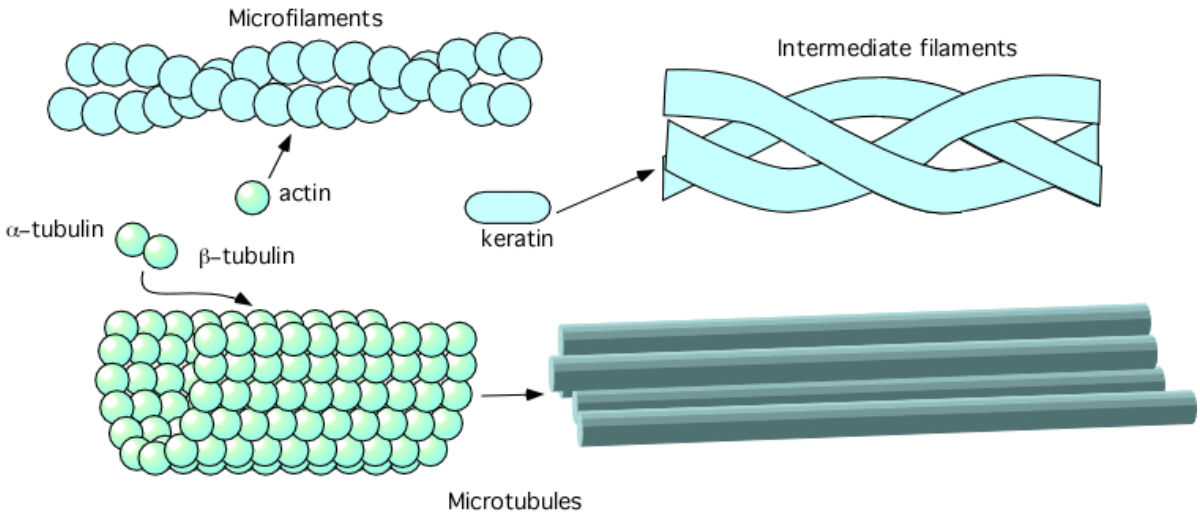


Figure Cytoskeletal elements. Eukaryotic cells have several different types of scaffolding proteins to help them keep their shape. Microfilaments, microtubules, and centrioles are all important structural elements.

A second type of small filamentous organelle in the cytoplasmic matrix is shaped like a thin cylinder about 25 nm in diameter. Because of its tubular nature this organelle is called a **microtubule.** Microtubules are complex structures constructed of two slightly different spherical protein subunits named tubulins, each of which is approximately 4 to 5 nm in diameter. These subunits are assembled in a helical arrangement to form a cylinder with an average of 13 subunits in one turn or circumference (**figure**). Microtubules serve at least three purposes: (1) they help maintain cell shape, (2) are involved with microfilaments in cell movements, and (3) participate in intracellular transport processes.

Microtubules also are present in structures that participate in cell or organelle movements—the mitotic spindle, cilia, and flagella. For example, the mitotic spindle is constructed of microtubules; when a dividing cell is treated with colchicine, the spindle is disrupted and chromosome separation blocked. Microtubules also are essential to the movement of eucaryotic cilia and flagella. Other kinds of filamentous components also are present in the matrix, the most important of which are the **intermediate filaments**

(about 8 to 10 nm in diameter). The microfilaments, microtubules, and intermediate filaments are major components of a vast, intricate network of interconnected filaments called the **cytoskeleton**. As mentioned previously, the cytoskeleton plays a role in both cell shape and movement. Procaryotes lack a true, organized cytoskeleton and may not possess actinlike proteins.

Endoplasmic reticulum

The endoplasmic reticulum (ER) is a finely divided system of interconnected membranes, consisting of tubules and vesicles that loop through the cell and are contiguous with the nuclear membrane. A drawing of the ER is shown in Figure. It functions in the synthesis of membranes and membrane proteins and is also involved in protein secretion. Not surprisingly, the ER is especially prominent in cells doing a large amount of protein secretion. The ER works very closely with the **Golgi apparatus** to carry out these functions. There is no structure in bacterial cells that is analogous to the ER, but many of the same functions are carried out on the inside surface of the cellular membrane in bacteria. ER comes in two types: rough ER and smooth ER.

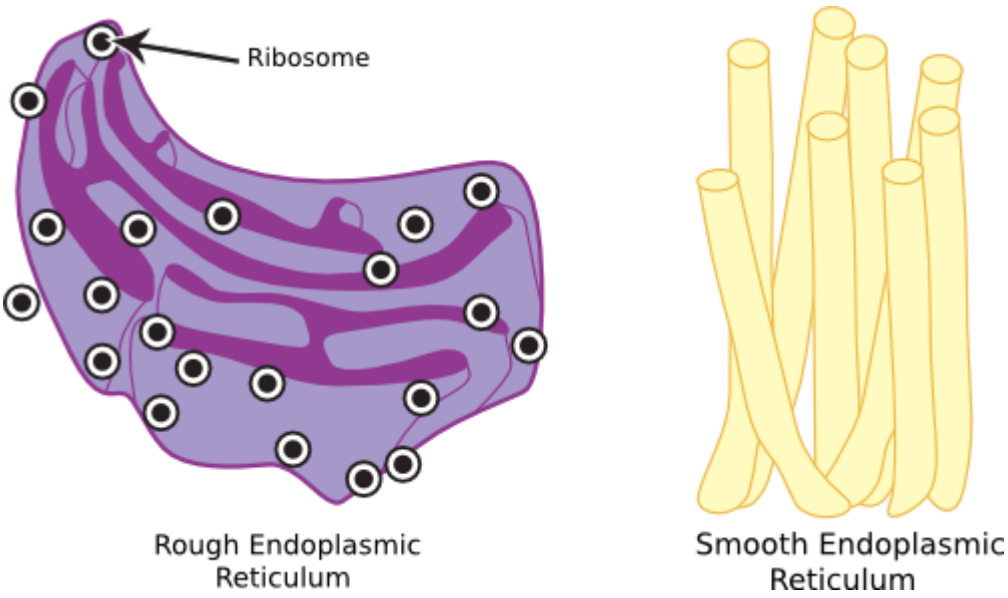


Figure The endosplasmic reticulum. Eukaryotic cells contain a network of passages that connect various elements of the cell and are also important in secretion.

Rough ER gets its appearance from the presence of ribosomes on its surface as seen in the electron microscope, and its function is the production, processing and export of proteins. During translation an appropriate signal guides the ribosome to the ER membrane and causes the protein to be synthesized directly across the membrane into the lumen of the ER. There proteins may be processed or modified by the addition of carbohydrates to form glycoproteins. After processing proteins move slowly through the ER and are packaged into vesicles of ER membrane called transition vesicles. These release from the ends of the ER and move by elements of the cytoskeleton either to the Golgi apparatus or to the plasma membrane. Once contact is made between the transition vesicle and the Golgi or the plasma membrane, the two fuse and release the contents of the vesicle into the target compartment. Smooth ER does not contain ribosomes and the lumen and membrane of smooth ER contain a variety of enzymes that perform many functions including modification of toxins and synthesis of steroids.

Golgi apparatus

The Golgi apparatus is an organelle containing a double membrane and it is mainly devoted to the processing of proteins synthesized in the ER. A drawing of the Golgi apparatus is shown in Figure. It is found in many eukaryotic cells, but it lacks a well-formed structure in many fungi and ciliate protozoa. It consists of regions of stacked contiguous membranes containing no ribosomes. Each membrane sac is 15 to 20 µm thick and separated from the next stack by about 30 µm. A complex network of tubes and vesicles extend from the edges of these sacs into the surrounding cytoplasm. The stack of membranes has a definite polarity with those near the ER (the cis face) having a different shape and enzyme content than those at the opposite end (the trans or maturing face). Studies of the Golgi apparatus appear to show material flowing into the cis face from vesicles, through the apparatus and then exiting at the trans face.

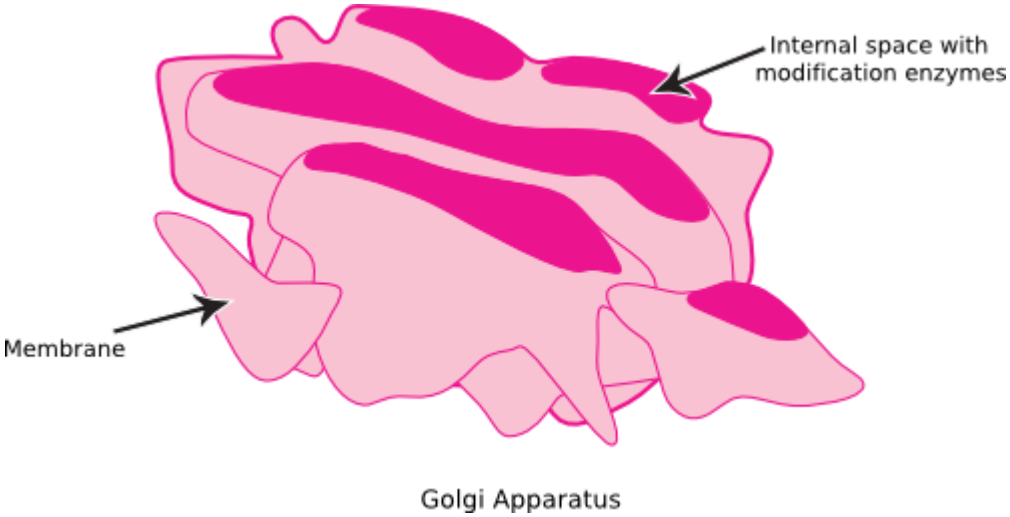


Figure The Golgi Apparatus. The Golgi apparatus is involved in the glycosylation and proteolytic processing of proteins that are to be secreted into various cellular organelles or to the outside environment. Proteins often pass through the Golgi apparatus as part of their maturation.

The role of the Golgi apparatus is to package material for export, but its exact function varies depending upon the organism. For example, *Giardia* and *Entamoeba* utilize Golgi apparatus to form cell walls during cyst formation. It often participates in the synthesis of cell membranes and the final processing of proteins before export. In many cases the Golgi apparatus contains glycosylation enzymes that add sugars to proteins as they move through its lumen. The type of glycosylation that takes place is dependent upon signals contained within the protein sequence. The Golgi also processes enzymes using proteases, which clip at specific amino acids sequences to form mature proteins and hormones. These mature proteins then move to their final destinations, which may be in the membrane, in lysosomes or secreted into the environment.

Lysosomes and Endocytosis

A very important function of the Golgi apparatus and endoplasmic reticulum is the synthesis of another organelle, the **lysosome.** This organelle (or a structure very much like it) is found in a variety of microorganismsprotozoa, some algae, and fungi as well as in plants and animals. Lysosomes are roughly spherical and enclosed in a single membrane; they average about 500 nm in diameter, but range from 50 nm to several µm

in size. They are involved in intracellular digestion and contain the enzymes needed to digest all types of macromolecules. These enzymes, called hydrolases, catalyze the hydrolysis of molecules and function best under slightly acid conditions (usually around pH 3.5 to 5.0). Lysosomes maintain an acidic environment by pumping protons into their interior. Digestive enzymes are manufactured by the RER and packaged to form lysosomes by the Golgi apparatus. A segment of smooth ER near the Golgi apparatus also may bud off lysosomes. Lysosomes are particularly important in those cells that obtain nutrients through **endocytosis.** In this process a cell takes up solutes or particles by enclosing them in vacuoles and vesicles pinched off from its plasma membrane. Vacuoles and vesicles are membranedelimited cavities that contain fluid, and often solid material. Larger cavities will be called vacuoles, and smaller cavities, vesicles. There are two major forms of endocytosis: phagocytosis and pinocytosis. During **phagocytosis** large particles and even other microorganisms are enclosed in a phagocytic vacuole or phagosome and engulfed (**figure**).

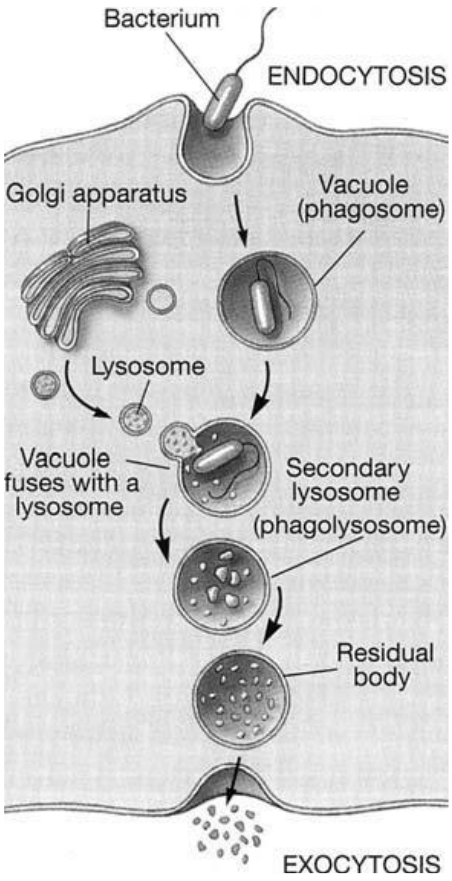


Figure Endocytosis. Membrane-bound vacuoles surround a food particle and internalize it in the form of a *phagosome*. This fuses with a lysosome, which releases digestive enzymes, resulting in the breakdown of the contents. The process of endocytosis is unique to eucaryotic cells.

In **pinocytosis** small amounts of the surrounding liquid with its solute molecules are pinched off as tiny pinocytotic vesicles (also called pinocytic vesicles) or pinosomes. Often phagosomes and pinosomes are collectively called **endosomes** because they are formed by endocytosis. The type of pinocytosis, receptormediated endocytosis, that produces coated vesicles is important in the entry of animal viruses into host cells. Material in endosomes is digested with the aid of lysosomes. Newly formed lysosomes, or **primary lysosomes,** fuse with phagocytic vacuoles to yield **secondary lysosomes,** lysosomes with material being digested. These phagocytic

vacuoles or secondary lysosomes often are called food vacuoles. Digested nutrients then leave the secondary lysosome and enter the cytoplasm. When the lysosome has accumulated large quantities of indigestible material, it is known as a **residual body.**

Lysosomes join with phagosomes for defensive purposes as well as to acquire nutrients. Invading bacteria, ingested by a phagocytic cell, usually are destroyed when lysosomes fuse with the phagosome. This is commonly seen in leukocytes (white blood cells) of vertebrates. Cells can selectively digest portions of their own cytoplasm in a type of secondary lysosome called an **autophagic vacuole**. It is thought that these arise by lysosomal engulfment of a piece of cytoplasm, or when the ER pinches off cytoplasm to form a vesicle that subsequently fuses with lysosomes. Autophagy probably plays a role in the normal turnover or recycling of cell constituents. A cell also can survive a period of starvation by selectively digesting portions of itself to remain alive. Following cell death, lysosomes aid in digestion and removal of cell debris. A most remarkable thing about lysosomes is that they accomplish all these tasks without releasing their digestive enzymes into the cytoplasmic matrix, a catastrophe that would destroy the cell. The lysosomal membrane retains digestive enzymes and other macromolecules while allowing small digestion products to leave. The intricate complex of membranous organelles composed of the Golgi apparatus, lysosomes, endosomes, and associated structures seems to operate as a coordinated whole whose main function is the import and export of materials. Christian de Duve (Nobel Prize, 1974) has suggested that this complex be called the vacuome in recognition of its functional unity. The ER manufactures secretory proteins and membrane, and contributes these to the Golgi apparatus.

The Golgi apparatus then forms secretory vesicles that fuse with the plasma membrane and release material to the outside. It also produces lysosomes that fuse with endosomes to digest material acquired through phagocytosis and pinocytosis. Membrane movement in the region of the vacuome lying between the Golgi apparatus

and the plasma membrane is two-way. Empty vesicles often are recycled and returned to the Golgi apparatus and plasma membrane rather than being destroyed. These exchanges in the vacuome occur without membrane rupture so that vesicle contents never escape directly into the cytoplasmic matrix.

Mitochondria and plastids are organelles of energy generation in eukaryotic cells

- Mitochondria are found in almost all eukaryotic cells and convert high-energy electrons into ATP.
- Plastids are factories for photosynthesis, converting light energy into high-energy electrons and ATP.
- Both of these structures trace their ancestry back to free-living prokaryotes.

Mitochondria are involved in energy generation through respiration. Mitochondria have no fixed shape, but often look like short rods in transmission electron micrographs when viewed along their long axis (Figure). Each mitochondrion contains two membranes. The outer membrane is smooth and serves as a selective barrier. The inner membrane is highly convoluted and folded and contains high numbers of membrane complexes. Nutrients are oxidized inside the mitochondria by catabolic enzymes and the high-energy electrons extracted are donated to a respiratory chain in the inner membrane. These enzymes then create a proton gradient and this gradient is then used to synthesize ATP. ATP leaves the mitochondria and it serves as a source of energy for the rest of the cell's machinery.

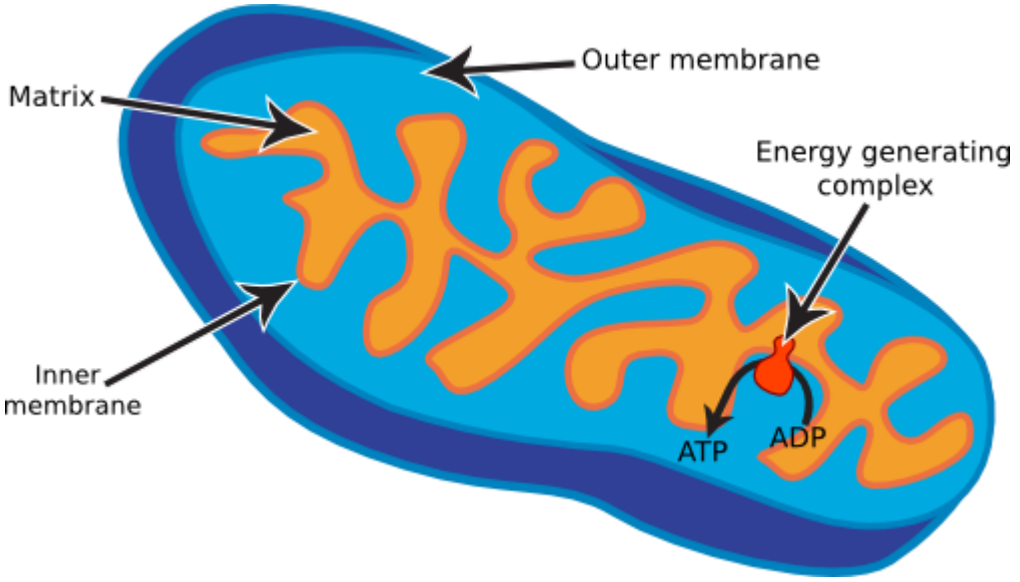


Figure Mitochondria structure. Mitochondria are rod-shaped structures that resemble the shape of common bacteria. They contain two membranes, similar to what is found in gram-negative bacteria, and 70S ribosomes. Energy generation occurs at the inner membrane.

Plastids are specialized organelles involved in metabolism that are unique to plants and come in several forms. Amyloplasts are starch storage containers found in some plants. Chloroplasts are oval-shaped structures inside of plant and algal cells that contain an outer and inner membrane as shown in Figure. The outer membrane serves a similar function to the outer membrane of mitochondria, while the inner membrane consists of a network of stacks of membranous disks, called thykaloids, which are attached together by narrow tubes of membrane. The thykaloid membranes are the centers of photosynthesis in eukaryotes. They contain enzyme complexes that capture light and produce ATP and high-energy electrons that are used to form sugar from carbon dioxide.

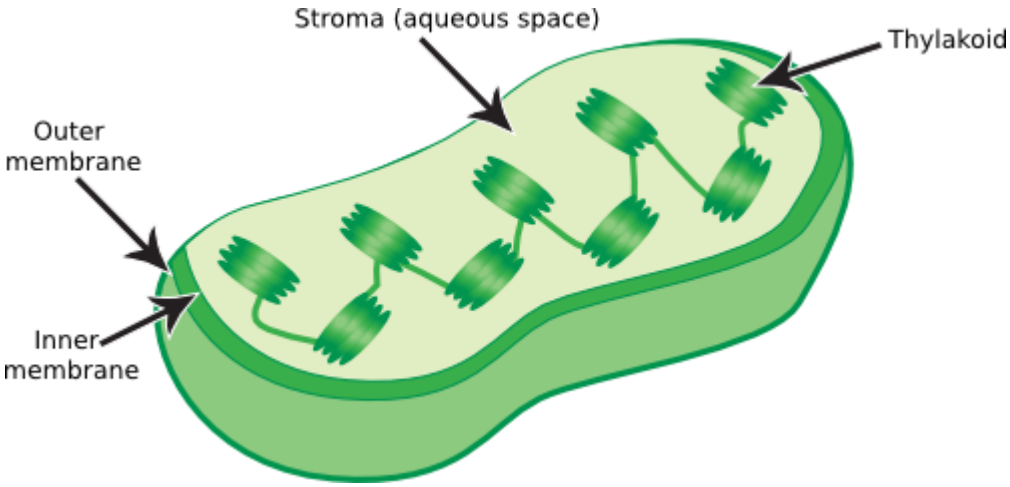


Figure Chloroplast structure. Chloroplasts are the site of the light reactions of photosynthesis. Light striking the chloroplast is converted into a proton motive force and this is used to generate energy. Chloroplasts contain two membranes, again similar to gram-negative bacteria, and the chloroplast itself is a relative of cyanobacteria.

Mitochondria and chloroplasts each have a single circular chromosome and large numbers of ribosomes that are of bacterial (70S) and not eukaryotic (80S) form. The presence of two lipid bilayers, a circular chromosome and 70S ribosomes is consistent with the evolutionary hypothesis.

The nucleus holds the cells genetic material in eukaryotes

- The genome of eukaryotes is sequestered to a membrane bound organelle called the nucleus.
- The nucleus is the site of replication and transcription.
- Most eukaryotes have more than one chromosome in their nucleus and replication of these chromosomes proceeds through a sequence of steps that are visible with a light microscope.
- The nucleus contains visible spots called nucleoli, which are the location or ribosome synthesis.

While the bacterial cell does seem to sequester its chromosome to a portion of the cytoplasm, there is no demarcation that divides the nucleoid from the rest of the cell. In eukaryotes the nuclear membrane separates the cell's DNA from the cytoplasm. The nucleus is the largest and most clearly visible organelle of eukaryotic cells. It contains

almost all the cell's DNA and is the site of chromosome replication and transcription. It has two layers of membrane encircling it called the nuclear envelope, with the outer layer being contiguous with the ER. Scattered throughout this nuclear envelope are circular openings known as nuclear pores. These pores are highly discriminatory, allowing easy movement in and out of the nucleus of only appropriate macromolecules such as proteins with specific sequences.

In eukaryotes, the chromosome is not a single circular piece of DNA as in most prokaryotes. Rather, it is split into a number of linear chromosomes with each cell containing at least two copies of each chromosome. The exceptions are those cells that specialize in reproduction and only contain one copy of the cell's chromosomes. Each piece of DNA is complexed with special basic structural proteins called histones that seem to be important in keeping the DNA organized. The DNA is also bound by other proteins involved in its maintenance and the entire set of DNA and associated proteins is called chromatin. For much of the cell cycle chromatin consists of long DNA strands formed into beads by association of histones along it length. Figure shows a nucleus in the midst of mitosis, with the chromosomes visible.

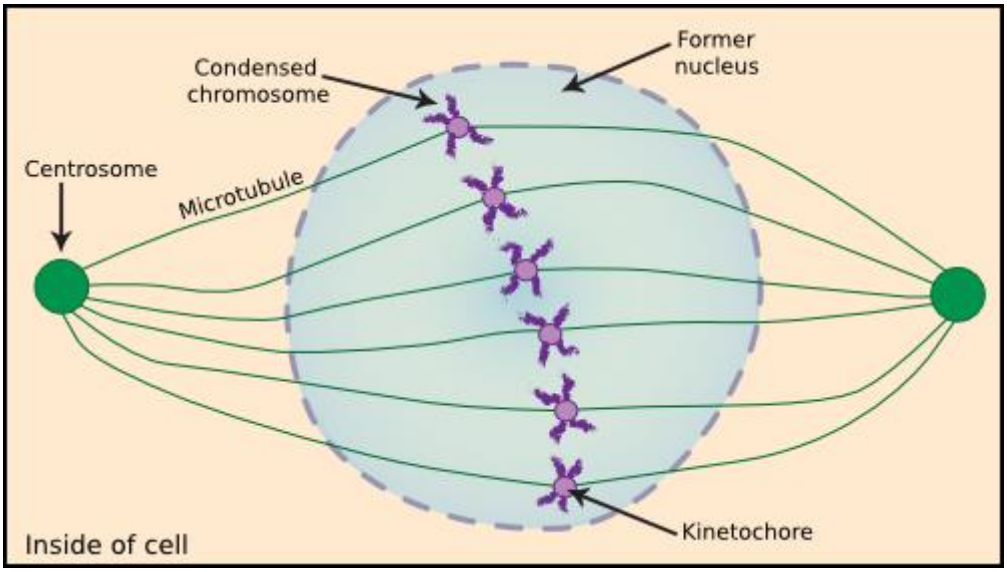


Figure A eukaryotic cell in the middle of mitosis. Eukaryotic DNA replication takes place during the cell phase called mitosis. At this time, protein synthesis is halted and the chromosomes condense. The sister chromatids meet at the middle of the cell and then migrate to two separate poles. This movement is coordinated by centrosomes, kinetochores and microtubules.

In actively growing cells the DNA is replicated from numerous sites, rather than the single bi-directional origin in prokaryotes. This is necessary due to the much larger amount of DNA found in most eukaryotic cells. During division in prokaryotes, the cell appears to simply split in two with each daughter cell receiving a chromosome. In contrast, eukaryotic cells go through a morphologically distinct phase, mitosis, to achieve separation of the chromosomes. One of the more important events of mitosis is the binding of additional histones and the contraction of the chromatin into compact structures that were called chromosomes due to their staining properties. (The original meaning of the term chromosomes is a colored body, but is now synonymous with a cell's DNA). The two daughter chromosomes formed during replication are physically separated into the separate daughter cells by the filaments called microtubules. These attach at one end to the chromosome at a region termed the kinetochores and at the other end they attach to one of two regions of the cell called a centrosome. By depolymerization of the microtubules at each centrosome, each daughter chromosome is pulled away from its partner and toward a region that eventually reforms as a new nucleus.

There are also a number of important differences in transcription between eukaryotes and prokaryotes. In eukaryotes, mRNA transcription takes place in the nucleus and the finished mRNA moves through the nuclear pores and into the cytoplasm for translation by the ribosomes. The genes of eukaryotic cells also contain regions of largely unimportant DNA, termed introns, that do not code for protein. After a gene is transcribed into mRNA these introns are removed before translation. One set of nuclear proteins removes these sequences and splices the actual coding sequences (exons) together to make the finished mRNA. The finished mRNA then travels from the nucleus to ribosomes in the cytoplasm. The mRNA of eukaryotic cells is also decorated

with modifications at each end that affect the stability of the mRNA. At the front end is usually a 5-cap made of 7-methylguanine attached to the mRNA by a triphosphate linkage. At the 3' end of the mRNA is a long stretch of A bases (a poly-A tail) that have a role in mRNA stability as mentioned earlier in this chapter. Finally, while it is quite common in bacteria to have a number of genes on each mRNA transcript, the vast majority of mRNAs in eukaryotes code for only a single protein product. Figure shows the steps in gene expression in eukaryotes.

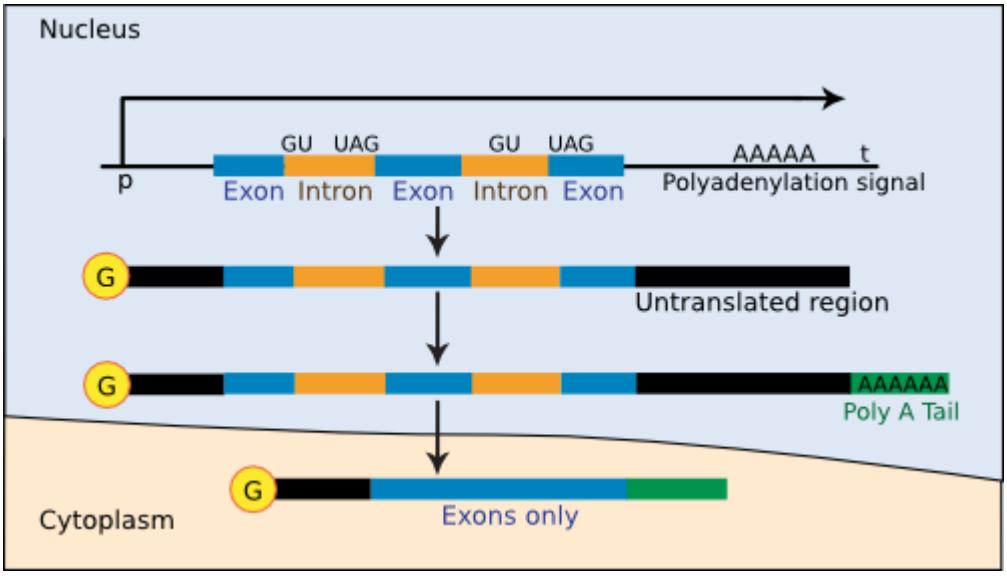


Figure The steps of gene expression in eukaryotes. Processing genetic information into protein is more complex in eukaryotes than in prokaryotes. In part this is due to the existence of introns in most genes of "higher eukaryotes" (though introns are rare in yeast). These have to be removed, a poly-A tail added to the 3' end and a cap added to the 5' end of the mRNA before it reaches the cytoplasm for translation.

Eukaryotic cells also contain one or more dark-staining structures within the nucleus called nucleoli. Although they are not enclosed within a separate membrane the nucleoli are complexes with separate granular and fibrillar regions. They are present in non-dividing cells, but frequently disappear during mitosis and again reappear after cell division is over. The nucleolus is the initial site of ribosome synthesis. This structure contains the DNA that codes for the ribosomal RNA genes. The ribosomal RNAs are

synthesized and then processed to form the final rRNA molecules. These are then combined with several ribosomal proteins synthesized in the cytoplasm to form an initial ribosomal complex. The entire complex then migrates out of the nucleus into the cytoplasm where it combines with other proteins to form a ribosome.

General properties of viruses

A. Infectious agents of both prokaryotes and eukaryotes

B. Acellular organization

C. Contains either DNA or RNA surrounded by a protein coat

D. Requires host cell to reproduce

E. Are viruses living? What is life?: Viruses can be considered exceptionally simple living organisms or exceptionally complex group of nonliving macromolecules, depending on your point of view.

III. Size

A. Range from 0.01 µm to 0.4 µm in diameter

B. smallest are the size of ribosomes and largest are the size of very small bacteria

IV. Virus structure

A. Complete, fully assembled virus particle in the extracellular phase = virion

B. Nucleic acids: contains only one kind that can be linear or circular; segmented or one molecule) a) ssDNA = single stranded DNA b) dsDNA = double stranded DNA c) ssRNA = single stranded RNA (1) plus stranded (same as mRNA) (2)

minus stranded (complementary to mRNA) d) dsRNA = double stranded RNA 2. genome is SMALL a) smallest = encode 3-4 proteins b) largest = encode 100 proteins

C. Capsid.

1. Nucleic acid is surrounded by a protein coat called the capsid.

2. Function a) Capsid protects the virion in the external environment b) Aids in transfer between host cells

3. Capsid structure

a) Formed from individual protein subunits called protomers

b) Usually there are several different types of protomers.

2 c) Several of the protomers assemble to form a capsomere (basic unit of the capsid) using many noncovalent interactions ‡ strong but can be disassembled

4**. Capsid symmetry:**

capsid are constructed in a highly symmetrical manner

a) Icosohedral Fig. 16-12, 13 (ex. poliovirus) (1) Generally composed of (a) 20 equilateral triangular faces made from capsomers called hexons which containing 6 protomers each

(b) 12 vertices made from capsomers called pentons which contain 5 protomers each

(2) Possible to have icosohedron composed of all pentons joined by flexible "arms"

(3) appear spherical b) Helical Fig. 16-11 (ex. tobacco mosaic virus – TMV) (1) hollow, cylinder shape

(2) can be rigid or flexible c) Complex = several types of symmetry in one virus (ex. pox viruses and bacteriophage T7)

D. Nucleocapsid = capsid + nucleic acid E. Envelope 1. Some viruses have membranous envelopes around nucleocapsid .

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POSSIBLE QUESTION

2marks

1. Write short notes on plasma membrane.
2. What is the special feature of endospores
3. Write the function of capsule
4. What is the role of ribosome in the protein synthesis
5. Write note on classification of purple bacteria with examples
6. State the assembly of flagella in bacteria.
7. Write short notes on Pili?
8. Give short notes on Flagella structure.
9. Comment on energy generation organelles in Eukaryotes ?
10. Write short notes on functions of Flagella.

8 marks

1. Elaborate the structural details of an Eukaryotic cell?
2. Explain the differences between Prokaryotic and Eukaryotic cells?

3. Enumerate the characteristic features of a Prokaryotic cell ?
4. Cell organization of gram positive cell wall ?
5. Give a brief notes on cytoplasmic matrix , Ribosome, Nucleoid , inclusion bodies?
6. Describe the Structural organization of eukaryotic cell.
7. Brief notes on Prokaryotic cell structure ?
8. Describe the property of Lichens and microalgae
9. Describe the ultra Structure of viruse?
10. Detailed notes on Flagella, cellwall, fimbriae?

Unit I Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
_____ acts as O antigen in Gram negative bacteria	Protein	Polysaccharide	Lipid	PG layer	Polysaccharide
_____ contains terminal endospore	*Penicillium*	*Streptocuccus*	*Clostridium*	*Mycobacterium*	*Clostridium*
_____ is a capsulated bacteria	*Staphylococcus*	*Klebsiella*	*Salmonella*	*Shigella*	*Klebsiella*
_____ controls the movement of substances in and out of a cell	Cell wall	PG layer	Capsule	Plasma membrane	Plasma membrane
_____ found in eukaryotes for motility/locomotion	Pili	Cilia	Fimbriae	Tail	Cilia
_____ required for conjugation in bacteria	Pili	Flagella	Fimbriae	Capsules	Pili
_____ is the largest organelle in a eukaryotic cell	Golgi apparatus	Chloroplast	Mitochondria	Nucleus	Nucleus

_____ are capable of self-replication as they possess their own DNA	Mitochondria	Chloroplast	Golgi apparatus	Endoplasmic reticulum	Mitochondria
_____ is not an example for eukaryote	Yeast	Animals	Plants	Bacteria	Bacteria
_____ __ are armed with enzymes that break down dangerous oxygen free radicals	Golgi Body	Vesicles	Peroxisomes	Lysosomes	Peroxisomes
A DNA containing virus	Measles	Rabies	Chickenpox	Hepatitis C	Chickenpox
A double membrane bound organelle that contain the green pigment chlorophyll for capturing sunlight energy	Chloroplasts	Mitochondria	Endoplasmic Reticulum	Golgi Body	Chloroplast
A membrane bound organelles that contain	Ribosome	Mesosome	Protosome	Lysosome	Lysosome

degradative enzymes.					
A viral capsid is composed of	Carbohydrates	Proteins	Lipids	Nucleic acid	Proteins
Atypical pneumonia is caused by	*Mycoplasma*	*E.coli*	*Bacillus*	*Aspergillus*	*Mycoplasma*
Bacteria anchor to the cell wall by means of	Filament	Hook	Basal body	Flagellin	Basal body
Bacteria store excess carbon in the form of	Glucose	Glycerol	Starch	Polyhydroxyalkonates	Polyhydroxyalkonates
Bacterium having a single flagellum at each pole	Atrichous	Peritrichous	Amphitrichous	Monotrichous	Amphitrichous
Common algal partner in lichens	*Dyrmecia*	*Chlamydomonas*	*Trebouxia*	*Volvox*	*Trebouxia*
Enveloped viruses are	Herpes virus	Parvovirus	Bacteriophages	Adenovirus	Herpes virus
Eukaryotic ribosomes are made up of _____ ribosomes	50S + 40S	60S + 40S	60S + 50S	70S + 40S	60S + 40S
Flagella are composed of sub units of	Flagellin	Flagellum	Pili	Cilia	Flagellin

Fluid mosaic model of plasma membrane was put forth by	Singer and Nicolson	Sanger and Neelson	Simon and Nicolas	Sanger and Nicolas	Singer and Nicolson
Gas vesicles are mostly present in _____	Gram positive bacteria	Gram negative bacteria	Photosynthetic bacteria	Aquatic bacteria	Aquatic bacteria
Gliding motility in bacteria is aided by	Pili	Fimbriae	Capsules	Flagella	Pili
If the glycocalyx is unorganized and loosely attached to the cell, it is called as	Slime layer	Pili	Capsule	Fimbriae	Slime layer
In aerobic bacteria pellicle formation in broth is attributed to	Capsules	Flagella	Fimbriae	Pili	Fimbriae
In *Bacillus anthracis*, the capsule is made up of polypeptide containing	L-glucosamine	D-glucosamine	D-glutamic acid	L-glutamic acid	D-glutamic acid
In the passive diffusion, solute molecules cross	Pressure difference	Ionic difference	Concentration difference	Solute difference	Concentration difference

the membrane as a result of					
LPS is found in	*Bacillus*	*Escherichia*	*Streptomyces*	*Staphylococcus*	*Escherichia*
Magnetostomes in bacteria	Help cells attach to metal objects	Help cells to orient in the earth's magnetic field	Help cells to magnetically attach to each other	Help cells to float on the surface of fresh water ponds	Help cells to orient in the earth's magnetic field
Manufacturing and movement of protein and lipids takes place in	Nucleus	Endoplasmic Reticulum	Golgi Body	Vesicles	Endoplasmic Reticulum
Members of archaeobacteria that requires high salinity are called as	Blue extreme halophiles	Green extreme halophiles	Yellow extreme halophiles	Red extreme halophiles	Red extreme halophiles
Membrane invaginations in to the bacterial cytoplasm are known as	Liposomes	Cytosomes	Mesosomes	Hydroxysomes	Mesosomes
Metechromatic granules in bacteria is made up of	Butyric acid	Polymer of glucose	Glycogen	Inorganic polyphosphate	Inorganic polyphosphate
Most fungal partner in lichen belongs to	*Sapromycetes*	*Phycomycetes*	*Ascomycetes*	*Deutromycetes*	*Ascomycetes*

Most part of the cytosol is made up of	Vitamins	Water	Minerals	Lipids	Water
Movement toward chemical attractants and away from repellents is called	Chemotaxis.	Tumbling.	Run	Gliding motility.	Chemotaxis.
Mutations occur frequently in _____ viruses	Enveloped	Non-enveloped	RNA	DNA	RNA
Mycoplasma is	Gram negative	Gram positive	Cell wall less	Acid fast	Cell wall less
Periplasmic space is found	Between cell wall and cell membrane	Within outer membrane	Below cell membrane	In between outer membrane and peptidoglycon	Between cell wall and cell membrane
Phycobiont refers to _____ component of a lichen.	Fungal	Photosynthetic	Environmental	Nutritional	Photosynthetic
Prokaryotes lack	Defined nucleus	Mitochondria	Golgi Apparatus	All of the above	All of the above
Ribosome is the site of	Transcription	Translation	Degradation	Energy generation	Transcription
Rough endoplasmic reticulum is covered with	Liposomes	Lysosomes	Mesosomes	Ribosomes	Ribosomes

S layer is found on _____ of bacteria	Surface	Spore	Cytoplasm	Nucleoid	Surface
Single strand, non-enveloped virus family containing RNA	*Rhabdoviridae*	*Picarnoviridae*	*Poxviridae*	*Hepadnaviridae*	*Picarnoviridae*
Sterols are absent in	Prokaryotes	Plant	Fungi	Higher animals	Prokaryotes
Taxonomic group of *Salmonella* are classified by O and H antigens. H antigens refers to	Capsular antigens	LPS antigens	Flagellar antigens	Fimbrial antigens	Flagellar antigens
Teichoic acid in Gram positive bacteria are polymers of	Glucose	Glyceraldehyde	Glutamine	Glycerol	Glycerol
The 70S prokaryotic ribosomes consist of	two 40S subunits	a 40S and a 30S subunit	a 50S and a 20S subunit	a 50S and a 30S subunit.	a 50S and a 30S subunit.
The antibiotic penicillin acts on	Cell membrane	Cell wall	DNA	Ribosomes	Cell wall
The backbone of Plasma Membrane is	Membrane proteins	Phospholipids	Glycolipids	Lipoproteins	Phospholipids

The cell wall of *Archaea* is made up of	Muramic acid	Pseudomurein	Mucopeptin	N-acetyl glucosamine	Pseudomurein
The central core of a virus particle consists of	Envelope	Nucleic acid	Sheath	Flagella	Nucleic acid
The main function of the Golgi apparatus is			Processing and modification of proteins		Processing and modification of proteins
The presence of D-amino acids in the crosslinks of the peptidoglycan layer is most likely because	D-amino acids fit the structural constrains of the cell wall better than L-amino acids.	most L-amino acids have already been used for protein synthesis.	D-amino acids are easier to crosslink in the absence of ribosomes.	Most peptidases can only cleave L-amino acids.	Most peptidases can only cleave L-amino acids.
The resistance of a bacterial spore is implicated by	Dipicolonic acid	Acetic acid	Muramic acid	Phosphoric acid	Dipicolonic acid
What is the function of ribosomes present in cytoplasm of bacteria?	Site of protein synthesis	Site of energy production	Site of genetic reproduction	Nucleotide synthesis	Site of protein synthesis
Which of the following is not true about	They consist of secreted material lying outside of	They can prevent desiccation of	They are required for bacteria to	They help bacteria resist phagocytosis by macrophages.	They are required for bacteria to grow normally in culture.

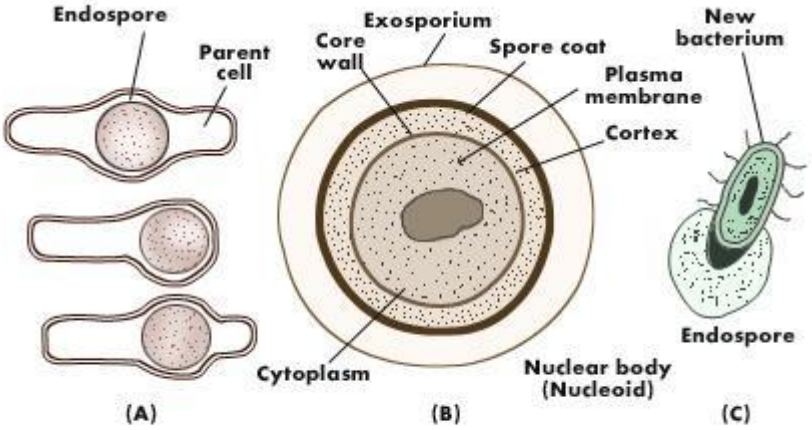
capsules and slime layers?	the bacterial cell wall.	bacteria cells.	grow normally in culture.		

Unit II –

The Bacterial Endospore

A number of gram-positive bacteria can form a special resistant, dormant structure called an **endospore.** Endospores develop within vegetative bacterial cells of several genera: *Bacillus* and *Clostridium* (rods), *Sporosarcina* (cocci), and others. These structures are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants, and desiccation. In fact, some endospores have remained viable for around 100,000 years, and actinomycete spores (which are not true endospores) have been recovered alive after burial in the mud for 7,500 years. Because of their resistance and the fact that several species of endospore-forming bacteria are dangerous pathogens, endospores are of great practical importance in food, industrial, and medical microbiology. This is because it is essential to be able to sterilize solutions and solid objects.

Endospores often survive boiling for an hour or more; therefore autoclaves must be used to sterilize many materials. Endospores are also of considerable theoretical interest. Because bacteria manufacture these intricate entities in a very organized fashion over a period of a few hours, spore formation is well suited for research on the construction of complex biological structures. In the environment, endospores aid in survival when moisture or nutrients are scarce. Endospores can be examined with both light and electron microscopes. Because spores are impermeable to most stains, they often are seen as colorless areas in bacteria treated with methylene blue and other simple stains; special spore stains are used to make them clearly visible. Spore position in the mother cell or **sporangium** frequently differs among species, making it of considerable value in identification. Spores may be centrally located, close to one end (subterminal), or definitely terminal. Sometimes a spore is so large that it swells the sporangium.



Endospore formation. A, Endospores according to their position in parent cells. B, An edospore in cross-section. C, Germination of endospore

The spore often is surrounded by a thin, delicate covering called the **exosporium.**A **spore coat** lies beneath the exosporium, is composed of several protein layers, and may be fairly thick. It is impermeable and responsible for the spore's resistance to chemicals. The **cortex,** which may occupy as much as half the spore volume, rests beneath the spore coat. It is made of a peptidoglycan that is less cross-linked than that in vegetative cells. The **spore cell wall** (or core wall) is inside the cortex and surrounds the protoplast or **core.** The core has the normal cell structures such as ribosomes and a nucleoid, but is metabolically inactive. It is still not known precisely why the endospore is so resistant to heat and other lethal agents. As much as 15% of the

spore's dry weight consists of **dipicolinic acid** complexed with calcium ions, which is located in the core. It has long been thought that dipicolinic acid was directly involved in

spore heat resistance, but heat-resistant mutants lacking dipicolinic acid now have been isolated. Calcium does aid in resistance to wet heat, oxidizing agents, and

sometimes dry heat. It may be that calcium-dipicolinate often stabilizes spore nucleic acids.

Recently specialized small, acid-soluble DNA-binding proteins have been discovered in the endospore. They saturate spore DNA and protect it from heat, radiation, dessication, and chemicals. Dehydration of the protoplast appears to be very important in heat resistance. The cortex may osmotically remove water from the protoplast, thereby protecting it from both heat and radiation damage. The spore coat also seems to protect against enzymes and chemicals such as hydrogen peroxide. Finally, spores contain some DNA repair enzymes. DNA is repaired during germination and outgrowth after the core has become active once again. In summary, endospore heat resistance probably is due to several factors: calcium-dipicolinate and acid-soluble protein stabilization of DNA, protoplast dehydration, the spore coat, DNA repair, the greater stability of cell proteins in bacteria adapted to growth at high temperatures, and others. Spore formation, **sporogenesis** or **sporulation,** normally commences when growth ceases due to lack of nutrients. It is a complex process and may be divided into seven stages.

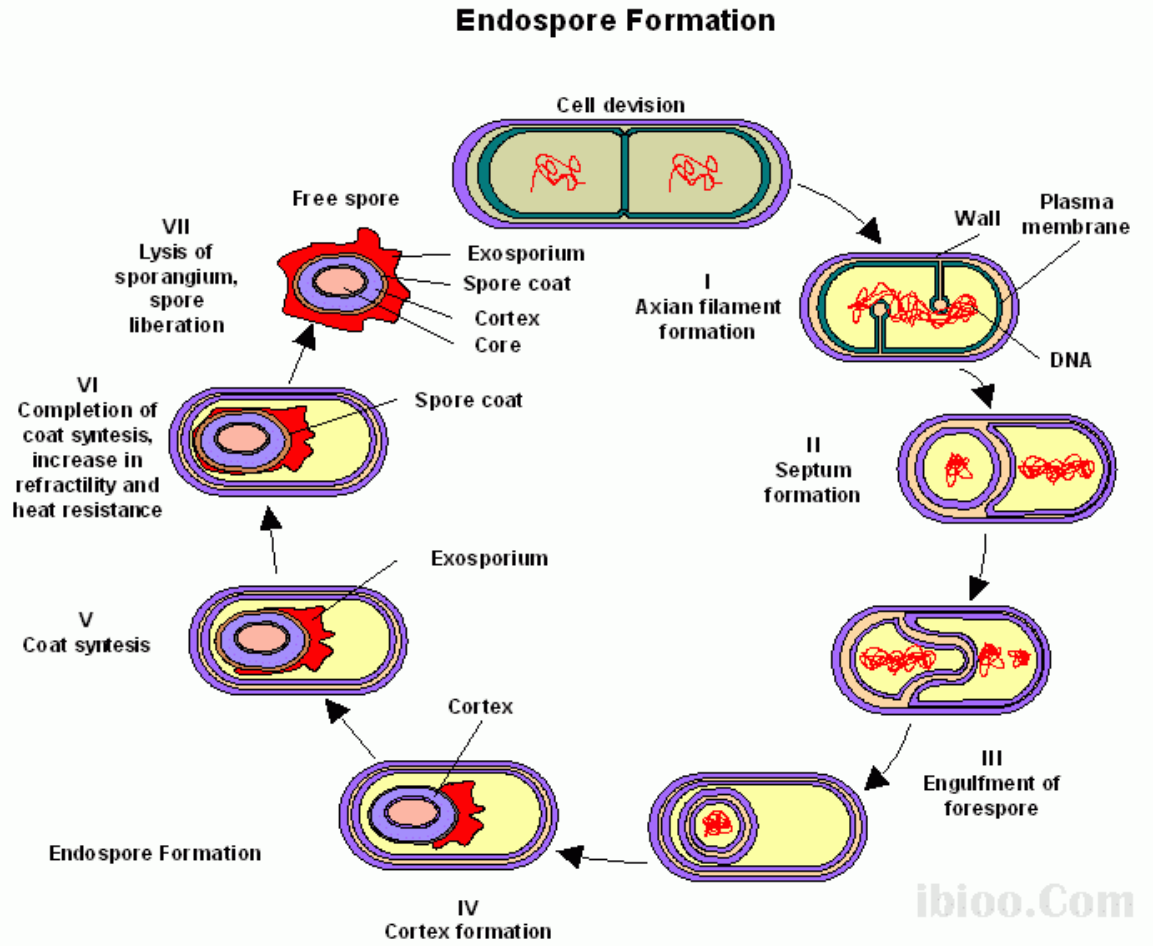


Figure A typical sporulation cycle in *Bacillus* species from the active vegetative cell to release and germination.

An axial filament of nuclear material forms (stage I), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum (stage II). The membrane continues to grow and engulfs the immature spore in a second membrane (stage III). Next, cortex is laid down in the space between the two membranes, and both calcium and dipicolinic acid are accumulated (stage IV). Protein coats then are formed around the cortex (stage V), and maturation of the spore occurs (stage VI). Finally, lytic enzymes destroy the sporangium releasing the spore (stage

VII). Sporulation requires only about 10 hours in *Bacillus megaterium.* The transformation of dormant spores into active vegetative cells seems almost as complex a process as sporogenesis. It occurs in three stages: (1) activation, (2) germination, and (3) outgrowth. Often an endospore will not germinate successfully, even in a nutrient-rich medium, unless it has been activated.

Activation is a reversible process that prepares spores for germination and usually results from treatments like heating. It is followed by **germination,** the breaking of the spore's dormant state. This process is characterized by spore swelling, rupture or absorption of the spore coat, loss of resistance to heat and other stresses, loss of refractility, release of spore components, and increase in metabolic activity. Many normal

metabolites or nutrients (e.g., amino acids and sugars) can trigger germination after activation. Germination is followed by the third stage, outgrowth. The spore protoplast makes new components, emerges from the remains of the spore coat, and develops again into an active bacterium.

Microbial Nutrition

The Common Nutrient Requirements

Analysis of microbial cell composition shows that over 95% of cell dry weight is made up of a few major elements: carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. These are called **macroelements** or macronutrients because they are required by microorganisms in relatively large amounts. The first six (C, O, H, N, S, and P) are components of carbohydrates, lipids, proteins, and nucleic acids. The remaining four macroelements exist in the cell as cations and play a variety of roles. For example, potassium is required for activity by a number of enzymes, including some of those involved in protein synthesis. Calcium, among other functions, contributes to the heat resistance of bacterial endospores.

Magnesium serves as a cofactor for many enzymes, complexes with ATP, and stabilizes ribosomes and cell membranes. Iron is a part of cytochromes and a cofactor for enzymes and electron-carrying proteins. All organisms, including microorganisms, require several **micronutrients** or **trace elements** besides macroelements.

The micronutrients manganese, zinc, cobalt, molybdenum, nickel, and copper are needed by most cells. However, cells require such small amounts that contaminants in water, glassware, and regular media components often are adequate for growth. Therefore it is very difficult to demonstrate a micronutrient requirement. In nature, micronutrients are ubiquitous and probably do not usually limit growth. Micronutrients are normally a part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure. For example, zinc is present at the active site of some enzymes but is also involved in the association of regulatory and catalytic subunits in *E. coli* aspartate carbamoyltransferase. Manganese aids many enzymes catalyzing the transfer of phosphate groups. Molybdenum is required for nitrogen fixation, and cobalt is a component of vitamin B12. Besides the common macroelements and trace elements, microorganisms may have particular requirements that reflect the special nature of their morphology or environment. need silicic acid to construct their beautiful cell walls of silica. Although most bacteria do not require large amounts of sodium, many bacteria growing in saline lakes and oceans depend on the presence of high concentrations of sodium ion. Finally, it must be emphasized that microorganisms require a balanced mixture of nutrients. If an essential nutrient is in short supply, microbial growth will be limited regardless of the concentrations of other nutrients.

Requirements for Carbon, Hydrogen, and Oxygen

The requirements for carbon, hydrogen, and oxygen often are satisfied together. Carbon is needed for the skeleton or backbone of all organic molecules, and molecules serving as carbon sources normally also contribute both oxygen and hydrogen atoms.

They are the source of all three elements. Because these organic nutrients are almost always reduced and have electrons that they can donate to other molecules, they also can serve as energy sources. Indeed, the more reduced organic molecules are, the higher their

energy content (e.g., lipids have a higher energy content than carbohydrates). This is because, as we shall see later, electron transfers release energy when the electrons move from reduced donors with more negative reduction potentials to oxidized electron acceptors with more positive potentials. Thus carbon sources frequently also serve as energy sources, although they don't have to. One important carbon source that does not supply hydrogen or energy is carbon dioxide. This is because CO2 is oxidized and lacks hydrogen. Probably all microorganisms can fix CO2 that is, reduce it and incorporate it into organic molecules. However, by definition, only **autotrophs** can use CO2 as their sole or principal source of carbon. Many microorganisms are autotrophic, and most of these carry out photosynthesis and use light as their energy source. Some autotrophs oxidize inorganic molecules and derive energy from electron transfers

The reduction of CO2 is a very energy-expensive process. Thus many microorganisms cannot use CO2 as their sole carbon source but must rely on the presence of more reduced, complex molecules such as glucose for a supply of carbon. Organisms that use reduced, preformed organic molecules as carbon sources are **heterotrophs.** For example, the glycolytic pathway produces carbon skeletons for use in biosynthesis and also releases energy as ATP and NADH. A most remarkable nutritional characteristic of microorganisms is their extraordinary flexibility with respect to carbon sources. Laboratory experiments indicate that there is no naturally occurring organic molecule that cannot be used by some microorganism. Actinomycetes will degrade amyl alcohol, paraffin, and even rubber. Some bacteria seem able to employ almost anything as a carbon source; for example, *Burkholderia cepacia* can use over 100 different carbon compounds. In contrast to these bacterial omnivores, some

bacteria are exceedingly fastidious and catabolize only a few carbon compounds. Cultures of methylotrophic bacteria metabolize methane, methanol, carbon monoxide, formic acid, and related one-carbon molecules. Parasitic members of the genus *Leptospira* use only long-chain fatty acids as their major source of carbon and energy. It appears that in natural environments complex populations of microorganisms often will metabolize even relatively indigestible

human-made substances such as pesticides. Indigestible molecules sometimes are oxidized and degraded in the presence of a growthpromoting nutrient that is metabolized at the same time, a process called cometabolism. The products of this breakdown process can then be used as nutrients by other microorganisms.

Nutritional Types of Microorganisms

In addition to the need for carbon, hydrogen, and oxygen, all organisms require sources of energy and electrons for growth to take place. Microorganisms can be grouped into nutritional classes based on how they satisfy all these requirements. There are only two sources of energy available to organisms: (1) light energy, and (2) the energy derived from oxidizing organic or inorganic molecules. **Phototrophs** use light as their energy source; **chemotrophs** obtain energy from the oxidation of chemical compounds (either organic or inorganic). Microorganisms also have only two sources for bacteria cannot oxidize water but extract electrons from inorganic donors like hydrogen, hydrogen sulfide, and elemental sulfur.

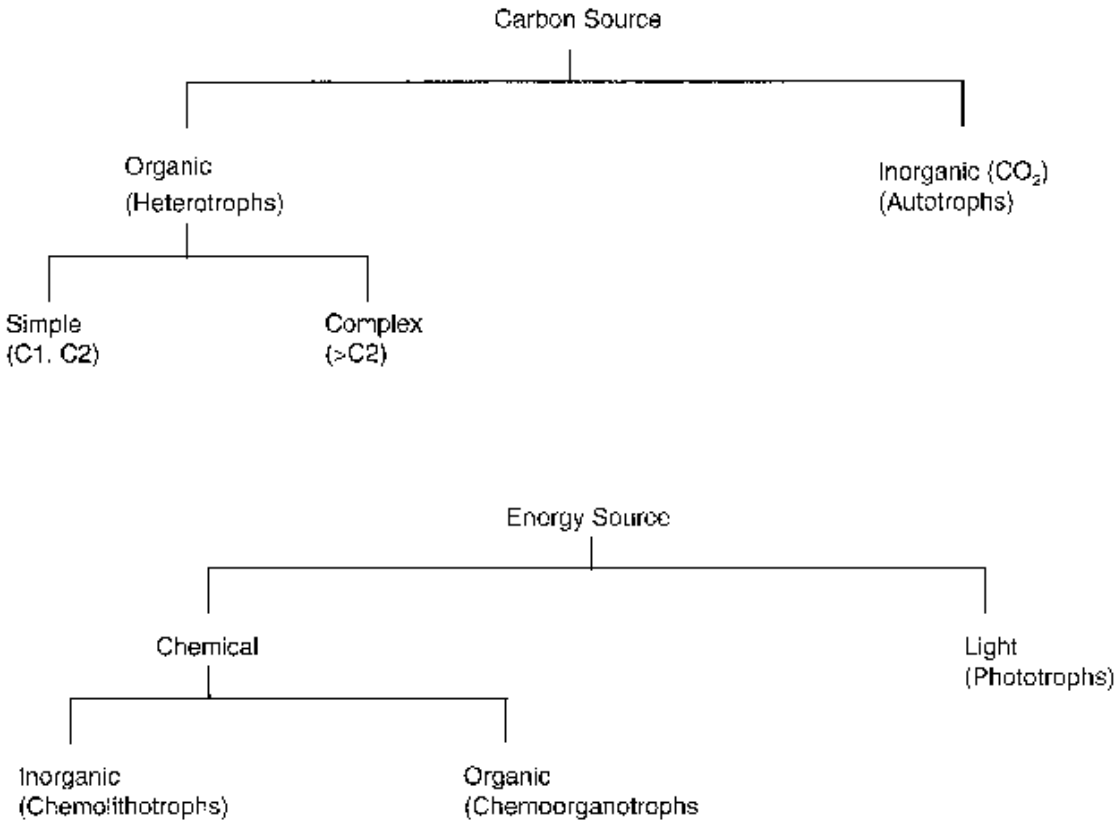


Fig. Classification of bacteria based on nutritional requirement

Chemoorganotrophic heterotrophs (often called **chemoheterotrophs,** chemoorganoheterotrophs, or even heterotrophs) use organic compounds as sources of energy, hydrogen, electrons, and carbon. Frequently the same organic nutrient will satisfy

all these requirements. It should be noted that essentially all pathogenic microorganisms are chemoheterotrophs. The other two nutritional classes have fewer microorganisms but often are very important ecologically. Some purple and green bacteria are photosynthetic and use organic matter as their electron donor and carbon source. These **photoorganotrophic heterotrophs** (photoorganoheterotrophs) are common inhabitants of polluted lakes and streams. Some of these bacteria also can grow

as photoautotrophs with molecular hydrogen as an electron donor. The fourth group, the **chemolithotrophic autotrophs** (chemolithoautotrophs), oxidizes reduced inorganic compounds such as iron, nitrogen, or sulfur molecules to derive both energy and electrons for biosynthesis. Carbon dioxide is the carbon source. A few chemolithotrophs can derive their carbon from organic sources and thus are heterotrophic. Chemolithotrophs contribute greatly to the chemical transformations of elements (e.g., the conversion of ammonia to nitrate or sulfur to sulfate) that continually occur in the ecosystem.

Although a particular species usually belongs in only one of the four nutritional classes, some show great metabolic flexibility and alter their metabolic patterns in response to environmental changes. For example, many purple nonsulfur bacteria act as photoorganotrophic heterotrophs in the absence of oxygen but oxidize organic molecules and function chemotrophically at normal oxygen levels. When oxygen is low, photosynthesis and oxidative metabolism may function simultaneously.

Another example is provided by bacteria such as *Beggiatoa* that rely on inorganic energy sources and organic (or sometimes CO2) carbon sources. These microbes are sometimes called **mixotrophic** because they combine chemolithoautotrophic and heterotrophic metabolic processes. This sort of flexibility seems complex and confusing, yet it gives its possessor a definite advantage if environmental conditions frequently change.

Requirements for Nitrogen, Phosphorus, and Sulfur

To grow, a microorganism must be able to incorporate large quantities of nitrogen, phosphorus, and sulfur. Although these elements may be acquired from the same nutrients that supply carbon, microorganisms usually employ inorganic sources as well. Nitrogen is needed for the synthesis of amino acids, purines, pyrimidines, some carbohydrates and lipids, enzyme cofactors, and other substances. Many microorganisms can use the nitrogen in amino acids, and ammonia often is directly

incorporated through the action of such enzymes as glutamate dehydrogenase or glutamine synthetase and glutamate synthase. Most phototrophs and many nonphotosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction.

A variety of bacteria (e.g., many cyanobacteria and the symbiotic bacterium *Rhizobium*) can reduce and assimilate atmospheric nitrogen using the nitrogenase system. Phosphorus is present in nucleic acids, phospholipids, nucleotides like ATP, several cofactors, some proteins, and other cell components. Almost all microorganisms use inorganic phosphate as their phosphorus source and incorporate it directly. Low phosphate levels actually limit microbial growth in many aquatic environments. Phosphate uptake by *E. coli* has been intensively studied. This bacterium can use both organic and inorganic phosphate. Some organophosphates such as hexose 6-phosphates can be taken up directly by transport proteins. Other organophosphates are often hydrolyzed in the periplasm by the enzyme alkaline phosphatase to produce inorganic phosphate, which then is transported across the plasma membrane. When inorganic phosphate is outside the bacterium, it crosses the outer membrane by the use of a porin protein channel. One of two transport systems subsequently moves the phosphate across the plasma membrane. At high phosphate concentrations, transport probably is due to the Pit system. When phosphate concentrations are low, the PST, (*p*hosphate-specific *t*ransport) system is more important. The PST system has higher affinity for phosphate; it is an ABC transporter and uses a periplasmic binding protein. Sulfur is needed for the synthesis of substances like the amino acids cysteine and methionine, some carbohydrates, biotin, and thiamine. Most microorganisms use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction; a few require a reduced form of sulfur such as cysteine.

Growth Factors

Microorganisms often grow and reproduce when minerals and sources of energy, carbon, nitrogen, phosphorus, and sulfur are supplied. These organisms have the enzymes and pathways nec essary to synthesize all cell components required for their wellbeing. Many microorganisms, on the other hand, lack one or more essential enzymes. Therefore they cannot manufacture all indispensable constituents but must obtain them or their precursors from the environment. Organic compounds required because they are essential cell components or precursors of such components and cannot be synthesized by the organism are called **growth factors.**

There are three major classes of growth factors: (1) amino acids, (2) purines and pyrimidines, and (3) vitamins. Amino acids are needed for protein synthesis, purines and pyrimidines for nucleic acid synthesis. **Vitamins** are small organic molecules that usually make up all or part of enzyme cofactors, and only very small amounts sustain growth. Some microorganisms require many vitamins; for example, *Enterococcus faecalis* needs eight different vitamins for growth. Other growth factors are also seen; heme (from hemoglobin or cytochromes) is required by *Haemophilus influenzae,* and some mycoplasmas need cholesterol. Knowledge of the specific growth factor requirements of

many microorganisms makes possible quantitative growthresponse assays for a variety of substances. For example, species from the bacterial genera *Lactobacillus* and *Streptococcus* can be used in microbiological assays of most vitamins and amino acids. The appropriate bacterium is grown in a series of culture vessels, each containing medium with an excess amount of all required components except the growth factor to be assayed.

A different amount of growth factor is added to each vessel. The standard curve is prepared by plotting the growth factor quantity or concentration against the total extent of bacterial growth. Ideally the amount of growth resulting is directly proportional to the quantity of growth factor present; if the growth factor concentration doubles, the final extent of bacterial growth doubles. The quantity of the growth factor

in a test sample is determined by comparing the extent of growth caused by the unknown sample with that resulting from the standards. Microbiological assays are specific, sensitive, and simple. They still are used in the assay of substances like vitamin B12 and biotin, despite advances in chemical assay techniques. The observation that many microorganisms can synthesize large quantities of vitamins has led to their use in industry. Several water-soluble and fat-soluble vitamins are produced partly or completely using industrial fermentations. Good examples of such vitamins and the microorganisms that synthesize them are riboflavin (*Clostridium, Candida, Ashbya, Eremothecium*), coenzyme A (*Brevibacterium*), vitamin B12 (*Streptomyces, Propionibacterium, Pseudomonas*), vitamin C (*Gluconobacter, Erwinia, Corynebacterium*), carotene (*Dunaliella*), and vitamin D (*Saccharomyces*).

Uptake of Nutrients by the Cell

The first step in nutrient use is uptake of the required nutrients by the microbial cell. Uptake mechanisms must be specific that is, the necessary substances, and not others, must be acquired. It does a cell no good to take in a substance that it cannot use. Since microorganisms often live in nutrient-poor habitats, they must be able to transport nutrients from dilute solutions into the cell against a concentration gradient. Finally, nutrient molecules must pass through a selectively permeable plasma membrane that will not permit the free passage of most substances. In view of the enormous variety of nutrients and the complexity of the task, it is not surprising that microorganisms make use of several different transport mechanisms. The most important of these are facilitated diffusion, active transport, and group translocation. Eucaryotic microorganisms do not appear to employ group translocation but take up nutrients by the process of endocytosis.

Facilitated Diffusion

A few substances, such as glycerol, can cross the plasma membrane by **passive diffusion.** Passive diffusion, often simply called diffusion, is the process in which molecules move from a region of higher concentration to one of lower concentration because of random thermal agitation. The rate of passive diffusion is dependent on the size of the concentration gradient between a cell's exterior and its interior.

A fairly large concentration gradient is required for adequate nutrient uptake by passive diffusion (i.e., the external nutrient concentration must be high), and the rate of uptake decreases as more nutrient is acquired unless it is used immediately. Very small molecules such as H2O, O2, and CO2 often move across membranes by passive diffusion. Larger molecules, ions, and polar substances do not cross membranes by passive or simple diffusion. The rate of diffusion across selectively permeable membranes is greatly increased by using carrier proteins, sometimes called **permeases,** which are embedded in the plasma membrane. Because a carrier aids the diffusion process, it is called **facilitated diffusion.** The rate of facilitated diffusion increases with the concentration gradient much more rapidly and at lower concentrations of the diffusing molecule than that of passive diffusion (figure).

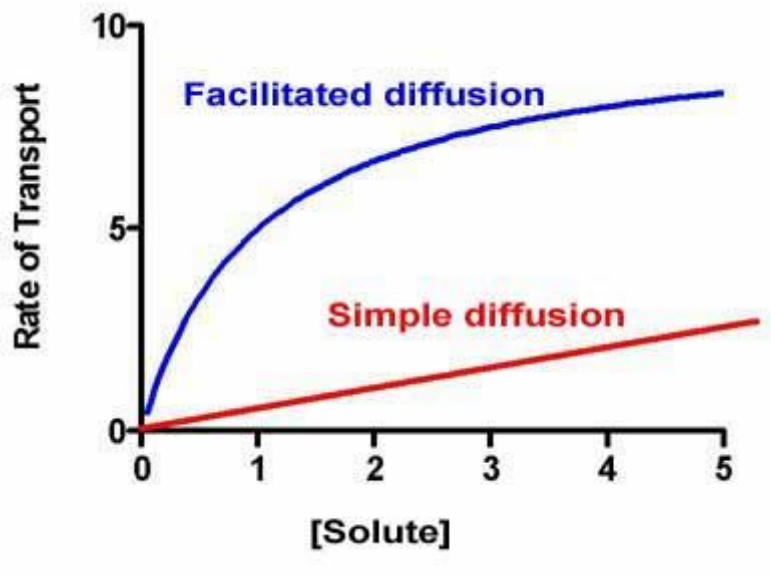


Figure Passive or simple and Facilitated Diffusion. The dependence of diffusion rate on the size of the solute's concentration gradient. Note the saturation effect or plateau above a specific gradient value when a

facilitated diffusion carrier is operating. This saturation effect is seen whenever a carrier protein is involved in transport.

Note that the diffusion rate levels off or reaches a plateau above a specific gradient value because the carrier is saturated that is, the carrier protein is binding and transporting as many solute molecules as possible. The resulting curve resembles an enzyme-substrate curve and is different from the linear response seen with passive diffusion. Carrier proteins also resemble enzymes in their specificity for the substance to be transported; each carrier is selective and will transport only closely related solutes. Although a carrier protein is involved, facilitated diffusion is truly diffusion. A concentration gradient spanning the membrane drives the movement of molecules, and

no metabolic energy input is required. If the concentration gradient disappears, net inward movement ceases. The gradient can be maintained by transforming the transported nutrient to another compound or by moving it to another membranous compartment in eucaryotes. Interestingly, some of these carriers are related to the major intrinsic protein of mammalian eye lenses and thus belong to the MIP family of proteins. The two most widespread MIP channels in bacteria are aquaporins that transport water and glycerol facilitators, which aid glycerol diffusion. Although much work has been done on the mechanism of facilitated diffusion, the process is not yet understood completely. It appears that the carrier protein complex spans the membrane (**figure**).

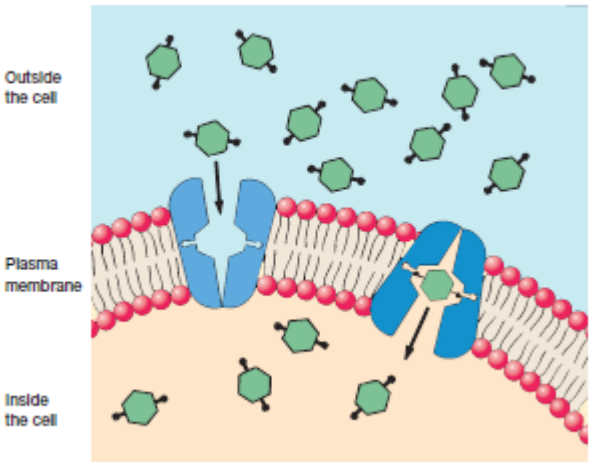


Figure A Model of Facilitated Diffusion. The membrane carrier can change conformation after binding an external molecule and subsequently release the molecule on the cell interior. It then returns to the outward oriented position and is ready to bind another solute molecule. Because there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside.

After the solute molecule binds to the outside, the carrier may change conformation and release the molecule on the cell interior. The carrier would subsequently change back to its original shape and be ready to pick up another molecule. The net effect is that a lipid-insoluble molecule can enter the cell in response to its concentration gradient. Remember that the mechanism is driven by concentration gradients and therefore is reversible. If the solute's concentration is greater inside the cell, it will move outward. Because the cell metabolizes nutrients upon entry, influx is favored. Facilitated diffusion does not seem to be important in prokaryotes because nutrient concentrations often are lower outside the cell so that facilitated diffusion cannot be used in uptake. Glycerol is transported by facilitated diffusion in *E. coli, Salmonella typhimurium, Pseudomonas, Bacillus,* and many other bacteria. The process

is much more prominent in eucaryotic cells where it is used to transport a variety of sugars and amino acids.

Active Transport

Although facilitated diffusion carriers can efficiently move molecules to the interior when the solute concentration is higher on the outside of the cell, they cannot take up solutes that are already more concentrated within the cell (i.e., against a concentration gradient). Microorganisms often live in habitats characterized by very dilute nutrient sources, and, to flourish, they must be able to transport and concentrate these nutrients. Thus facilitated diffusion mechanisms are not always adequate, and other

approaches must be used. The two most important transport processes in such situations are active transport and group translocation, both energy-dependent processes.

Active transport is the transport of solute molecules to higher concentrations, or against a concentration gradient, with the use of metabolic energy input. Because active transport involves protein carrier activity, it resembles facilitated diffusion in some ways. The carrier proteins or permeases bind particular solutes with great specificity for the molecules transported. Similar solute molecules can compete for the same carrier protein in both facilitated diffusion and active transport. Active transport is also characterized by the carrier saturation effect at high solute concentrations. Nevertheless, active transport differs from facilitated diffusion in its use of metabolic energy and in its ability to concentrate substances. Metabolic inhibitors that block energy production will inhibit active transport but will not affect facilitated diffusion (at least for a short time). Binding protein transport systems or ***A*TP-*b*inding *c*assette transporters (ABC transporters)** are active in bacteria, archaea, and eucaryotes. Usually these transporters consist of two hydrophobic membrane-spanning domains associated on their cytoplasmic surfaces with two nucleotide-binding domains (**figure**).

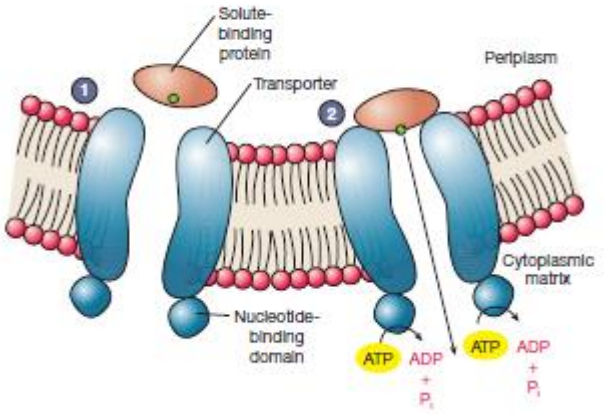


Figure ABC Transporter Function. (*1*) The solute binding protein binds the substrate to be transported and approaches the ABC transporter complex. (*2*) The solute binding protein attaches to the transporter and releases the substrate, which is moved across the membrane with the aid of ATP hydrolysis.

The membrane-spanning domains form a pore in the membrane and the nucleotide-binding domains bind and hydrolyze ATP to drive uptake. ABC transporters employ special substrate binding proteins, which are located in the periplasmic space of gram-negative bacteria or are attached to membrane lipids on the external face of the gram-positive plasma membrane. These binding proteins, which also may participate in chemotaxis, bind the molecule to be transported and then interact with the membrane transport proteins to move the solute molecule inside the cell. *E. coli* transports a variety of sugars (arabinose, maltose, galactose, ribose) and amino acids (glutamate, histidine, leucine) by this mechanism. Substances entering gram-negative bacteria must pass through the outer membrane before ABC transporters and other active transport systems can take action. There are several ways in which this is accomplished. When the substance is small, a generalized porin protein such as OmpF can be used; larger molecules require specialized porins. In some cases (e.g., for uptake of iron and vitamin

B12), specialized high-affinity outer membrane receptors and transporters are used. It should be noted that eucaryotic ABC transporters are sometimes of great medical importance. Some tumor cells pump drugs out using these transporters. Cystic fibrosis results from a mutation that inactivates an ABC transporter that acts as a chloride ion channel in the lungs.

Bacteria also use proton gradients generated during electron transport to drive active transport. The membrane transport proteins responsible for this process lack special periplasmic solute binding proteins. The lactose permease of *E. coli* is a well-studied example. The permease is a single protein having a molecular weight of about 30,000. It transports a lactose molecule inward as a proton simultaneously enters the cell (a higher concentration of protons is maintained outside the membrane by electron transport chain activity). Such linked transport of two substances in the same direction is called **symport.** Here, energy stored as a proton gradient drives solute transport. Although the mechanism of transport is not completely understood, it is thought that binding of a proton to the transport protein changes its shape and affinity for the solute to be transported. *E. coli* also uses proton symport to take up amino acids and organic acids like succinate and malate.

A proton gradient also can power active transport indirectly, often through the formation of a sodium ion gradient (**figure**). Such linked transport in which the transported substances move in opposite directions is termed **antiport.** The sodium gradient generated by this proton antiport system then drives the uptake of sugars and amino acids. A sodium ion could attach to a carrier protein, causing it to change shape. The carrier would then bind the sugar or amino acid tightly and orient its binding sites toward the cell interior. Because of the low intracellular sodium concentration, the sodium ion would dissociate from the carrier, and the other molecule would follow. *E. coli* transport proteins carry the sugar melibiose and the amino acid glutamate when sodium simultaneously moves inward.

Sodium symport or cotransport also is an important process in eucaryotic cells where it is used in sugar and amino acid uptake. ATP, rather than proton motive force, usually drives sodium transport in eucaryotic cells. Often a microorganism has more than one transport system for each nutrient, as can be seen with E. coli. This bacterium has at least five transport systems for the sugar galactose, three systems each for the amino acids glutamate and leucine, and two potassium transport complexes. When there are several transport systems for the same substance, the systems differ in such properties as their energy source, their affinity for the solute transported, and the nature of their regulation. Presumably this diversity gives its possessor an added competitive advantage in a variable environment.

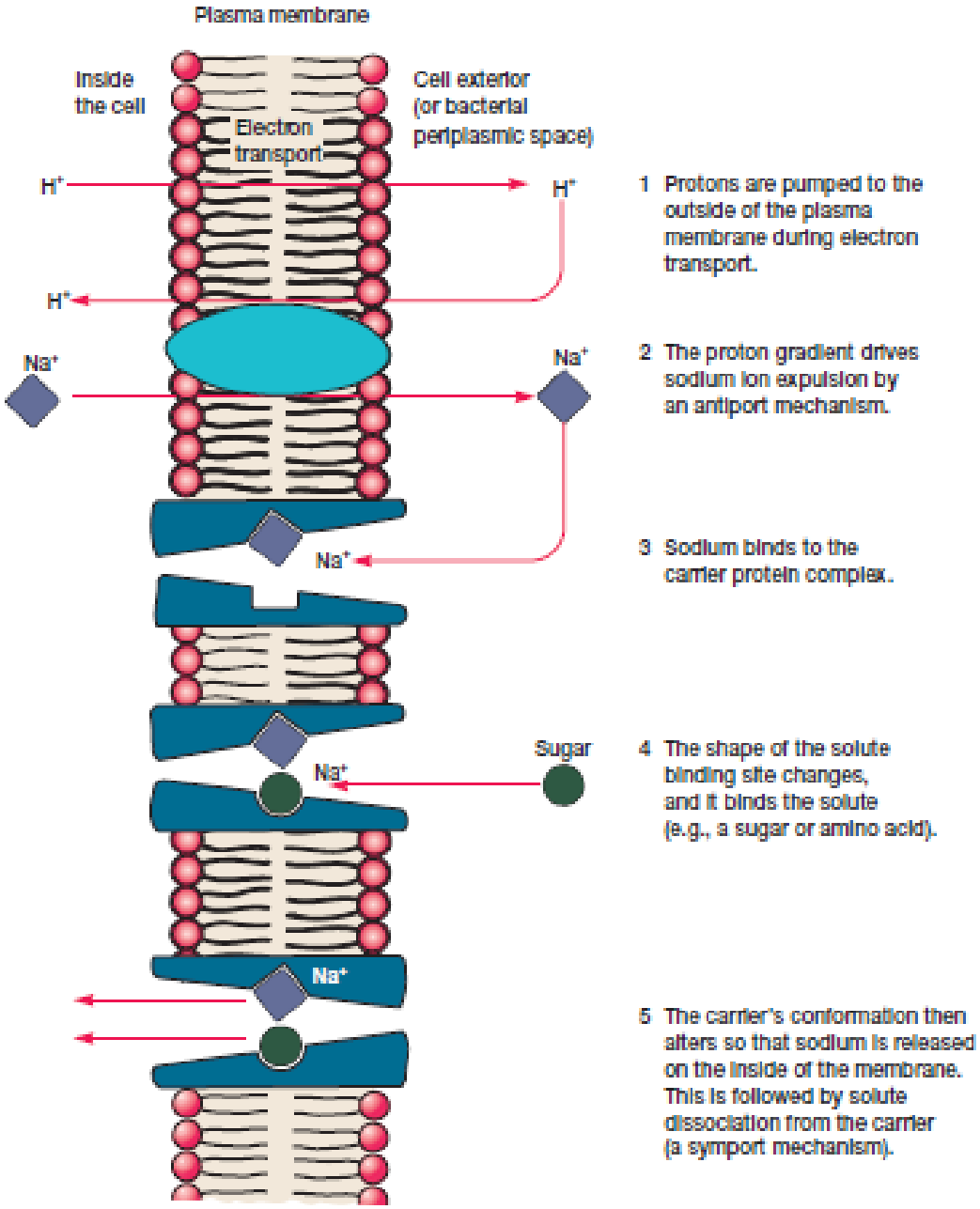


Figure Active Transport Using Proton and odium Gradients. (1) Protons are pumped to the outside of the plasma membrane during electron transport. (2) The proton gradient drives sodium ion expulsion by an antiport mechanism. (3) Sodium binds to the

carrier protein complex. (4) The shape of the solute binding site changes, and it binds the solute (e.g., a sugar or amino acid). (5) The carrier's conformation then alters so that sodium is released on the inside of the membrane. This is followed by solute dissociation from the carrier (a symport mechanism).

Group Translocation

In active transport, solute molecules move across a membrane without modification. Many procaryotes also take up molecules by **group translocation,** a process in which a molecule is transported into the cell while being chemically altered (this can be classified as a type of energy-dependent transport because metabolic energy is used). The best-known group translocation system is the **phosphoenolpyruvate: sugar phosphotransferase system (PTS).** It transports a variety of sugars into procaryotic cells

while phosphorylating them using phosphoenolpyruvate (PEP) as the phosphate donor. The PTS is quite complex. In *E. coli* and *Salmonella typhimurium*, it consists of two enzymes and a low molecular weight heat-stable protein (HPr). HPr and enzyme I (EI) are cytoplasmic. Enzyme II (EII) is more variable in structure and often composed of three subunits or domains. EIIA (formerly called EIII) is cytoplasmic and soluble. EIIB also is hydrophilic but frequently is attached to EIIC, a hydrophobic protein that is embedded in the membrane. A high-energy phosphate is transferred from PEP to enzyme II with the aid of enzyme I and HPr (**figure**).

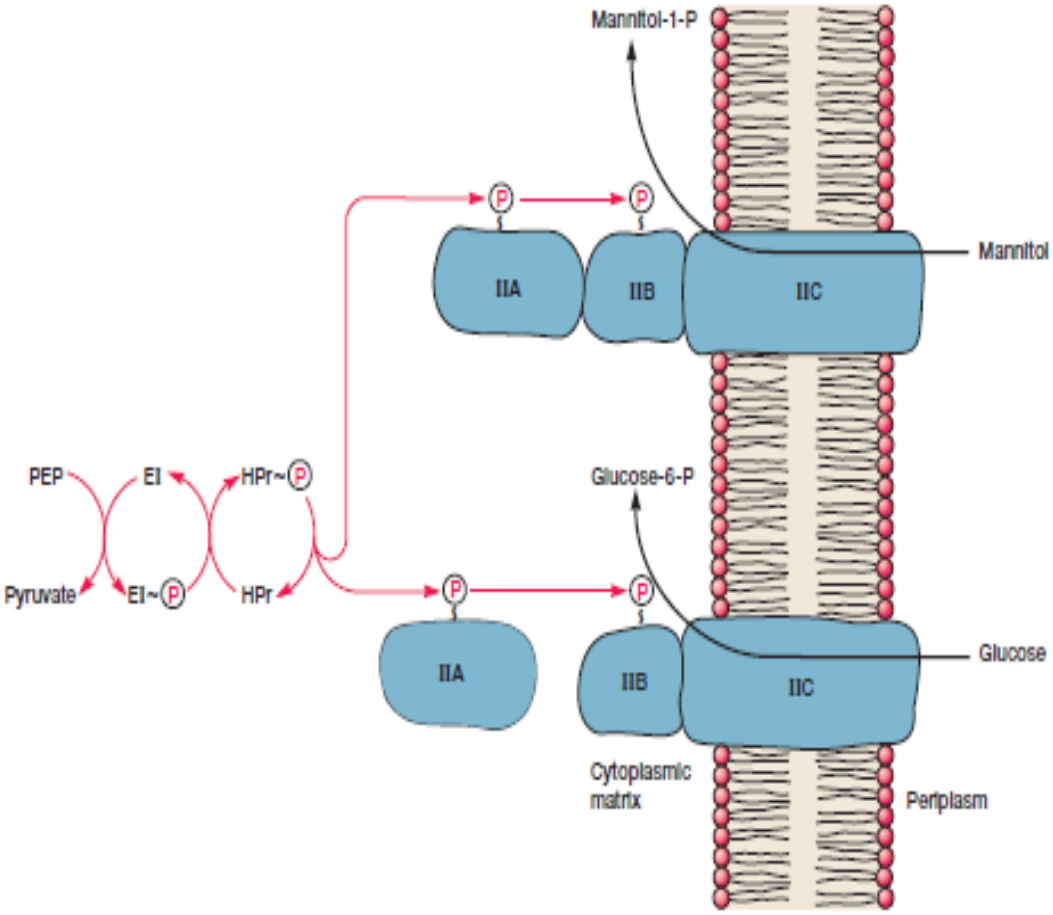


Figure Group Translocation: Bacterial PTS Transport. Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated. The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). The high-energy phosphate is transferred from HPr to the soluble EIIA. EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system. In either case the phosphate moves from EIIA to EIIB, and then is transferred to the sugar during transport through the membrane. Other relationships between the EII

components are possible. For example, IIA and IIB may form a soluble protein separate from the membrane complex; the phosphate still moves from IIA to IIB and then to the membrane domain(s).

Then, a sugar molecule is phosphorylated as it is carried across the membrane by enzyme II. Enzyme II transports only specific sugars and varies with PTS, whereas enzyme I and HPr are common to all PTSs. PTSs are widely distributed in procaryotes. Except for some species of *Bacillus* that have both glycolysis and the phosphotransferase

system, aerobic bacteria seem to lack PTSs. Members of the genera *Escherichia, Salmonella, Staphylococcus,* and other facultatively anaerobic bacteria have phosphotransferase systems; some obligately anaerobic bacteria (e.g., *Clostridium*) also have PTSs. Many carbohydrates are transported by these systems. *E. coli* takes up glucose, fructose, mannitol, sucrose, *N*acetylglucosamine, cellobiose, and other carbohydrates by group translocation. Besides their role in transport, PTS proteins can act

as chemoreceptors for chemotaxis.

Iron Uptake

Almost all microorganisms require iron for use in cytochromes and many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric iron and its derivatives, which leaves little free iron available for transport. Many bacteria and fungi have overcome this difficulty by secreting siderophores [Greek for iron bearers]. **Siderophores** are low molecular weight molecules that are able to complex with ferric iron and supply it to the cell. These iron-transport molecules are normally either hydroxamates or phenolatescatecholates. Ferrichrome is a hydroxamate produced by many fungi; enterobactin is the catecholate formed by *E. coli*. It appears that three siderophore groups complex with iron orbitals to form a six-coordinate, octahedral

complex. Microorganisms secrete siderophores when little iron is available in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a siderophore-receptor protein. Then the iron is either released to enter the cell directly or

the whole iron-siderophore complex is transported inside by an ABC transporter. In *E. coli* the siderophore receptor is in the outer membrane of the cell envelope; when the iron reaches the periplasmic space, it moves through the plasma membrane with the aid of the transporter. After the iron has entered the cell, it is reduced to the ferrous form. Iron is so crucial to microorganisms that they may use more than one route of iron uptake to ensure an adequate supply.

Microbial Growth

Growth may be defined as an increase in cellular constituents. It leads to a rise in cell number when microorganisms reproduce by processes like budding or binary fission. In the latter, individual cells enlarge and divide to yield two progeny of approximately equal size. Growth also results when cells simply become longer or larger. If the microorganism is **coenocytic** that is, a multinucleate organism in which nuclear divisions are not accompanied by cell divisions growth results in an increase in cell size but not cell number. It is usually not convenient to investigate the growth and reproduction of individual microorganisms because of their small size. Therefore, when studying growth, microbiologists normally follow changes in the total population number.

The Growth Curve

Population growth is studied by analyzing the growth curve of a microbial culture. When microorganisms are cultivated in liquid medium, they usually are grown in a **batch culture** or closed system that is, they are incubated in a closed culture vessel

with a single batch of medium. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases (**figure**).

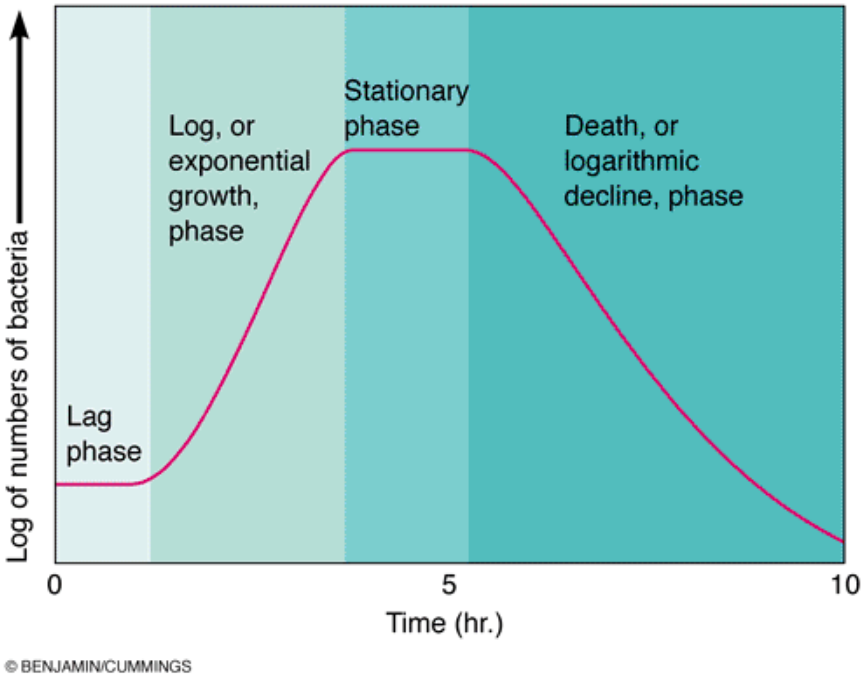


Figure Microbial Growth Curve in a Closed System

Lag Phase

When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs, and therefore this period is called the **lag phase.** Although cell division does not take place right away and there is no net increase in mass, the cell is synthesizing new components. A lag phase prior to the start of cell division can be necessary for a variety of reasons. The cells may be old and depleted of ATP, essential cofactors, and ribosomes; these must be synthesized before growth can

begin. The medium may be different from the one the microorganism was growing in previously. Here new enzymes would be needed to use different nutrients. Possibly the microorganisms have been injured and require time to recover. Whatever the causes, eventually the cells retool, replicate their DNA, begin to increase in mass, and finally divide. The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase. On the other hand, when a young, vigorously growing exponential phase culture is transferred to fresh medium of the same composition, the lag phase will be short or absent.

Exponential Phase

During the **exponential** or **log phase,** microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing. Their rate of growth is constant during the exponential phase; that is, the microorganisms are dividing and doubling in number at regular intervals. Because each individual divides at a slightly different moment, the growth curve rises smoothly rather than in discrete jumps. The population is most uniform in terms of chemical and physiological properties during this phase; therefore exponential phase cultures are usually used in biochemical and physiological studies. Exponential growth is **balanced growth.** That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, **unbalanced growth** results. This is growth during which the rates of synthesis of cell components vary relative to one another until a new balanced state is reached. This response is readily observed in a shift-up experiment in which bacteria are transferred from a nutritionally poor medium to a richer one.

The cells first construct new ribosomes to enhance their capacity for protein synthesis. This is followed by increases in protein and DNA synthesis. Finally, the

expected rise in reproductive rate takes place. Unbalanced growth also results when a bacterial population is shifted down from a rich medium to a poor one. The organisms may previously have been able to obtain many cell components directly from the medium. When shifted to a nutritionally inadequate medium, they need time to make the enzymes required for the biosynthesis of unavailable nutrients. Consequently cell division and DNA replication continue after the shift-down, but net protein and RNA synthesis slow. The cells become smaller and reorganize themselves metabolically until they are able to grow again. Then balanced growth is resumed and the culture enters the exponential phase. These shift-up and shift-down experiments demonstrate that microbial growth is under precise, coordinated control and responds quickly to changes in environmental conditions. When microbial growth is limited by the low concentration of a required nutrient, the final net growth or yield of cells increases with the initial amount of the limiting nutrient present. This is the basis of microbiological assays for vitamins and other growth factors. The rate of growth also increases with nutrient concentration, but in a hyperbolic manner much like that seen with many enzymes. The shape of the curve seems to reflect the rate of nutrient uptake by microbial transport proteins. At sufficiently high nutrient levels the transport systems are saturated, and the growth rate does not rise further with increasing nutrient concentration.

Stationary Phase

Eventually population growth ceases and the growth curve becomes horizontal. This **stationary phase** usually is attained by bacteria at a population level of around 109 cells per ml. Other microorganisms normally do not reach such high population densities, protozoan and algal cultures often having maximum concentrations of about 106 cells per ml. Of course final population size depends on nutrient availability and other factors, as well as the type of microorganism being cultured. In the stationary phase the total number of viable microorganisms remains constant. This may result from a balance between cell division and cell death, or the population may simply cease

to divide though remaining metabolically active. Microbial populations enter the stationary phase for several reasons. One obvious factor is nutrient limitation; if an essential nutrient is severely depleted, population growth will slow. Aerobic organisms often are limited by O2 availability. Oxygen is not very soluble and may be depleted so quickly that only the surface of a culture will have an O2 concentration adequate for growth.

The cells beneath the surface will not be able to grow unless the culture is shaken or aerated in another way. Population growth also may cease due to the accumulation of toxic waste products. This factor seems to limit the growth of many anaerobic cultures (cultures growing in the absence of O2). For example, streptococci can produce so much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and growth is inhibited. Streptococcal cultures also can enter the stationary phase due to depletion of their sugar supply. Finally, there is some evidence that growth may cease when a critical population level is reached. Thus entrance into the stationary phase may result from several factors operating in concert. This probably often occurs in nature

as well because many environments have quite low nutrient levels. Starvation can be a positive experience for bacteria. Many do not respond with obvious morphological changes such as endospore formation, but only decrease somewhat in overall size, often accompanied by protoplast shrinkage and nucleoid condensation. The more important changes are in gene expression and physiology. Starving bacteria frequently produce a variety of **starvation proteins,** which make the cell much more resistant to damage in a variety of ways. They increase peptidoglycan cross-linking and cell wall strength. The Dps (*D*NA-binding *p*rotein from *s*tarved cells) protein protects DNA. Chaperones prevent protein denaturation and renature damaged proteins. As a result of these and many other mechanisms, the starved cells become harder to kill and more resistant to starvation itself, damaging temperature changes, oxidative and osmotic damage, and toxic chemicals such as chlorine. These changes are so effective that some

bacteria can survive starvation for years. Clearly, these considerations are of great practical importance in medical and industrial microbiology. There is even evidence that *Salmonella typhimurium* and some other bacterial pathogens become more virulent when starved.

Death Phase

Detrimental environmental changes like nutrient deprivation and the buildup of toxic wastes lead to the decline in the number of viable cells characteristic of the **death phase.** The death of a microbial population, like its growth during the exponential phase,

is usually logarithmic (that is, a constant proportion of cells dies every hour). This pattern in viable cell count holds even when the total cell number remains constant because the cells simply fail to lyse after dying. Often the only way of deciding whether a bacterial cell is viable is by incubating it in fresh medium; if it does not grow and reproduce, it is assumed to be dead. That is, death is defined to be the irreversible loss of the ability to reproduce. Although most of a microbial population usually dies in a logarithmic fashion, the death rate may decrease after the population has been drastically reduced. This is due to the extended survival of particularly resistant cells. For this and other reasons, the death phase curve may be complex.

Measuring Microbial Growth

Direct Methods of Measurement

1. Plate count:

- Most frequently used method of measuring bacterial populations.

- Inoculate plate with a sample and count number of colonies.

Assumptions:

- Each colony originates from a single bacterial cell.
- Original inoculum is homogeneous.
- No cell aggregates are present.

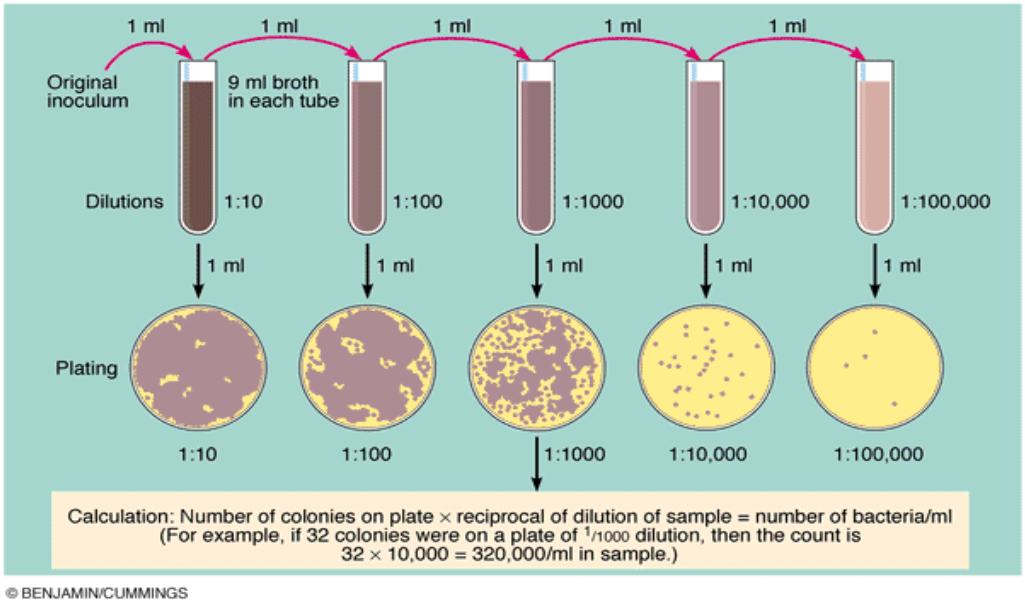
Advantages:

- Measures **viable** cells

Disadvantages:

- Takes 24 hours or more for visible colonies to appear.
- Only counts between 25 and 250 colonies are accurate.
- Must perform **serial dilutions** to get appropriate numbers/plate.

- **Serial Dilutions are Used with the Plate Count Method to Measure Numbers of Bacteria**
- **Serial Dilutions are Used with the Plate Count Method to Measure Numbers of Bacteria**



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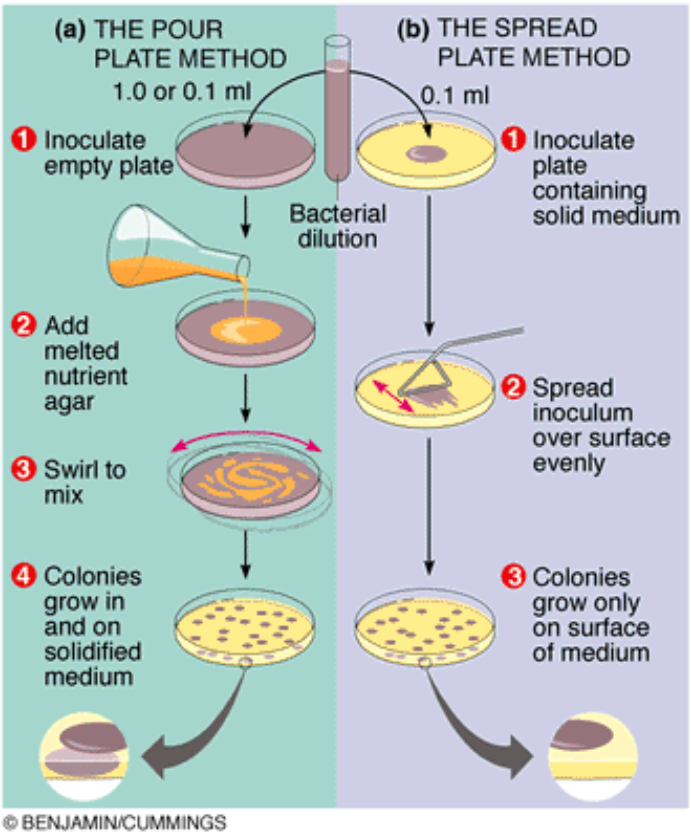
Serial Dilutions are Used with the Plate Count Method to Measure Numbers of Bacteria

Pour Plates versus Spread Plates



Pour Plate:

- Introduce a 1.0 or 0.1 ml inoculum into an **empty** Petri dish.
- Add liquid nutrient medium kept at 50°C.
- Gently mix, allow to solidify, and incubate.

Disadvantages:

- Not useful for heat sensitive organisms.
- Colonies appear under agar surface.

B. Spread Plate:

- Introduce a 0.1 ml inoculum onto the **surface** of Petri dish.

- Spread with a sterile glass rod.
- **Advantages**: Colonies will be on surface and not exposed to melted agar.

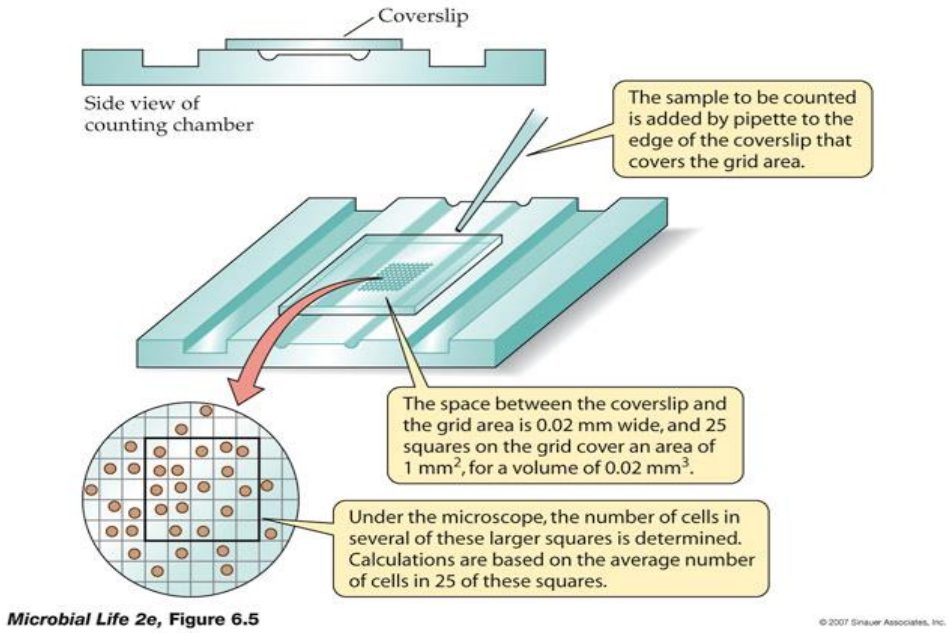
2. Filtration:

- Used to measure small quantities of bacteria.
    - **Example**: Fecal bacteria in a lake or in ocean water.
- A large sample (100 ml or more) is filtered to retain bacteria.
- Filter is transferred onto a Petri dish.
- Incubate and count colonies.

3. Most Probable Number (MPN):

- Used mainly to measure bacteria that will not grow on solid medium.
- Dilute a sample repeatedly and inoculate several broth tubes for each dilution point.
- Count the number of positive tubes in each set.
- **Statistical method**: Determines 95% probability that a bacterial population falls within a certain range.

4. Direct Microscopic Count:



- A specific volume of a bacterial suspension (0.01 ml) is placed on a microscope slide with a special grid.
- Stain is added to visualize bacteria.
- Cells are counted and multiplied by a factor to obtain concentration.

Advantages:

- No incubation time required.

Disadvantages:

- Cannot always distinguish between live and dead bacteria.
- Motile bacteria are difficult to count.
- Requires a high concentration of bacteria (10 million/ml).

Indirect Methods of Measurement

1. Turbidity:

4 As bacteria multiply in media, it becomes turbid.

4 Use a spectrophotometer to determine % transmission or absorbance.

4 Multiply by a factor to determine concentration.

Advantages:

- No incubation time required.

Disadvantages:

- Cannot distinguish between live and dead bacteria.
- Requires a high concentration of bacteria (10 to 100 million cells/ml).

Spectrophotometer

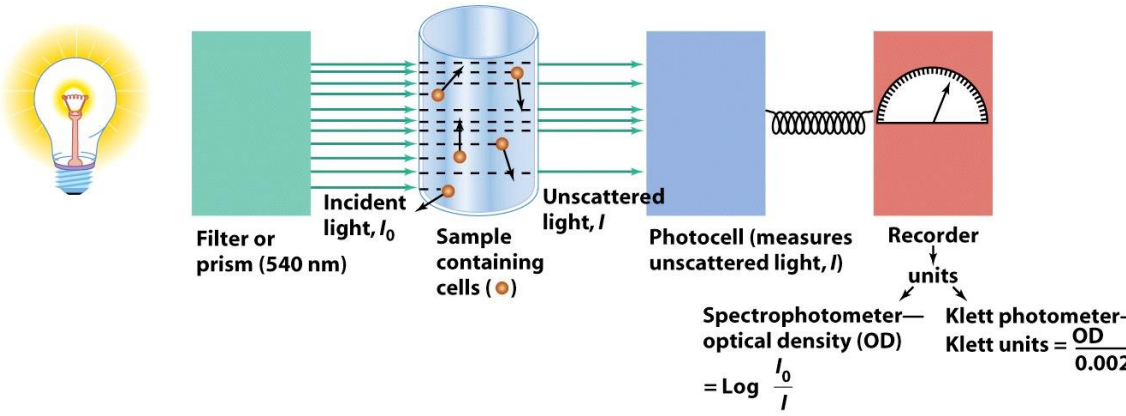


Figure 6-12a Brock Biology of Microorganisms 11/e
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2. Metabolic Activity:

- As bacteria multiply in media, they produce certain products:
  - Carbon dioxide
  - Acids
- Measure metabolic products.
- Expensive

3. Dry Weight:

- Bacteria or fungi in liquid media are centrifuged.
- Resulting cell pellet is weighed.
- Doesn't distinguish live and dead cells.

The Mathematics of Growth

Knowledge of microbial growth rates during the exponential phase is indispensable to microbiologists. Growth rate studies contribute to basic physiological and ecological research and the solution of applied problems in industry. Therefore the quantitative aspects of exponential phase growth will be discussed. During the exponential phase each microorganism is dividing at constant intervals. Thus the population will double in number during a specific length of time called the **generation time** or **doubling time.** This situation can be illustrated with a simple example. Suppose that a culture tube is inoculated with one cell that divides every 20 minutes (**table**).

Table An Example of Exponential Growth

Time[a]	Division Number	2^a	Population ($N_0 \times 2^a$)	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

The population will be 2 cells after 20 minutes, 4 cells after 40 minutes, and so forth. Because the population is doubling every generation, the increase in population is always 2*n* where *n* is the number of generations. The resulting population increase is exponential or logarithmic (**figure**).

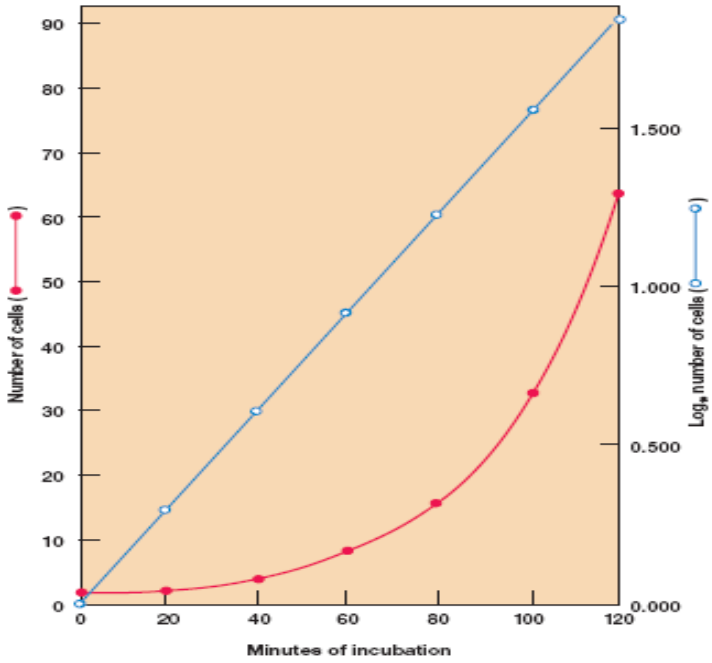


Figure Exponential Microbial Growth. The data from table for six generations of growth are plotted directly (•–•) and in the logarithmic form (°–°). The growth curve is exponential as shown by the linearity of the log plot. generation time.

Let *N*0 = the initial population number

Nt = the population at time

t n = the number of generations in time *t*

Then inspection of the results in table will show that

$$N_t = N_0 \times 2^n.$$

Solving for *n*, the number of generations, where all logarithms are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the **mean growth rate constant *(k)*.** This is the number of generations per unit time, often expressed as the generations per hour.

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

The time it takes a population to double in size—that is, the **mean generation time** or mean doubling time (g), can now be calculated. If the population doubles ($t = g$), then

$$N_t = 2N_0.$$

Substitute $2N0$ into the mean growth rate equation and solve for k.

$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The mean generation time is the reciprocal of the mean growth rate constant.

$$g = \frac{1}{k}$$

The mean generation time (g) can be determined directly from a semilogarithmic plot of the growth data (**figure**) and the growth rate constant calculated from the g value.

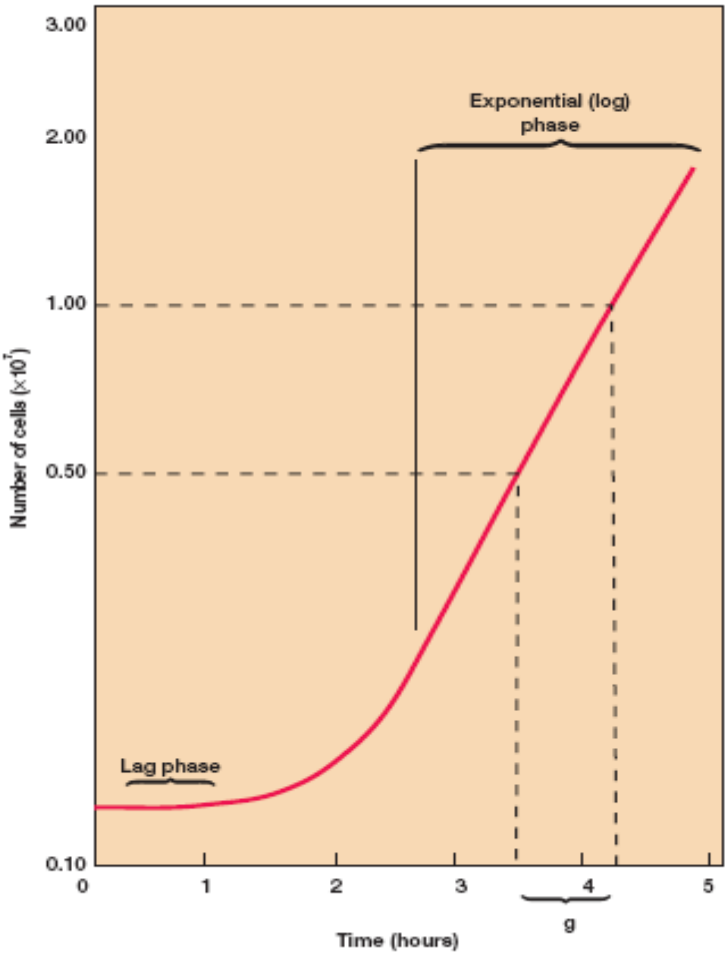


Figure Generation Time Determination. The generation time can be determined from a microbial growth curve. The population data are plotted with the logarithmic axis used for the number of cells. The time to double the population number is then read directly from the plot. The log of the population number can also be plotted against time on regular axes. The generation time also may be calculated directly from the previous equations. For example, suppose that a bacterial population increases from 103 cells to 109 cells in 10 hours.

$$k = \frac{\log 10^9 - \log 10^3}{(0.301)(10\ \text{hr})} = \frac{9 - 3}{3.01\ \text{hr}} = 2.0\ \text{generations/hr}$$

$$g = \frac{1}{2.0\ \text{gen./hr}} = 0.5\ \text{hr/gen. or 30 min/gen.}$$

Generation times vary markedly with the species of microorganism and environmental conditions. They range from less than 10 minutes (0.17 hours) for a few bacteria to several days with some eucaryotic microorganisms. Generation times in nature are usually much longer than in culture.

The Continuous Culture of Microorganisms

Up to this point the focus has been on closed systems called batch cultures in which nutrient supplies are not renewed nor wastes removed. Exponential growth lasts for only a few generations and soon the stationary phase is reached. However, it is possible to grow microorganisms in an open system, a system with constant environmental conditions maintained through continual provision of nutrients and removal of wastes. These conditions are met in the laboratory by a **continuous culture system.** A microbial

population can be maintained in the exponential growth phase and at a constant biomass concentration for extended periods in a continuous culture system. **The Chemostat** Two major types of continuous culture systems commonly are used: (1) chemostats and (2) turbidostats. A **chemostat** is constructed so that sterile medium is fed into the culture vessel at the same rate as the media containing microorganisms is removed (**figure**).

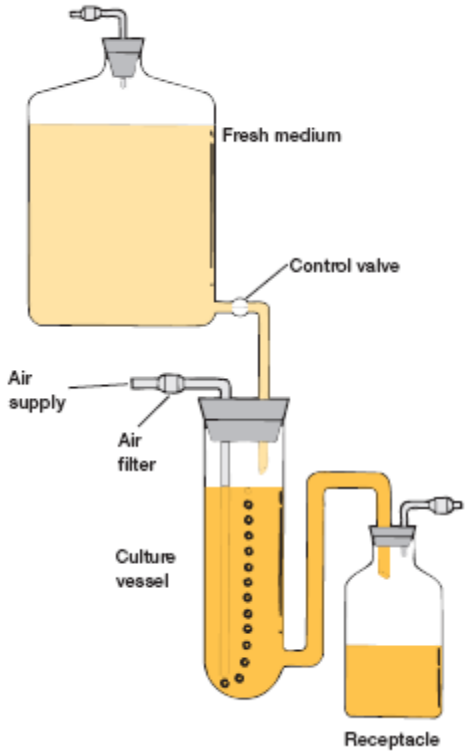


Figure A Continuous Culture System: The Chemostat.

Schematic diagram of the system. The fresh medium contains a limiting amount of an essential nutrient. Growth rate is determined by the rate of flow of medium through the culture vessel. The culture medium for a chemostat possesses an essential nutrient (e.g., an amino acid) in limiting quantities. Because of the presence of a limiting nutrient, the growth rate is determined by the rate at which new medium is fed into the growth chamber, and the final cell density depends on the concentration of the limiting nutrient. The rate of nutrient exchange is expressed as the dilution rate (*D*), the rate at which medium flows through the culture vessel relative to the vessel volume, where *f* is the flow

rate (ml/hr) and *V* is the vessel volume (ml).

$$D = f/V$$

For example, if *f* is 30 ml/hr and *V* is 100 ml, the dilution rate is 0.30 hr^{-1}. Both the microbial population level and the generation time are related to the dilution rate (**figure**).

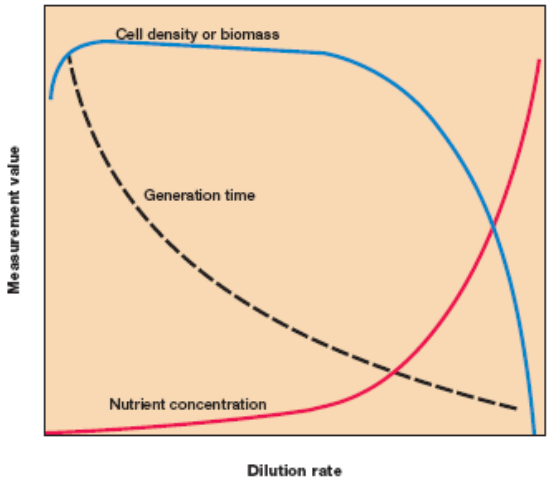


Figure Chemostat Dilution Rate and Microbial Growth.

The effects of changing the dilution rate in a chemostat. The microbial population density remains unchanged over a wide range of dilution rates. The generation time decreases (i.e., the growth rate rises) as the dilution rate increases. The limiting nutrient will be almost completely depleted under these balanced conditions. If the dilution rate rises too high, the microorganisms can actually be washed out of the culture vessel before reproducing because the dilution rate is greater than the maximum growth rate. The limiting nutrient concentration rises at higher dilution rates because fewer microorganisms are present to use it. At very low dilution rates, an increase in *D*

causes a rise in both cell density and the growth rate. This is because of the effect of nutrient concentration on the growth rate, sometimes called the

Monod relationship (figure). Only a limited supply of nutrient is available at low dilution rates. Much of the available energy must be used for cell maintenance, not for growth and reproduction. As the dilution rate increases, the amount of nutrients and the resulting cell density rise because energy is available for both maintenance and growth. The growth rate increases when the total available energy exceeds the **maintenance energy.**

The Turbidostat

The second type of continuous culture system, the **turbidostat,** has a photocell that measures the absorbance or turbidity of the culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity or cell density. The turbidostat differs from the chemostat in several ways. The dilution rate in a turbidostat varies rather than remaining constant, and its culture medium lacks a limiting nutrient. The turbidostat operates best at high dilution rates; the chemostat is most stable and effective at lower dilution rates. Continuous culture systems are very useful because they provide a constant supply of cells in exponential phase and growing at a known rate. They make possible the study of microbial growth at very low nutrient levels, concentrations close to those present in natural environments. These systems are essential for research in many areas for example, in studies on interactions between microbial species under environmental conditions resembling those in a freshwater lake or pond. Continuous systems also are used in food and industrial microbiology.

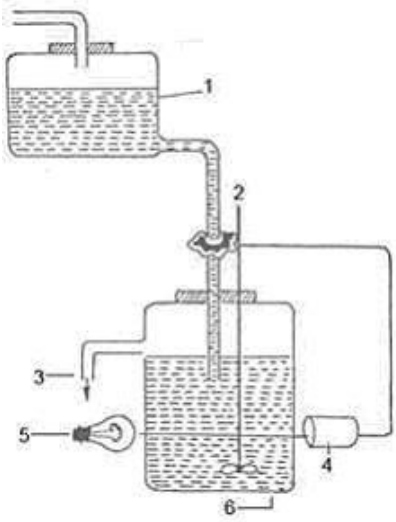


Fig: Turbidostat

1. Reservoir of Sterile Medium

4. Photo cell

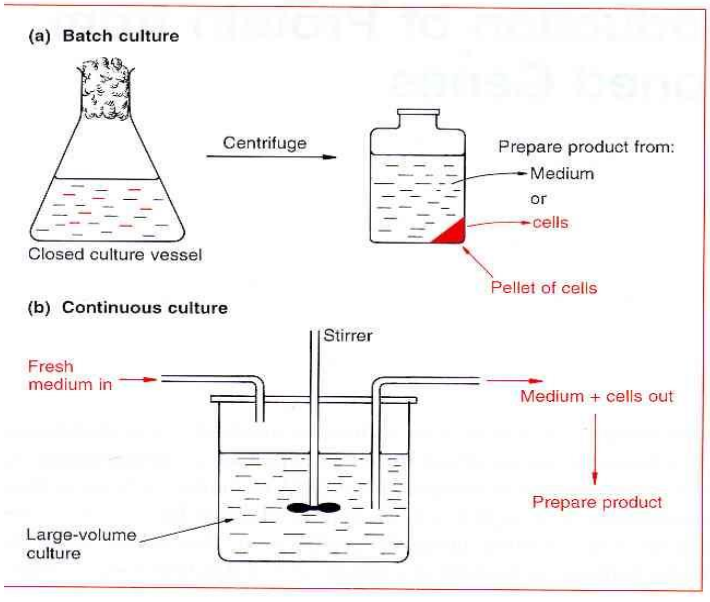
2. Valve Controlling Flow of Medium

5. Light Source

3. Outlet for Spent Medium

6. Turbidostat

BATCH CULTURE and CONTUINUOUS REACTORS



1. Batch culture is where biomass is added to a reactor containing the substrate consisting of a carbon source, an energy source and nutrients. The cells

grow using these and at some time later, one of these is exhausted. This is referred to as the "limiting substrate" – all other substrates are considered "in excess"

2. If the carbon and energy source runs out, then the biomass stops growing and the remaining excess nutrients are not uptaken (unless they are an energy source as well). If the nutrient runs out first, then (after this) the remaining excess carbon and energy source may continue to be utilised (at a slower arte than when the biomass is growing). Thus further products may be formed. Since there are no nutrients remaining , there can be no further biomass growth. These two differing profiles are important in wastewater treatment.
3. Batch culture consists of six phase the first two of which are the lag phases, periods of time where the cell adjusts to its new environment and is not yet capable of growing at it maximum possible rate (μ_{MAX}). Following growth at the maximum rate, there is a **very rapid** decline form the maximum growth rate to zero **over a very short time period**. Because of this short time, the cell is unable to adjust its macromolecular composition, enzyme levels etc, since at one time there is excess substrate and then, a few moments later, there is substrate limitation and then, a few moments later no substrate remaining. This situation is unlike continuous culture, where we are able to sustain biomass at any specific growth arte for 0 to μ_{MAX} **indefinitely by an appropriate feeding policy.** Consequently, these cells come to a steady state, in which there macromolecular composition and their enzymes levels have been adjusted to best suit the specific growth rate at which they are growing.

4. In batch culture, microorganisms go through a number of growth phases. Initially, the specific growth rate is less than the maximum specific growth rate possible and the growth rate increases as the microorganism adapts to

the growth environment. At some point during the process, the microorganism will grow at the maximum specific growth arte until the limiting substrate becomes limiting. At this point, the specific growth rate rapidly declines from the maximum specific rate to zero. This process occurs in a very short time and , consequently, the microorganism is unable to make any significant adjustments to enzyme levels, pH and osmotic gradients, macromolecular composition etc.

5. In continuous culture, on the other hand, microorganisms are placed in an environment where the feed rate to the system and from the system is fixed. Thus, microorganisms experience a constant, and steady supply of limiting substrate and nutrients. Consequently, they can (over time) adjust their enzyme levels, pH and osmotic gradients, macromolecular composition etc. to achieve an "optimal growth". This situation is generally referred to as "steady state" and may take up to 10-20 generations to achieve.

6. When a mass balance for biomass and limiting substrate is undertaken in a continuous culture, there are three major outcomes:
   a. The specific growth rate is equal to the feed rate divided by the reactor volume. This is also referred to as the dilution rate.

$$D = F / V = \mu$$

   b. The biomass concentration in the outlet stream is equal to the biomass from substrate yield times the difference between the feed concentration and outlet concentration of the limiting substrate:

$$X = y_{XS} * (S_0 - S)$$

Since the outlet concentration of limiting substrate is generally far less than the feed concentration, this reduces to:

$$X \approx y_{XS} * S_o$$

c. The concentration of biomass in the reactor is the same as the concentration of biomass in the effluent.

7. Continuous systems are, consequently, not very effective in the case of slow growing organisms. Under such conditions, either the federate must be very small (meaning that the throughput of waste is very small) or the reactor volume must be very large (which is expensive).

8. To overcome this problem, cell recycle is introduced. Under this condition, the biomass density in the reactor becomes very large and, since the feed concentration of limiting substrate remains the same, each cell gets less and less of this limiting substrate. Consequently, the specific growth rate reduces rapidly. Such systems are also referred to a "high biomass density systems" or "high density, low growth rate systems"

9. The importance of low growth rate systems lies in the fact that specific (limiting) substrate uptake is described by the relationship:

$$Q_S = \alpha\mu + \beta$$

Where α is the "growth associated" specific substrate uptake rate (mmol substrate / g biomass) and β is the "non-growth associated" specific substrate uptake rate (mmol substrate / g biomass / h). "Non-growth associated" processes include the maintance of concentration and osmotiv gradients, DNA repair and other cellular process requiring ATP in the absence of growth. As the growth rate becomes lower and lower, the "non-growth associated" specific substrate uptake rate is a larger proportion of the total specific substrate uptake. In addition, for the case where the limiting substrate is used to generate energy via catabolism, under lower and lower

specific growth rates, the proportion of the total specific substrate uptake that is channeled through the catabolic process is increased. This has major consequences for wastewater treatment, since it means that at low growth rates the amount of sludge production (anabolism) will be less than under higher growth rates.

10. For this reason high density systems are widely used.

11. When a mass balance for biomass and limiting substrate is undertaken in a continuous culture with biomass recycle, there are three major outcomes:
    d. The specific growth rate is no longer equal to the feed rate divided by the reactor volume. Hence, feed rates higher than the maximum feed rates possible in continuous systems are possible.

       The biomass concentration in the outlet stream is no longer equal to the biomass from substrate yield times the difference between the feed concentration and outlet concentration of the limiting substrate.
    e. The concentration of biomass in the effluent is not the same as the concentration of biomass in the reactor.

12. Sequencing Batch Reactors of Fill/Draw Reactors are semi batch reactors that are able to accumulate biomass to a high concentration by repeated cycles of supply if new growth medium (filling), allowing growth, settling, take off of spent liquor, and then REPEATING supply of new growth medium (filling), allowing growth, settling, take off of spent liquor. This is achieved by using a CSTR and having the reactor stirrer being able to be turned off and on and having both the influent and effluent pumps being also to also be turned on and off. When aeration to the tank is included and there is the provision for this to be turned on or off, then the reactor can be

operated in an aerobic operation, anaerobic operation and an anoxic operation. Such operational modes make this reactor very versatile and, it is for this reason, that it is gaining in popularity and application. Significant aeration savings can be obtained by operating a number of SBR's in parallel since the volume aerated can be significantly reduced by having the aeration cyclers of individual reactors staggered over the full operational cycle time.

Synchronous Growth of Bacteria

Studying the growth of bacterial populations in batch or continuous cultures does not permit any conclusions about the growth behavior of individual cells, because the distribution ofcell size (and hence cell age) among the members of the population is completely random. Information about the growth behavior of individual bacteria can, however, be obtained by the study of **synchronous cultures**. Synchronized cultures must be composed of cells which are all at the same stage of the **bacterial cell cycle**. Measurements made on synchronized cultures are equivalent to measurements made on individual cells. A number of clever techniques have been devised to obtain bacterial populations at the same stage in the cell cycle. Some techniques involve manipulation of environmental parameters which induces the population to start or stop growth at the same point in the cell cycle, while others are physical methods for selection of cells that have just completed the process of binary fission. Theoretically, the smallest cells in a bacterial population are those that have just completed the process of cell division. Synchronous growth of a population of bacterial cells is illustrated in Figure. Synchronous cultures rapidly lose synchrony because not all cells in the population divide at exactly the same size, age or time.

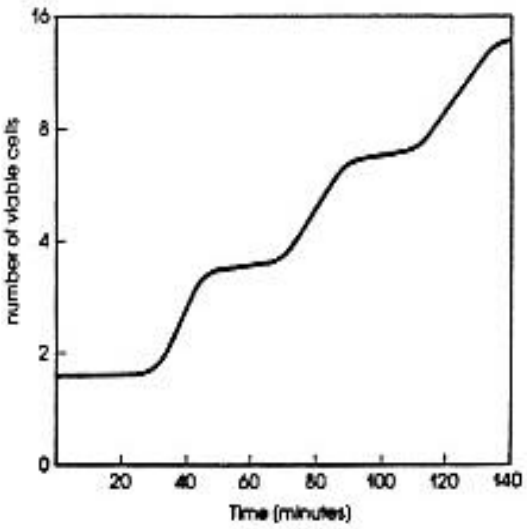


Figure . The synchronous growth of a bacterial population. By careful selection of cells that have just divided, a bacterial population can be synchronized in the bacterial cell division cycle. Synchrony can be maintained for only a few generations.

By careful selection of cells that have just divided, a bacterial population can be synchronized in the bacterial cell division cycle. Synchrony can be maintained for only a few generations.

Factors affecting microbial growth

Growth may be profoundly affected by a number of physical factors.

Temperature

Microorganisms as a group are able to grow over a wide range of temperatures, from around freezing to above boiling point. For any organism, the *minimum* and *maximum* growth temperatures define the range over which growth is possible; this is typically about 25–30 ◦C. Growth is slower at low temperatures because enzymes work less efficiently and also because lipids tend to harden and there is a loss of membrane

fluidity. Growth rates increase with temperature until the *optimum* temperature is reached, then the rate falls again. The optimum and limiting temperatures for an organism are a reflection of the temperature range of its enzyme systems, which in turn are determined by their three-dimensional protein structures. The optimum temperature is generally closer to the maximum growth temperature than the minimum. Once the optimum value is passed, the loss of activity caused by denaturation of enzymes causes the rate of growth to fall away sharply.

The majority of microorganisms achieve optimal growth at 'middling' temperatures of around 20–45 ◦C; these are called *mesophiles* (Figure 5.5). Contrast these with *thermophiles*, which have become adapted to not only surviving, but thriving at much higher temperatures. Typically, these would be capable of growth within a range of about 40–80 ◦C, with an optimum around 50–65 ◦C. *Extreme thermophiles* have optimum values in excess of this, and can tolerate temperatures in excess of 100°C. In 2003, a member of the primitive bacterial group called the Archaea was reported as growing at a temperature of 121 ◦C, a new world record! *Psychrophiles* occupy the other extreme of the temperature range; they can grow at 0◦C, with optimal growth occurring at 15 ◦C or below. Such organisms are not able to grow at temperatures

above 25 ◦C or so. *Psychrotrophs*, on the other hand, although they can also grow at 0 ◦C, have much higher temperature optima (20–30°C). Members of this group are often economically significant due to their ability to grow on refrigerated foodstuffs.In the laboratory, appropriate temperatures for growth are provided by culturing in an appropriate incubator. These come in a variety of shapes and sizes, but all are thermostatically controlled and generally hold the temperature within a degree or two of the desired value.

pH

Microorganisms are strongly influenced by the prevailing pH of their surroundings. As with temperature, we can define minimum, optimum and maximum values for growth of a particular type. The pH range between minimum and maximum values) is greater in fungi than it is in bacteria. Most microorganisms grow best around neutrality (pH 7). Many bacteria prefer slightly alkaline conditions but relatively few are tolerant of acid conditions, and fewer still are *acidophilic*.

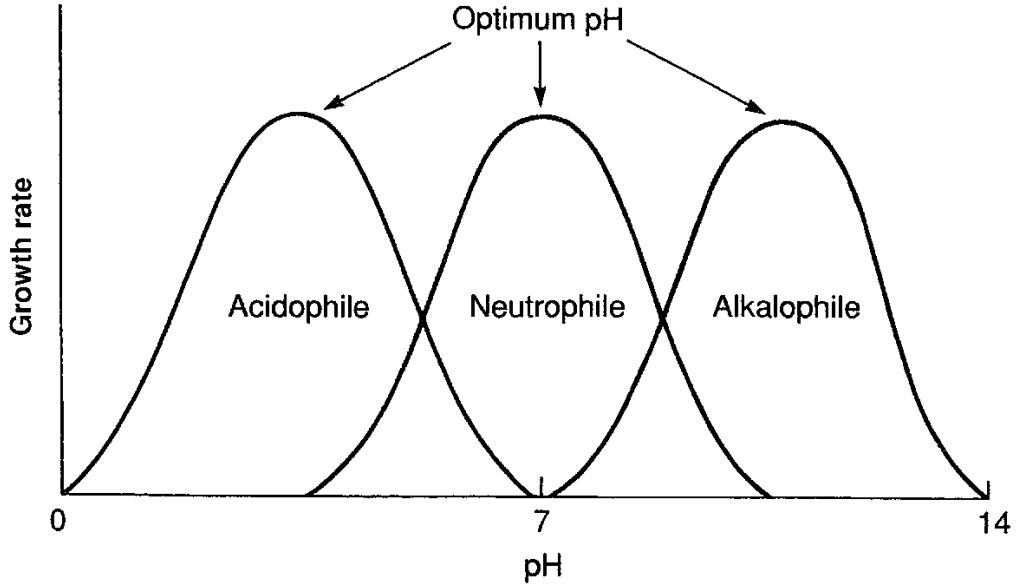


Figure Effect of pH on microbial growth rate. Individual species of microorganism occupy a relatively narrow range of pH. Although for most species this is around neutrality, both acidophilic and alkalophilic forms exist. The shape of the curve reflects the properties of a particular organism's enzymes and other proteins

Fungi, on the other hand, generally prefer slightly acid conditions and therefore tend to dominate bacteria when these prevail. The reason for the growth rate falling away either side of the optimum value is again due to alterations in three-dimensional protein structure. The pH value of growth media is adjusted to the desired value by the addition of acid or alkali during its preparation. The metabolic activities of

microorganisms often means that they change the pH of their environment as growth proceeds, so it is important in a laboratory growth medium that a desirable pH is not only set but maintained. This is achieved by the use of an appropriate *buffer* system. Phosphate buffers are widely used in the microbiology laboratory; they enable media to minimise changes in their pH when acid or alkali is produced

Oxygen

Oxygen is present as a major constituent (20 per cent) of our atmosphere, and most life forms are dependent upon it for survival and growth. Such organisms are termed *aerobes*. Not all organisms are aerobes however; some *anaerobes* are able to survive in the absence of oxygen, and for some this is actually a necessity. Aerobic organisms require oxygen to act as a terminal electron acceptor in their respiratory chains. Such organisms, when grown in laboratory culture, must therefore be provided with enough oxygen to satisfy their requirements. For a shallow layer of medium such as that in a petri dish, sufficient oxygen is available dissolved in surface moisture. In a deeper culture such as a flask of broth however, aerobes will only grow in the surface layers unless additional oxygen is provided (oxygen is poorly soluble in water). This is usually done by shaking or mechanical stirring.

Obligate anaerobes cannot tolerate oxygen at all. They are cultured in special anaerobic chambers, and oxygen excluded from all liquid and solid media. *Facultative anaerobes* are able to act like aerobes in the presence of oxygen, but have the added facility of being able to survive when conditions become anaerobic. *Aerotolerant anaerobes* are organisms that are basically anaerobic; although they are not inhibited by atmospheric oxygen, they do not utilise it. *Microaerophiles* require oxygen, but are only able to tolerate low concentrations of it (2–10 per cent), finding higher concentrations harmful. Organisms inoculated into a static culture medium will grow at positions that reflect their oxygen preferences.

Carbon dioxide

Heterotrophic bacteria also require small amounts of carbon dioxide, which is incorporated into various metabolic intermediates. This dependency can be demonstrated by the failure of these organisms to grow if carbon dioxide is deliberately removed from the atmosphere. Microorganisms have different oxygen requirements. In a static culture, microorganisms occupy different regions of the medium, reflecting their pattern of oxygen usage. (a) Obligate aerobes must grow at or near the surface, where oxygen is able to diffuse. (b) Facultative anaerobes are able to adjust their metabolism to the prevailing oxygen conditions. (c) Obligate anaerobes, in contrast, occupy those zones where no oxygen is present at all. (d) Aerotolerant anaerobes do not use oxygen, but neither are they inhibited by it. (e) Microaerophiles have specific oxygen requirements, and can only grow within a narrow range of oxygen tensions

Osmotic pressure

Osmosis is the diffusion of water across a semipermeable membrane from a less concentrated solution to a more concentrated one, equalising concentrations. The pressure

required to make this happen is called the *osmotic pressure*. If a cell were placed in a hypertonic solution (one whose solute concentration is higher), osmosis would lead to a loss of water from the cell (*plasmolysis*). This is the basis of using high concentrations of salt or other solutes in preserving foods against microbial attack. In the opposite situation, waterwould pass from a dilute (hypotonic) solution into the cell, causing it to swell and burst. The rigid cell walls of bacteria prevent them from bursting; this, together with their minute size, makes them less sensitive to variations in osmotic pressure than other types of cell. They are generally able to tolerate NaCl concentrations of between 0.5 and 3.0 per cent. *Haloduric* (‘salt-tolerant’) bacteria are able to tolerate concentrations ten times as high, but prefer lower concentrations, whereas *halophilic* (‘salt-loving’) forms are adapted to grow best in conditions of high salinity such as

those that prevail in the Dead Sea in the Middle East. In order to do this without plasmolysis occurring, they must build up a higher internal solute concentration, which they do by actively concentrating potassium ions inside the cell.

Light

Phototrophic organisms require light in order to carry out photosynthesis. In the laboratory, care must be taken that light of the correct wavelength is used, and that the source used does not also act as a heat source. Fluorescent light produces little heat, but does not provide the wavelengths in excess of 750 nm needed by purple and green photosynthetic bacteria.

Biofilm formation

A **biofilm** is any group of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed ofextracellular polymeric substances (EPS). The EPS components are produced by the cells within the biofilm and are typically a polymericconglomeration of extracellular DNA, proteins, and polysaccharides. Because they have three-dimensional structure and represent a community lifestyle for microorganisms, biofilms are frequently described metaphorically as "cities for microbes.

Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium.[7] Biofilms can be present on the teeth of most animals as dental plaque, where they may cause tooth decay andgum disease.

Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or in some cases, by exposure of planktonic cells to sub-inhibitory concentrations

of antibiotics.[9][10]When a cell switches to the biofilm mode of growth, it undergoes aphenotypic shift in behavior in which large suites of genes are differentially regulated

The formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. It is thought that the first colonist bacteria of a biofilm adhere to the surface initially through weak, reversible adhesion via van der Waals forces and hydrophobic effects.[12][13] If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.

Hydrophobicity can also affect the ability of bacteria to form biofilms. Bacteria with increased hydrophobicity have reduced repulsion between the extracellular matrix and the bacterium.[14] Some bacteria species are not able to attach to a surface on their own successfully due to their limited motility but are instead able to anchor themselves to the matrix or directly to other, earlier bacteria colonists. Non-motile bacteriacannot recognize surfaces or aggregate together as easily as motile bacteria.

During surface colonization bacteria cells are able to communicate using quorum sensing (QS) products such as N-acyl homoserine lactone (AHL). Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. Polysaccharide matrices typically enclose bacterial biofilms. In addition to the polysaccharides, these matrices may also contain material from the surrounding environment, including but not limited to minerals, soil particles, and blood components, such as erythrocytes and fibrin.[14] The final stage of biofilm formation is known as dispersion, and is the stage in which the biofilm is established and may only change in shape and size.

The development of a biofilm may allow for an aggregate cell colony (or colonies) to be increasingly resistant to antibiotics. Cell-cell communication or quorum sensing has been shown to be involved in the formation of biofilm in several bacterial species.

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POSSIBLE QUESTION

UNIT 2

2 marks

1. Define sporulation.
2. Write the function of spores
3. Example of spore forming bacteria?
4. Asexual sporulation in fungi?
5. Describe about Biofilm.
6. Write short notes on Biosurfactant.
7. Write about the factors affecting sporulation?
8. What is passive diffusion?
9. Mention the chemicals used for controlling the microorganism.
10. Difference between asexual and sexual sporulation?

8marks

1. Brief notes on sporulation cycle?
2. Explain the pattern of spore formation in Aspergillus?
3. Explain the pattern of spore formation in Penicillium?
4. Describe the Nutritional requirements of microorganisms?
5. Detail account on Biofilm formation in bacteria?
6. Write elaborately about the Production of biosurfactant?
7. Illustrate the stages of spore cycle in eukaryotes.
8. Detail account on factors Affecting spore formation?
9. Justify the Autoclaving kill the pathogens with suitable examples
10. Detail account on Physical method to control microorganism.

Unit II Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
_____ is a matrix of extracellular polymeric substance formed by a group of microbes for adhering on a surface.	Biofilm	Biosurfactant	Capsule	Polysaccharide	Biofilm
_____ frequently forms biofilms in cooling towers and air conditioned rooms causing disease	*Pseudomonas*	*Legionella*	*Streptococcus*	*Vibrio*	*Legionella*
_____ produces sporangiospores	*Leptospira*	*Cocci*	*Bacillus*	*Rhizopus*	*Rhizopus*
_____ forms subtermial endospore	*Aspergillus*	*Bacillus subtilis*	*Nocardia*	*Streptococcus*	*Bacillus subtilis*
_____ is a type of asexual spore	Metaspore	Endospores	Fusospores	Sporangiospores	Sporangiospores
_____ are formed by fragmentatio of hypha	Zoospores	Endospores	Arthrospores	Exospores	Arthrospores
_____ is the main method of communication between the bacterial cells within the biofilm	Mineral sensing	Protein sensing	Carbohydrate sensing	Quorum sensing	Quorum sensing
_____ helps in the formation of microbial biofilms	Extracellular glucose	Extracellular Lipids	Extracellular polysaccharides	Extracellular vitamins	Extracellular polysaccharides
A branching network of hypha	Monocelium	Dicelium	Mycelium	Tricelium	Mycelium
A class of biosurfactant	Sophorolipids	Surfactin	Rhamnolipids	Lichenysin	Rhamnolipids

produced by Pseudomonas					
A cross-wall in a hypha	Peptum	Septum	Pestum	Pectum	Septum
A female gametangium is called	Oogonium	Sporangium	Exosporium	Cortex	Oogonium
A form of asexual spore in fungi belonging to the Basidiomycota	Boidia	Coidia	Zooidia	Oidia	Oidia
A structure specialized for the production of gametes during sexual reproduction	Gametangium	Megaterium	Systrophium	Hypogium	Gametangium
A surface active antibiotic	Penicillin	Gramicidin S	Streptomycin	Tetracycline	Gramicidin S
Amino acid rich in spore coat of bacteria	Cystine	Arginine	Lysine	Leucine	Cystine
Ascus usually contain _____ number of ascospores	4 to 8	3 to 5	2 to 9	1 to 2	4 to 8
Aspergillus and *Penicillium* belongs to	Ascocetes	Mycocetes	Ascomycota	Mycoascota	Ascomycota
Blastospores are seen in	Sporulating Yeast	Bacteria	Moulds	Algae	Sporulating Yeast
Cell or nucleus containing two complete sets of chromosomes, one from each parent is	Polaroid	Amphoid	Diploid	Haploid	Diploid
Cell or nucleus having a single set of unpaired chromosomes is	Diploid	Polaroid	Amphoid	Haploid	Haploid
Clostridium tetani forms	No spore	Central endospore	Sub-terminal endospore	Terminal endospore	Terminal endospore

Compounds produced by microbes that reduce surface tension between molecules & their interface	Biofilm	Vitamins	Antibiotics	Biosurfactant	Biosurfactant
Dental plaque is an oral biofilm that adheres to the teeth ans is caused by _____	*Lactococcus*	*Streptococcus mutans*	*Cyanobacteria*	*Fusarium*	*Streptococcus mutan*
Endospores are chracteristics of	Bacteria	Fungi	Algae	Virus	Bacteria
Endospores can survive environmental stresses such as	High temperature	UV irradiation	Desiccation	All of the above	All of the above
Eukaryotic ribosomes are made up of _____ ribosomes	60S + 40S	50S + 40S	60S + 50S	70S + 40S	60S + 40S
Example for bacteria producing central spores	*Staphylococcus*	*Penicillium*	*Bacillus cereus*	*Actinomycetes*	*Bacillus cereus*
Extreme survival strategy of endospore formation is seen in _____ bacteria	High G+C	Low G+C	Moderate G+C	No G+C	Low G+C
Final stage of biofilm formation is	Dispersion	Expression	Repression	Opression	Dispersion
Fusion between hyphae is called	Oocystis	Endocystis	Cyanocystis	Anastomosis	Anastomosis
Fusion of two nuclei within a cell is called	Plasmogamy	Karyogamy	Heterothallic	Homothallic	Karyogamy
Hydrocarbon degradation can be enhanced by	Biosurfactants	Biofilms	Bio-oils	Biopolymers	Biosurfactants

In fungi, spores are enclosed/contained in a specialised structure called	Acremonium	Sterigmata	Sporangium	Spore coat	Sporangium
In *Streptomycetes*, spores develop from dedicated reproductive structures called	Mycelial hyphae	Conidiophore	Phialides	Aerial hyphae	Aerial hyphae
Kind of spores seen in fungi belonging to the Ascomycota and Basidiomycota	Conidia	Zooidia	Subterminal	Oocysts	Conidia
Microbial surfactants are complex molecules covering a wide range of chemical types including	Peptides	Fatty acids	Phospholipids	All of the above	All of the above
Model organism to study spore formation	*Mycobacterium*	*Bacillus subtilis*	*Streptomyces*	*Fusarium*	*Bacillus subtilis*
Mycelia or Hyphae of *Aspergillus* are	Haploid	Diploid	Polaroid	Amphoid	Haploid
Non motile spore is called	Metaspore	Aplanospore	Oospore	Zoospore	Aplanospore
Outer coat of a bacterial endospore is called	Exosporium	Spore coat	Cortex	Endosporium	Exosporium
Phialospores are seen in	*Aspergillus*	*Fusarium*	*Trichoderma*	*Penicillium*	*Penicillium*
Plasmogamy and Karyogamy are part of _____ in fungi	Asexual cycle	Sexual cycle	Budding cycle	Fission cycle	Sexual cycle
Prokaryotes lack	Mitochondria	Defined nucleus	Golgi Apparatus	All of the above	All of the above
Rhizopus and *Mucor* produces	Gyzospores	Zygospores	Sporozygs	Oospores	Zygospores
Size of an endospore in	1.2 mm	1.2 cm	12 µm	1.2 µm	1.2 µm

Bacillus					
Spore forming is mainly seen in _____ bacteria	Gram positive	Gram negative	Cell wall less	Acid-fast	Gram positive
Spores of Streptomyces accumulate the sugar	Rhamnose	Sucrose	Trehalose	Glucose	Trehalose
Spores of Streptomyces have an additional outer layer of proteins called _____ which renders hydrophobicity to aerial hyphae	Chaplins	Caplins	Duplins	Triplins	Chaplins
Staining technique for visualizing endospores	Grams	Alberts	Schaeffer-Fulton	Loefflers	Schaeffer-Fulton
Sterols are absent in	Plant	Fungi	Higher animals	Prokaryotes	Prokaryotes
Surfactin and Lichenysin are mainly produced by bacteria of the genus	*Lactobacillus*	*Bacillus*	*Bordetella*	*Torulopsis*	*Bacillus*
The cell wall of *Archaea* is made up of	Muramic acid	Pseudomurein	Mucopeptin	N-acetyl glucosamine	Pseudomurein
The developmental unit of a mycelium	Dyphae	Hyphae	Myphae	Syphae	Hyphae
The resistance of a bacterial spore is implicated by	Muramic acid	Acetic acid	Dipicolonic acid	Phosphoric acid	Dipicolonic acid
The yield of the biosurfactant greatly depends on the _____ environment of the growing microorganism	Coexistence	Atmospheric	Nutritional	Temperature	Nutritional
Type of spores produced by	Endospore	Exospore	Basidiospores	Zoospore	Basidiospores

mushrooms					
Which forms a complex with dipicolinic acid that is responsible for the resistance of spore?	Magnesium	Potassium	Sodium	Calcium	Calcium
Which of the following has the capability to form biofilm?	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	Both I & II	*Penicillium*	Both I & II
Yeast reproduce by	Fission	Spore	Budding	None	Budding

Unit III –

Microbial metabolism

An Overview of Metabolism

Metabolism may be divided into two major parts. In **catabolism** [Greek *cata,* down, and *ballein,* to throw] larger and more complex molecules are broken down into smaller, simpler molecules with the release of energy. Some of this energy is trapped and made available for work; the remainder is released as heat. The trapped energy can then be used in anabolism, the second area of metabolism. **Anabolism** [Greek *ana,* up] is the synthesis of complex molecules from simpler ones with the input of energy. An anabolic process uses energy to increase the order of a system. Although the division of metabolism into two major parts is convenient and commonly employed, not all energy-yielding processes are comfortably encompassed by the previous definition of catabolism unless it is expanded to include processes that do not involve the degradation of complex organic molecules. In a broader sense, microorganisms usually use one of three sources of energy. Phototrophs capture radiant energy from the sun. Chemoorganotrophs oxidize organic molecules to liberate energy, while chemolithotrophs employ inorganic nutrients as energy sources.

Microorganisms vary not only in their energy sources, but also in the electron acceptors used by chemotrophs. Three major kinds of acceptors are employed. In **fermentation** energy substrate is oxidized and degraded without the participation of an exogenous or externally derived electron acceptor. Usually the catabolic pathway produces an intermediate such as pyruvate that acts as the electron acceptor. Fermentation normally occurs under anaerobic conditions, but also occurs sometimes when oxygen is present. Of course, energy-yielding metabolism can make use of

exogenous or externally derived electron acceptors. This metabolic process is called **respiration** and may be divided into two different types.

In **aerobic respiration,** the final electron acceptor is oxygen, whereas the acceptor in **anaerobic respiration** is a different exogenous acceptor. Most often the acceptor in anaerobic respiration is inorganic, but organic acceptors such as fumarate may be used. Most respiration involves the activity of an electron transport chain. The amount of available energy is quite different for fermentation and respiration. The electron acceptor in fermentation is at the same oxidation state as the original nutrient and there is no overall net oxidation of the nutrient. Thus only a limited amount of energy is made available. The acceptor in respiratory processes has reduction potential much more positive that the substrate and thus considerably more energy will be released during respiration. In both aerobic and anaerobic respiration, ATP is formed as a result of electron transport chain activity.

Electrons for the chain can be obtained from inorganic nutrients, and it is possible to derive energy from the oxidation of inorganic molecules rather than from organic nutrients. This ability is confined to a small group of prokaryotes called chemolithotrophs It should be noted that these definitions of fermentation, aerobic respiration, and anaerobic respiration are slightly different from those often used by biologists and biochemists. Fermentation also may be defined as an energy-yielding process in which organic molecules serve as both electron donors and acceptors. Respiration is an energy-yielding process in which the acceptor is an inorganic molecule, either oxygen (aerobic respiration) or another inorganic acceptor (anaerobic respiration).

Before learning about some of the more important catabolic pathways, it is best to look at the “lay of the land” and get our bearings. Albert Lehninger, a biochemist who worked at Johns Hopkins medical school, helped considerably in this task for chemoorganoheterotrophs by pointing out that aerobic metabolism may be divided into three stages (**figure**).

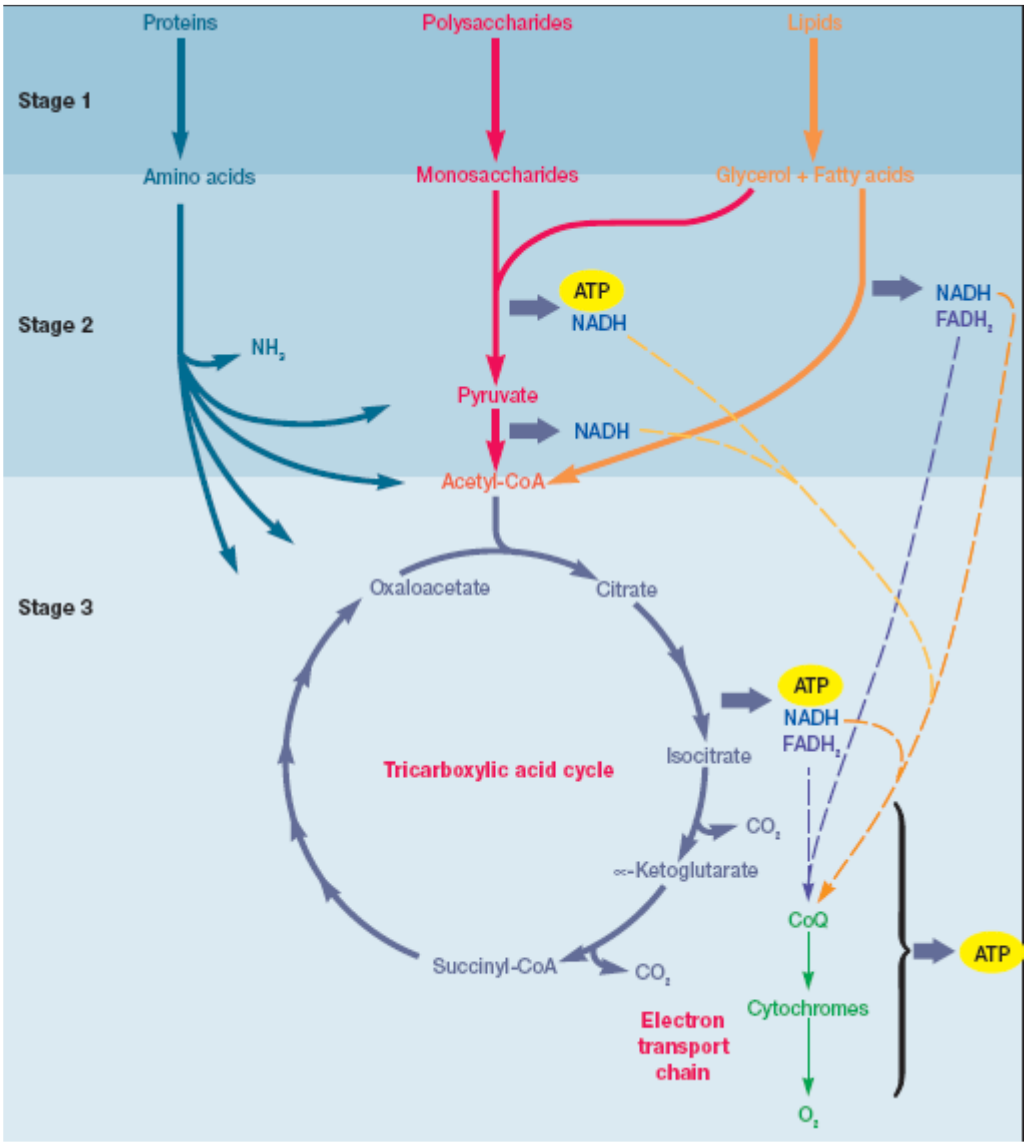


Figure Sources of Energy for Microorganisms.

In the first stage of catabolism, larger nutrient molecules (proteins, polysaccharides, and lipids) are hydrolyzed or otherwise broken down into their constituent parts. The chemical reactions occurring during this stage do not release much energy. Amino acids, monosaccharides, fatty acids, glycerol, and other products

of the first stage are degraded to a few simpler molecules in the second stage. Usually metabolites like acetyl coenzyme A, pyruvate, and tricarboxylic acid cycle intermediates are formed.The second-stage process can operate either aerobically or anaerobically and often produces some ATP as well as NADH and/or FADH2. Finally, nutrient carbon is fed into the tricarboxylic acid cycle during the third stage of catabolism, and molecules are oxidized completely to CO2 with the production of ATP, NADH, and FADH2. The cycle operates aerobically and is responsible for the release of much energy. Much of the ATP derived from the tricarboxylic acid cycle (and stage-two reactions) comes from the oxidation of NADH and FADH2 by the electron transport chain. Oxygen, or sometimes another inorganic molecule, is the final electron acceptor. Notice that the microorganism

begins with a wide variety of molecules and reduces their number and diversity at each stage. That is, nutrient molecules are funneled into ever fewer metabolic intermediates until they are finally fed into the tricarboxylic acid cycle. A common pathway often degrades many similar molecules (e.g., several different sugars). These metabolic pathways consist of enzyme catalyzed reactions arranged so that the product of one reaction serves as a substrate for the next. The existence of a few common catabolic pathways, each degrading many nutrients, greatly increases metabolic efficiency by avoiding the need for a large number of less metabolically flexible pathways. It is in the catabolic phase that microorganisms exhibit their nutritional diversity. Most microbial biosynthetic pathways closely resemble their counterparts in higher organisms. The uniqueness of microbial metabolism lies in the diversity of sources from which it generates ATP and NADH.

Carbohydrates and other nutrients serve two functions in the metabolism of heterotrophic microorganisms: (1) they are oxidized to release energy, and (2) they supply carbon or building blocks for the synthesis of new cell constituents. Although many anabolic pathways are separate from catabolic routes, there are **amphibolic pathways** [Greek *amphi*, on both sides] that function both catabolically and

anabolically. Two of the most important are the glycolytic pathway and the tricarboxylic acid cycle. Most reactions in these two pathways are freely reversible and can be used to synthesize and degrade molecules. The few irreversible catabolic steps are bypassed in biosynthesis with special enzymes that catalyze the reverse reaction. For example, the enzyme fructose bisphosphatase reverses the phosphofructokinase step when glucose is synthesized from pyruvate. The presence of two separate enzymes, one catalyzing the reversal of the other's reaction, permits independent regulation of the catabolic and anabolic functions of these amphibolic pathways.

The Breakdown of Glucose to Pyruvate

Microorganisms employ several metabolic pathways to catabolize glucose and other sugars. Because of this metabolic diversity, their metabolism is often confusing. To avoid confusion as much as possible, the ways in which microorganisms degrade sugars to pyruvate and similar intermediates are introduced by focusing on only three routes: (1) glycolysis, (2) the pentose phosphate pathway, and (3) the Entner-Doudoroff pathway. Next the pathways of aerobic and anaerobic pyruvate metabolism are described. For the sake of simplicity, the chemical structures of metabolic intermediates are not used in pathway diagrams

The Glycolytic Pathway

The **Embden-Meyerhof** or **glycolytic pathway** is undoubtedly the most common pathway for glucose degradation to pyruvate in stage two of catabolism. It is found in all major groups of microorganisms and functions in the presence or absence of O2. **Glycolysis** [Greek *glyco,* sweet, and *lysis,* a loosening] is located in the cytoplasmic

matrix of procaryotes and eucaryotes. The pathway as a whole may be divided into two parts (**figure**).

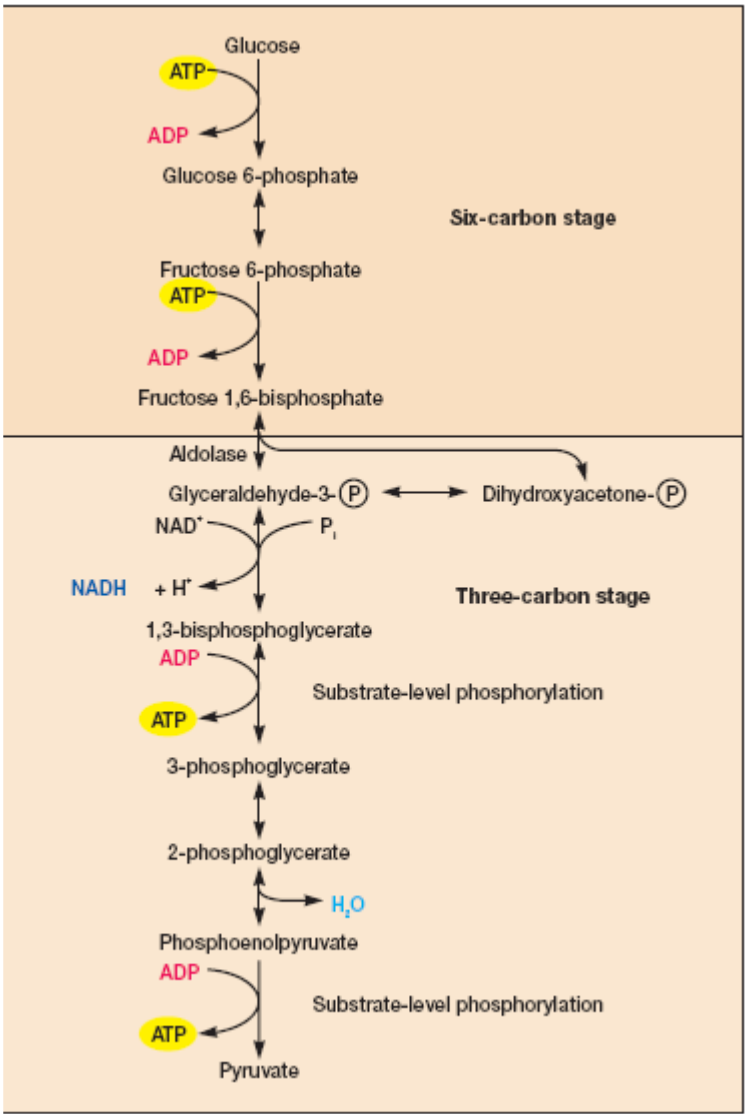


Figure 9.5 Glycolysis. The glycolytic pathway for the breakdown of glucose to pyruvate. The two stages of the pathway and their products are indicated.

In the initial six-carbon stage, glucose is phosphorylated twice and eventually converted to fructose 1,6- bisphosphate. Other sugars are often fed into the pathway by conversion to glucose 6-phosphate or fructose 6-phosphate. This preliminary stage does not yield energy; in fact, two ATP molecules are expended for each glucose. These initial steps "prime the

pump" by adding phosphates to each end of the sugar. The phosphates will soon be used to make ATP. The three-carbon stage of glycolysis begins when the enzyme fructose 1,6-bisphosphate aldolase catalyzes the cleavage of fructose 1,6-bisphosphate into two halves, each with a phosphate group. One of the products, glyceraldehyde 3-phosphate, is

converted directly to pyruvate in a five-step process. Because the other product, dihydroxyacetone phosphate, can be easily changed to glyceraldehyde 3-phosphate, both halves of fructose 1,6-bisphosphate are used in the three-carbon stage. Glyceraldehyde 3-phosphate is first oxidized with NAD_ as the electron acceptor, and a phosphate is simultaneously incorporated to give a high-energy molecule called 1,3-bisphosphoglycerate. The highenergy phosphate on carbon one is subsequently donated to ADP to produce ATP. This synthesis of ATP is called **substrate-level phosphorylation** because ADP phosphorylation is coupled with the exergonic breakdown of a high-energy substrate molecule. A somewhat similar process generates a second ATP by substrate-level phosphorylation. The phosphate group on 3-phosphoglycerate shifts to carbon two, and 2-phosphoglycerate is dehydrated to form a second high-energy molecule, phosphoenolpyruvate. This molecule donates its phosphate to ADP forming a second ATP and pyruvate, the final product of the pathway.

The glycolytic pathway degrades one glucose to two pyruvates by the sequence of reactions just outlined. ATP and NADH are also produced. The yields of ATP and NADH may be calculated by considering the two stages separately. In the six-carbon stage two ATPs are used to form fructose 1,6-bisphosphate. For each glyceraldehydes 3-

phosphate transformed into pyruvate, one NADH and two ATPs are formed. Because two glyceraldehyde 3-phosphates arise from a single glucose (one by way of dihydroxyacetone phosphate), the three-carbon stage generates four ATPs and two NADHs per glucose. Subtraction of the ATP used in the six-carbon stage from that produced in the three-carbon stage gives a net yield of two ATPs per glucose. Thus the catabolism of glucose to pyruvate in glycolysis can be represented by the following simple equation.

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i + 2\text{NAD}^+ \longrightarrow 2\text{ pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+$$

The Pentose Phosphate Pathway

A second pathway, the **pentose phosphate** or **hexose monophosphate pathway** may be used at the same time as the glycolytic pathway or the Entner-Doudoroff sequence. It can operate either aerobically or anaerobically and is important in biosynthesis as well as in catabolism. The pentose phosphate pathway begins with the oxidation of glucose 6-phosphate to 6-phosphogluconate followed by the oxidation of 6-phosphogluconate to the pentose ribulose 5-phosphate and CO2 (**figure**).

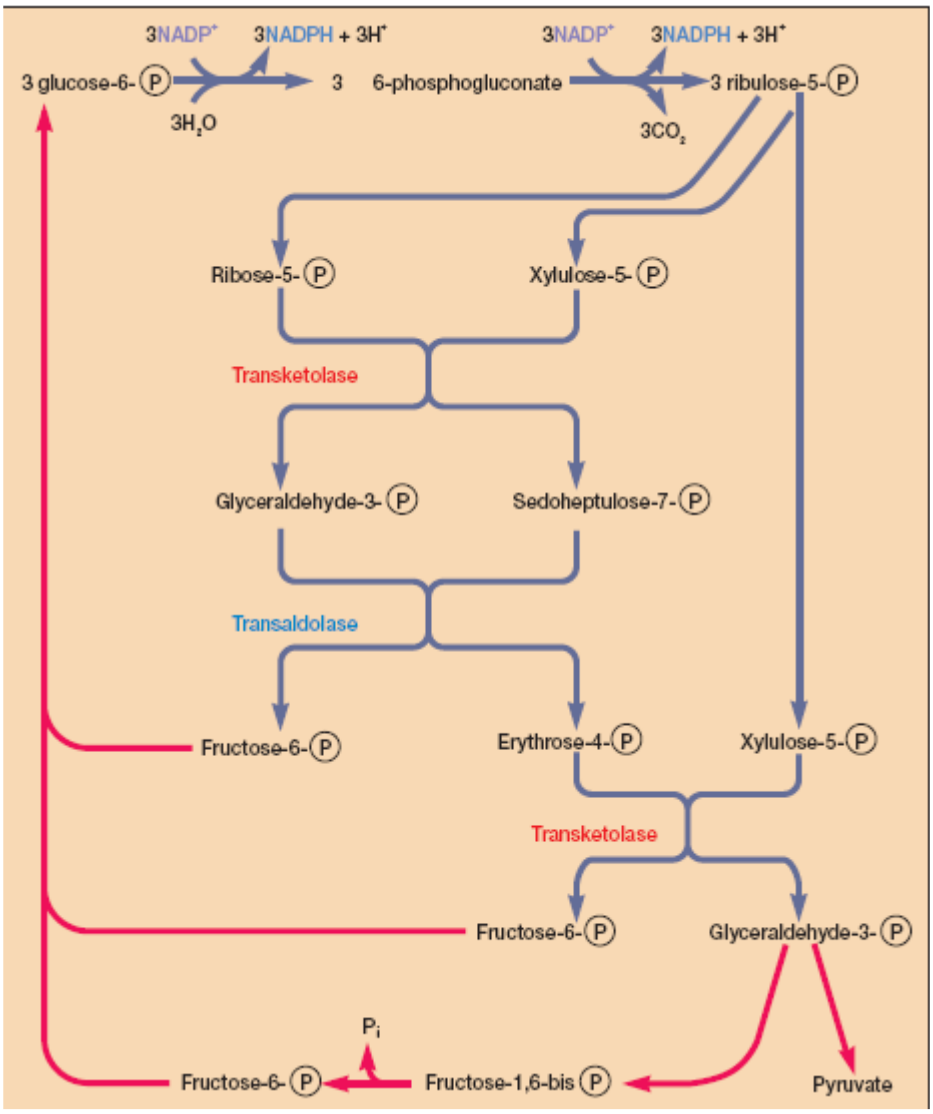


Figure The Pentose Phosphate Pathway.

The conversion of three glucose 6-phosphate molecules to two fructose 6-phosphates and a glyceraldehyde 3-phosphate is traced. The fructose 6-phosphates are changed back to glucose 6-phosphate. The glyceraldehyde 3-phosphate can be converted to pyruvate or combined with a molecule of dihydroxyacetone phosphate

(from the glyceraldehyde 3-phosphate formed by a second turn of the pathway) to yield fructose 6-phosphate.

NADPH is produced during these oxidations. Ribulose 5-phosphate is then converted to a mixture of three- through seven-carbon sugar phosphates. Two enzymes unique to this pathway play a central role in these transformations: (1) transketolase catalyzes the transfer of two-carbon ketol groups, and (2) transaldolase transfers a three-carbon group from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate (**figure**).

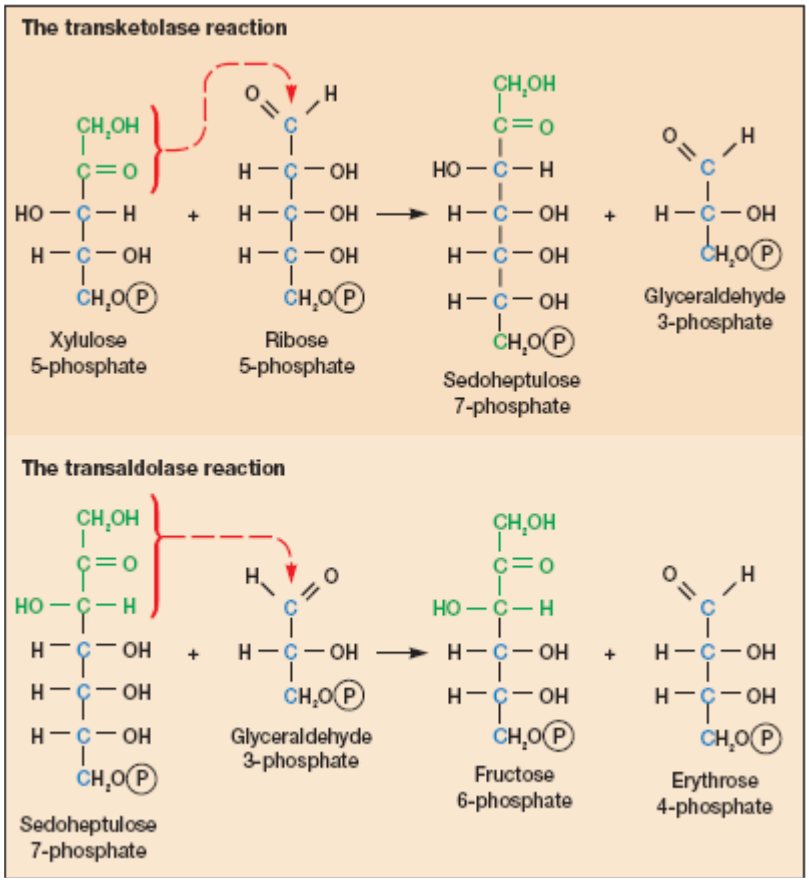


Figure Transketolase and Transaldolase. Examples of the transketolase and transaldolase reactions of the pentose phosphate pathway. The groups transferred in these reactions are in color.

The overall result is that three glucose 6-phosphates are converted to two fructose 6-phosphates, glyceraldehyde 3-phosphate, and three CO2 molecules, as shown in the following equation.

$$\begin{aligned}&3 \text{ glucose 6-phosphate} + 6NADP^+ + 3H_2O \longrightarrow \\ &2 \text{ fructose 6-phosphate} + \text{glyceraldehyde 3-phosphate} + \\ &3CO_2 + 6NADPH + 6H^+\end{aligned}$$

These intermediates are used in two ways. The fructose 6- phosphate can be changed back to glucose 6-phosphate while glyceraldehyde 3-phosphate is converted to pyruvate by glycolytic enzymes. The glyceraldehyde 3-phosphate also may be returned to the pentose phosphate pathway through glucose 6-phosphate formation. This results in the complete degradation of glucose 6- phosphate to CO2 and the production of a great deal of NADPH.

$$\begin{aligned}&\text{Glucose 6-phosphate} + 12NADP^+ + 7H_2O \longrightarrow \\ &6CO_2 + 12NADPH + 12H^+ + P_i\end{aligned}$$

The pentose phosphate pathway has several catabolic and anabolic functions that are summarized as follows:

1. NADPH from the pentose phosphate pathway serves as a source of electrons for the reduction of molecules during biosynthesis.

2. The pathway synthesizes four- and five-carbon sugars for a variety of purposes. The four-carbon sugar erythrose 4-phosphate is used to synthesize aromatic amino acids and vitamin B6 (pyridoxal). The pentose ribose 5-phosphate is a major component of nucleic acids, and ribulose 1,5-bisphosphate is the primary CO2 acceptor in photosynthesis. Note that when a microorganism is growing on a pentose carbon

source, the pathway also can supply carbon for hexose production (e.g., glucose is needed for peptidoglycan synthesis).

3. Intermediates in the pentose phosphate pathway may be used to produce ATP. Glyceraldehyde 3-phosphate from the pathway can enter the three-carbon stage of the glycolytic pathway and be converted to ATP and pyruvate. The latter may be oxidized in the tricarboxylic acid cycle to provide more energy. In addition, some NADPH can be converted to NADH, which yields ATP when it is oxidized by the electron transport chain. Because five-carbon sugars are intermediates in the pathway, the pentose phosphate pathway can be used to catabolize pentoses as well as hexoses. Although the pentose phosphate pathway may be a source of energy in many microorganisms, it is more often of greater importance in biosynthesis. Several functions of the pentose phosphate pathway are mentioned again in chapter 10 when biosynthesis is considered more directly.

The Entner-Doudoroff Pathway

Although the glycolytic pathway is the most common route for the conversion of hexoses to pyruvate, another pathway with a similar role has been discovered. The **Entner-Doudoroff pathway** begins with the same reactions as the pentose phosphate pathway, the formation of glucose 6-phosphate and 6-phosphogluconate (**figure**)

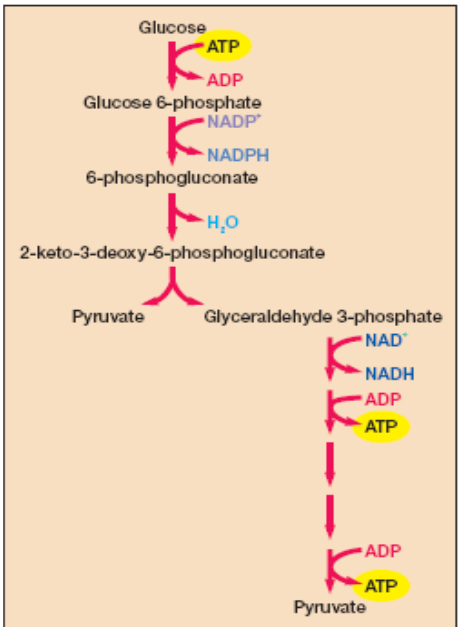


Figure The Entner-Doudoroff Pathway. The sequence leading from glyceraldehyde 3-phosphate to pyruvate is catalyzed by enzymes common to the glycolytic pathway.

Instead of being further oxidized, 6- phosphogluconate is dehydrated to form 2-keto-3-deoxy-6- phosphogluconate or KDPG, the key intermediate in this pathway. KDPG is then cleaved by KDPG aldolase to pyruvate and glyceraldehydes 3-phosphate. The glyceraldehyde 3-phosphate is converted to pyruvate in the bottom portion of the glycolytic pathway. If the Entner-Doudoroff pathway degrades glucose to pyruvate in this way, it yields one ATP, one NADPH, and one NADH per glucose metabolized. Most bacteria have the glycolytic and pentose phosphate pathways, but some substitute the Entner-Doudoroff pathway for glycolysis. The Entner-Doudoroff pathway is generally found in *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Agrobacterium*, and a few other gram-negative genera. Very few gram-positive bacteria have this pathway, with *Enterococcus faecalis* being a rare exception.

The Tricarboxylic Acid Cycle

Although some energy is obtained from the breakdown of glucose to pyruvate by the pathways previously described, much more is released when pyruvate is degraded aerobically to CO2 in stage three of catabolism. The multienzyme system called the pyruvate dehydrogenase complex first oxidizes pyruvate to form CO2 and **acetyl coenzyme A (acetyl-CoA),** an energy-rich molecule composed of coenzyme A and acetic acid joined by a highenergy thiol ester bond. Acetyl-CoA arises from the catabolism of many carbohydrates, lipids, and amino acids. It can be further degraded in the tricarboxylic acid cycle. The substrate for the **tricarboxylic acid (TCA) cycle, citric acid cycle,** or **Krebs cycle** is acetyl-CoA (figure).

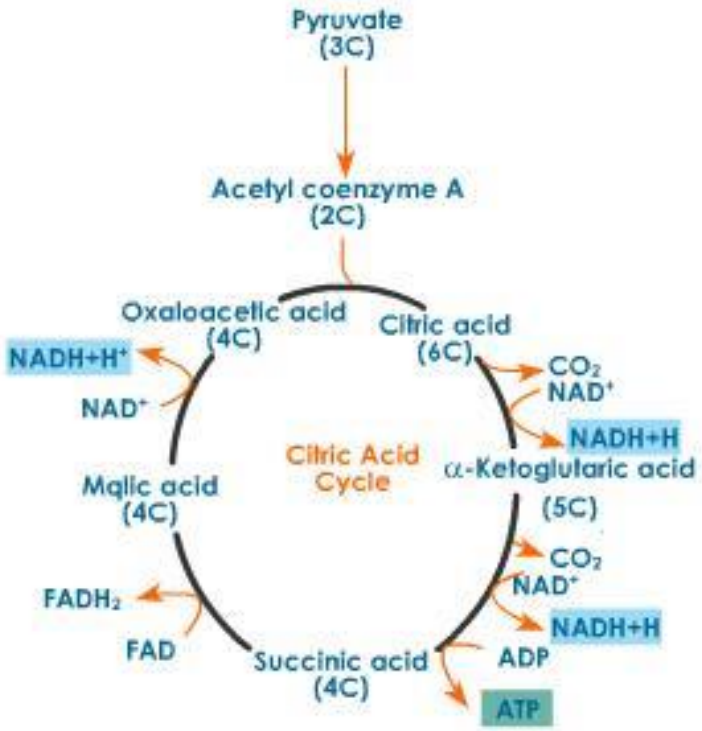


Figure The Tricarboxylic Acid Cycle

The traditional way to think about the cycle is in terms of its intermediates and products, and the chemistry involved in each step. In the first reaction acetyl-CoA is condensed with a fourcarbon intermediate, oxaloacetate, to form citrate and to begin the six-carbon stage. Citrate (a tertiary alcohol) is rearranged to give isocitrate, a more readily oxidized secondary alcohol. Isocitrate is subsequently oxidized and decarboxylated twice to yield α-ketoglutarate, then succinyl-CoA. At this point two NADHs are formed and two carbons are lost from the cycle as CO2. Because two carbons were added as acetyl-CoA at the start, balance is maintained and no net carbon is lost. The cycle now enters the four-carbon stage during which two oxidation steps yield one FADH2 and one NADH per acetyl-CoA. In addition, GTP (a high-energy molecule equivalent to ATP) is produced from succinyl-CoA by substrate-level phosphorylation. Eventually oxaloacetate is reformed and ready to join with another acetyl-CoA. Inspection of figure 9.12 shows that the TCA cycle generates two CO2s, three NADHs, one FADH2, and one GTP for each acetyl- CoA molecule oxidized.

Another way to think of the TCA cycle is in terms of its function as a pathway that oxidizes acetyl-CoA to CO2. From this perspective, the first step is the attachment of an acetyl group to the acetyl carrier, oxaloacetate, to form citrate. The second stage begins with citrate and ends in the formation of succinyl-CoA. Here, the acetyl carrier portion of citrate loses two carbons when it is oxidized to give two CO2s. The third and last stage converts succinyl-CoA back to oxaloacetate, the acetyl carrier, so that it can pick up another acetyl group. TCA cycle enzymes are widely distributed among microorganisms. The complete cycle appears to be functional in many aerobic bacteria, free-living protozoa, and most algae and fungi. This is not surprising because the cycle is such an important source of energy. However, the facultative anaerobe *E. coli* does not use the full TCA cycle under anaerobic conditions or when the glucose concentration is high but does at other times. Even those microorganisms that lack the complete TCA cycle usually have most of the cycle enzymes, because one of TCA cycle's major functions is to provide carbon skeletons for use in biosynthesis.

Glyoxylate cycle

Some bacteria, algae, fungi, and protozoa can grow with acetate as the sole carbon source by using it to synthesize TCA cycle intermediates in the **glyoxylate cycle** (**figure**).

This cycle is made possible by two unique enzymes, isocitrate lyase and malate synthase, that catalyze the following reactions.

$$\text{Isocitrate} \xrightarrow{\text{isocitrate lyase}} \text{succinate} + \text{glyoxylate}$$

$$\text{Glyoxylate} + \text{acetyl-CoA} \xrightarrow{\text{malate synthase}} \text{malate} + \text{CoA}$$

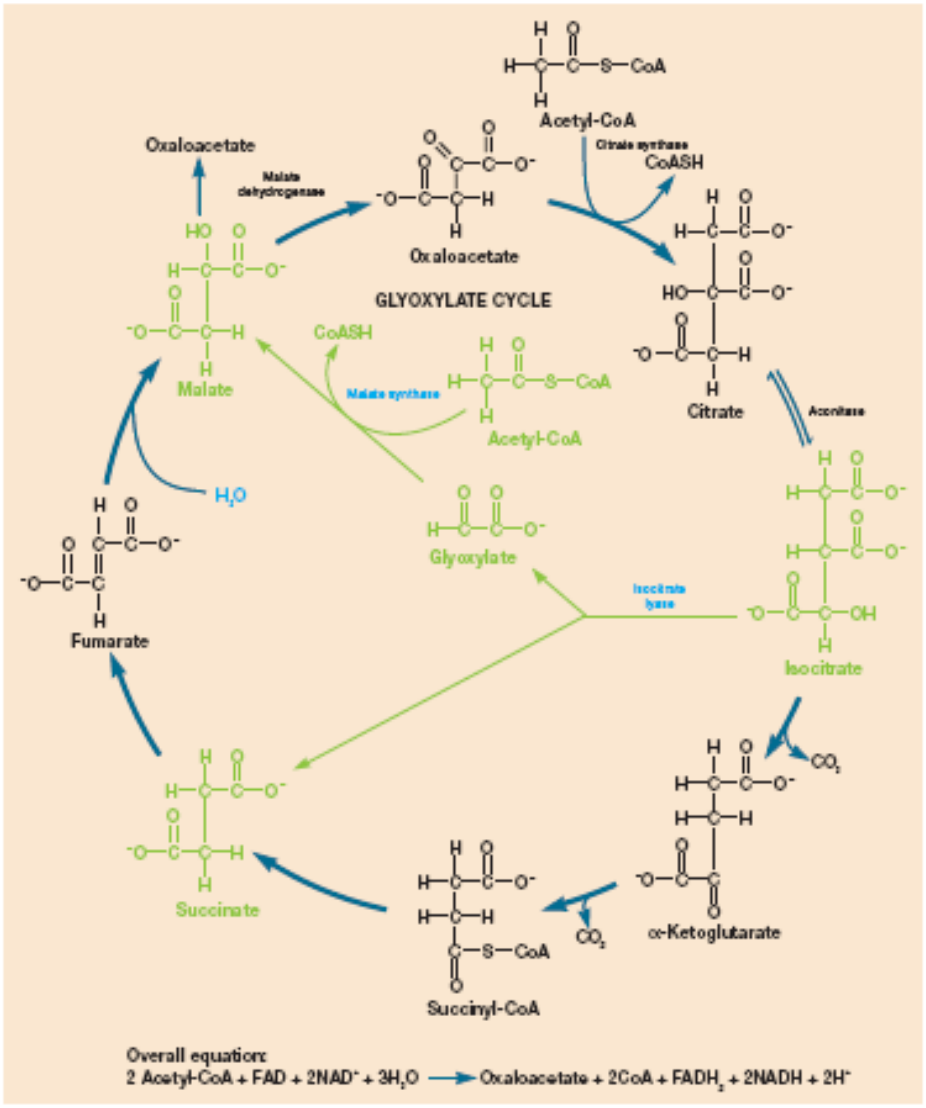


Fig: **The Glyoxylate Cycle**

The glyoxylate cycle is actually a modified TCA cycle. The two decarboxylations of the latter pathway (the isocitrate dehydrogenase and α-ketoglutarate dehydrogenase steps) are bypassed, making possible the conversion of acetyl-CoA to form oxaloacetate without loss of acetyl-CoA carbon as CO2. In this fashion acetate and

any molecules that give rise to it can contribute carbon to the cycle and support microbial growth.

Electron Transport and Oxidative Phosphorylation

Little ATP has been synthesized up to this point. Only the equivalent of four ATP molecules is directly synthesized when oneglucose is oxidized to six CO2 molecules by way of glycolysis and the TCA cycle. Most ATP generated comes from the oxidation of

NADH and FADH2 in the electron transport chain. The mitochondrial electron transport chain will be examined first because it has been so well studied. Then we will turn to bacterial chains, and finish with a discussion of ATP synthesis.

The Electron Transport Chain

The mitochondrial **electron transport chain** is composed of a series of electron carriers that operate together to transfer electrons from donors, like NADH and FADH2, to acceptors, such as O2 (**figure**).

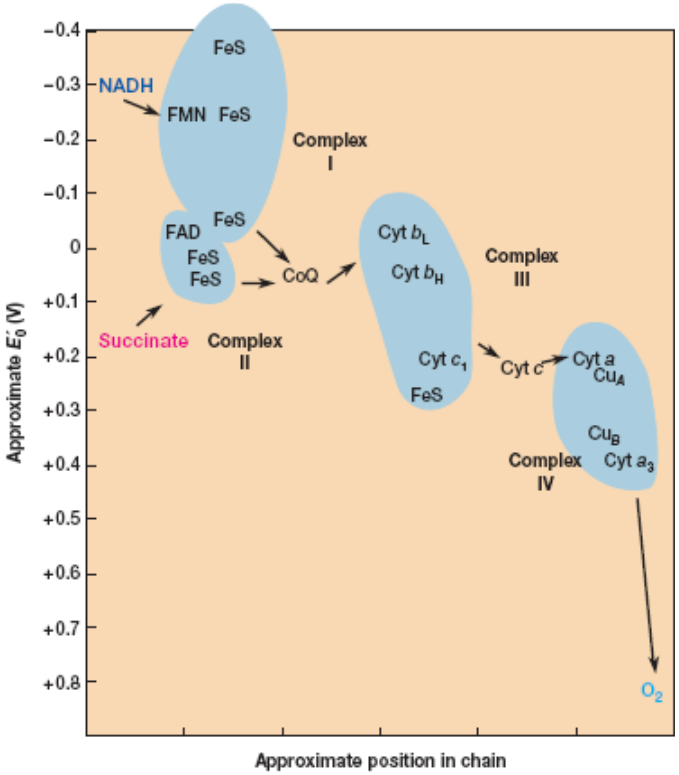


Figure The Mitochondrial Electron Transport Chain.

The electrons flow from carriers with more negative reduction potentials to those with more positive potentials and eventually combine with O2 and H+ to form water. The electrons move down this potential gradient much like water flowing down a series of rapids. The difference in reduction potentials between O2 and NADH is large, about 1.14 volts, and makes possible the release of a great deal of energy. The potential changes at several points in the chain are large enough to provide sufficient energy for ATP production, much like the energy from waterfalls can be harnessed by waterwheels and used to generate electricity. The electron transport chain breaks up the large overall energy release into small steps. Some of the liberated energy is trapped in the form of ATP. As will be seen shortly, electron transport at these points may generate proton and electrical gradients. These gradients can then drive ATP synthesis. The electron

transport chain carriers reside within the inner membrane of the mitochondrion or in the bacterial plasma membrane. The mitochondrial system is arranged into four complexes of carriers, each capable of transporting electrons part of the way to O2 (**figure**). Coenzyme Q and cytochrome *c* connect the

complexes with each other.

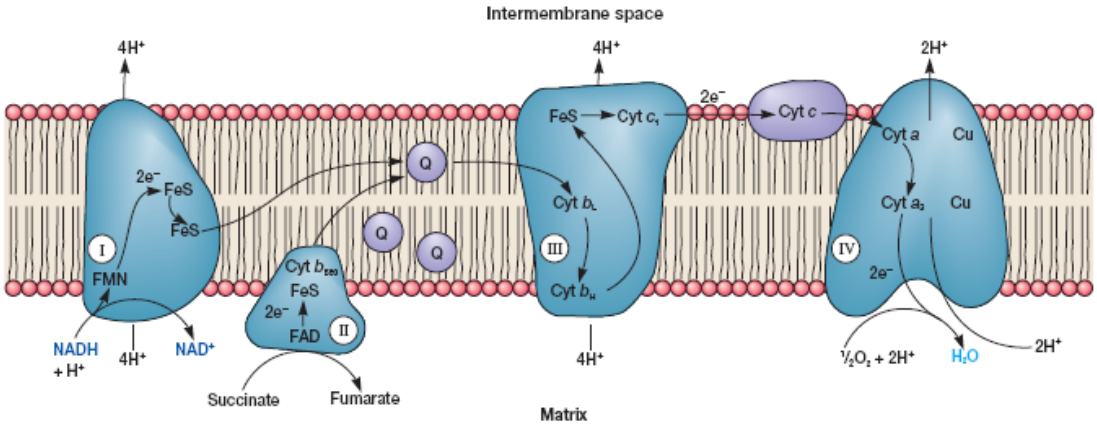


Figure The Chemiosmotic Hypothesis Applied to Mitochondria. In this scheme the carriers are organized asymmetrically within the inner membrane so that protons are transported across as electrons move along the chain. Proton release into the intermembrane space occurs when electrons are transferred from carriers, such as FMN and coenzyme Q (Q), that carry both electrons and protons to components like nonheme iron proteins (FeS proteins) and cytochromes (Cyt) that transport only electrons. Complex IV pumps protons across the membrane as electrons pass from cytochrome *a* to oxygen. Coenzyme Q transports electrons from complexes I and II to complex III. Cytochrome *c* moves electrons between complexes III and IV. The number of protons moved across the membrane at each site per pair of electrons transported is still somewhat uncertain; the current consensus is that at least 10 protons must move outward during NADH oxidation.

The process by which energy from electron transport is used to make ATP is called **oxidative phosphorylation.** Thus as many as three ATP molecules may be synthesized from ADP and Pi when a pair of electrons pass from NADH to an atom of O2. This is the same thing as saying that the phosphorus to oxygen (P/O) ratio is equal to 3. Because electrons from FADH2 only pass two oxidative phosphorylation points, the maximum P/O ratio for FADH2 is 2. The actual P/O ratios may be less than 3.0 and 2.0 in eucaryotic mitochondria. The preceding discussion has focused on the eucaryotic mitochondrial electron transport chain. Although some bacterial chains resemble the mitochondrial chain, they are frequently very different. They vary in their electron carriers (e.g., in their cytochromes) and may be extensively branched. Electrons often can

enter at several points and leave through several terminal oxidases. Bacterial chains also may be shorter and have lower P/O ratios than mitochondrial transport chains. Thus procaryotic and eucaryotic electron transport chains differ in details of construction although they operate using the same fundamental principles. The electron transport chains of *Escherichia coli* and *Paracoccus denitrificans* will serve as examples of these differences **figure.**

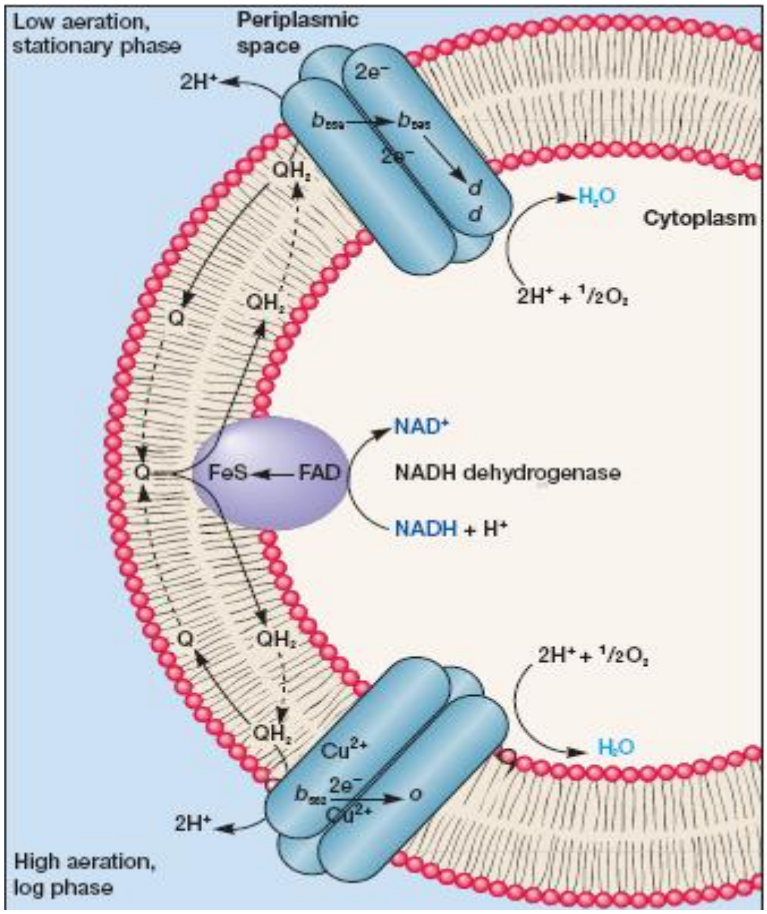


Figure The Aerobic Respiratory System of *E. coli*

Although it transports electrons from NADH to acceptors and moves protons across the plasma membrane, the *E. coli* chain is quite different from the mitochondrial chain. For example, it is branched and contains a quite different array of cytochromes. Coenzyme Q or ubiquinol donates electrons to both branches, but they operate under different growth conditions. The cytochrome *d* branch has very high affinity for oxygen and functions at low oxygen levels. It is not as efficient as the cytochrome *o* branch because it does not actively pump protons. The cytochrome *o* branch has moderately high affinity for oxygen, is a proton pump, and operates at higher oxygen concentrations.

Oxidative Phosphorylation

The mechanism by which oxidative phosphorylation takes place has been studied intensively for years. Currently the most widely accepted hypothesis about how oxidative phosphorylation occurs is the chemiosmotic hypothesis. According to the **chemiosmotic hypothesis,** first formulated in 1961 by the British biochemist Peter Mitchell, the electron

transport chain is organized so that protons move outward from the mitochondrial matrix and electrons are transported inward (**figure**).

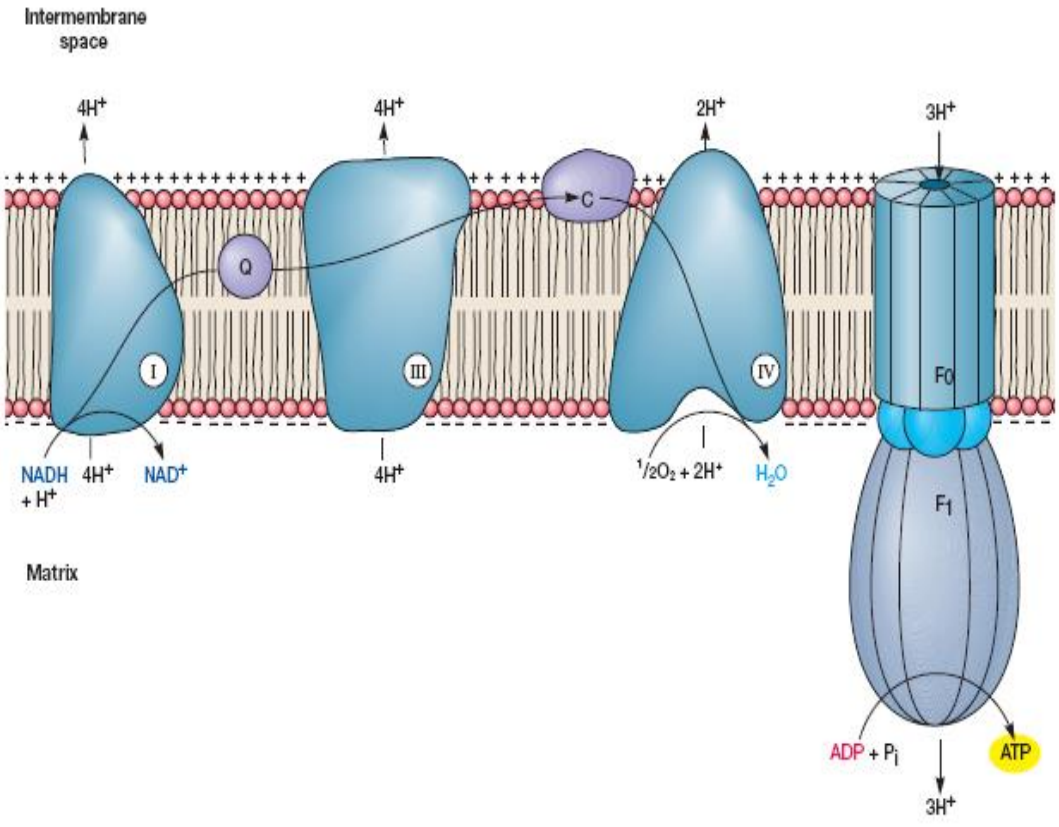


Figure Chemiosmosis. An overview of the chemiosmotic hypothesis as applied to mitochondrial function. The flow of electrons from NADH to oxygen causes protons to move from the mitochondrial matrix to the intermembrane space. This generates

proton and electrical gradients. When protons move back to the matrix through the F1F0 complex, F1 synthesizes ATP. In procaryotes, the process is similar except that the protonsmove from the cytoplasm to the periplasm.

Proton movement may result either from carrier loops, or from the action of special proton pumps that derive their energy from electron transport. The result is **proton motive force (PMF),** composed of a gradient of protons and a membrane potential due to the unequal distribution of charges. When protons return to the mitochondrial matrix driven by the proton motive force, ATP is synthesized in a reversal of the ATP hydrolysis reaction. A similar process takes place in procaryotes, with electron flow causing the protons to move outward across the plasma membrane. ATP synthesis occurs when these protons diffuse back into the cell. The proton motive force also may drive the transport of molecules across membranes and the rotation of bacterial flagella and thus plays a central role in procaryotic physiology (**figure**).

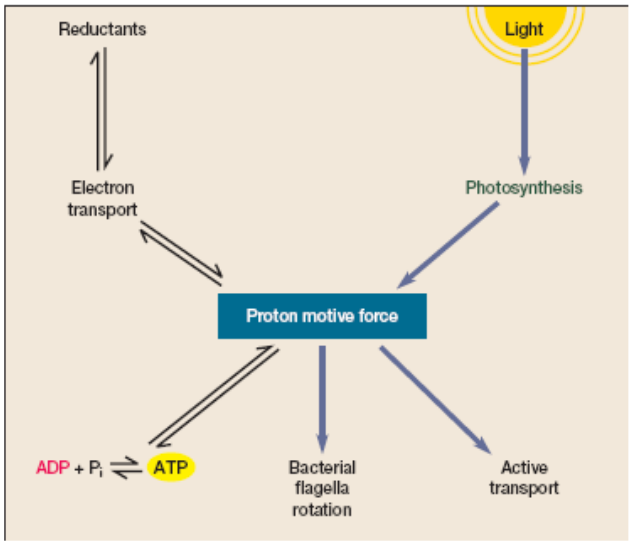


Figure The Central Role of Proton Motive Force.

It should be noted that active transport is not always driven by PMF.The chemiosmotic hypothesis is accepted by most microbiologists. There is considerable evidence for the generation of proton and charge gradients across membranes. However, the evidence for proton gradients as the direct driving force for oxidative phosphorylation is not yet conclusive. In some halophilic marine bacteria, sodium ions may be used to drive ATP synthesis. Whatever the precise mechanism, ATP synthesis takes place at the F1F0 ATPase or **ATP synthase** (**figure**).

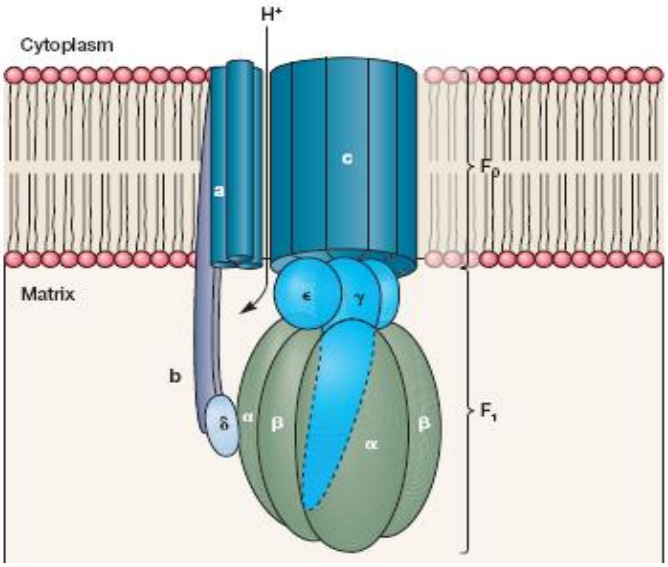


Figure ATP Synthase Structure and Function. (**a**) The major structural features of ATP synthase deduced from X-ray crystallography and other studies. F1 is a spherical structure composed largely of alternating α and β subunits; the three active sites are on the β subunits. The γ subunit extends upward through the center of the sphere and can rotate. The stalk (γ and ε subunits) connects the sphere to F0, the membrane embedded complex that serves as a proton channel. F0 contains one a subunit, two b subunits, and 9–12 c subunits. The stator arm is composed of subunit a, two b subunits, and the δ subunit; it is embedded in the membrane and attached to F1. A ring of c subunits in F0 is connected to the stalk and may act as a rotor and move past the a subunit of the stator. As the c subunit ring turns, it rotates the shaft (γε subunits).

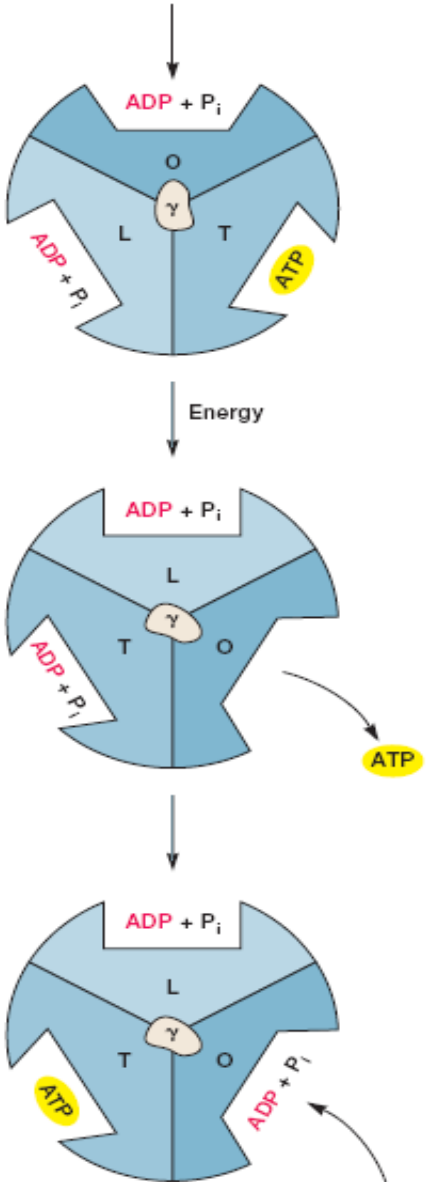


Figure The binding change mechanism of ATP synthesis is depicted in a simplified schematic diagram in which the F1 sphere is viewed from the membrane side. The three active sites appear able to exist in three different conformations: an

inactive open (O) conformation with low affinity for substrates, an inactive L conformation with fairly loose affinity for the substrates, and an active tight (T) conformation that has high affinity for substrates. In the first step, ADP and Pi bind to the O site. Then the γ subunit rotates 120° with the input of energy, presumably from proton flow through F0. This rotation causes conformational changes in all three subunits resulting in a release of newly formed ATP and a conversion of the L site to an active T conformation. Finally, ATP is formed at the new T site while more ADP and Pi bind to the unoccupied O site and everything is ready for another energy-driven γ subunit rotation.

The mitochondrial F1 component appears as a spherical structure attached to the inner membrane surface by a stalk and the F0 component, which is embedded in the membrane. The F1F0 ATPase is on the inner surface of the plasma membrane in bacteria. F0 participates in proton movement across the membrane, and this movement through a channel in F0 is believed to drive oxidative phosphorylation. F1 is a large complex in which three α subunits alternate with three β subunits. The γ subunit extends downward from the $\alpha_3\beta_3$ complex; it composes part of the stalk and interacts with F0. The δ subunit also is located in the stalk. Much of the γ subunit is positioned in the center of F1, surrounded by the α and β subunits. The γ subunit rotates rapidly in a counterclockwise direction within the $\alpha_3\beta_3$ complex much like a car's crankshaft and causes conformational changes that drive ATP synthesis at the active sites on the β subunits. Thus the ATP synthase is the smallest rotary motor known, much smaller than the bacterial flagellum.

Many chemicals inhibit the aerobic synthesis of ATP and can even kill cells at sufficiently high concentrations. These inhibitors generally fall into two categories. Some directly block the transport of electrons. The antibiotic piericidin competes with coenzyme Q; the antibiotic antimycin A blocks electron transport between cytochromes

b and *c;* and both cyanide and azide stop the transfer of electrons between cytochrome *a* and O2 because they are structural analogs of O2. Another group of inhibitors known as **uncouplers** stops ATP synthesis without inhibiting electron transport itself. Indeed, they may even enhance the rate of electron flow. Normally electron transport is tightly coupled with oxidative phosphorylation so that the rate of ATP synthesis controls the rate of electron transport. The more rapidly ATP is synthesized during oxidative phosphorylation, the faster the electron transport chain operates to supply the required energy. Uncouplers disconnect oxidative phosphorylation from electron transport; therefore the energy released by the chain is given off as heat rather than as ATP. Many uncouplers like dinitrophenol and valinomycin may allow hydrogen ions, potassium ions, and other ions to cross the membrane without activating the F1F0 ATPase. In this way they destroy the pH and ion gradients. Valinomycin also may bind directly to the F1F0 ATPase and inhibit its activity.

The Yield of ATP in Glycolysis and Aerobic Respiration

The maximum ATP yield in eucaryotes from glycolysis, the TCA cycle, and electron transport can be readily calculated. The conversion of glucose to two pyruvate molecules during glycolysis gives a net gain of two ATPs and two NADHs. Because each NADH can yield a maximum of three ATPs during electron transport and oxidative phosphorylation (a P/O ratio of 3), the total aerobic yield from the glycolytic pathway is eight ATP molecules. Under anaerobic conditions, when the NADH is not oxidized by the electron transport chain, only two ATPs will be generated during the degradation of glucose to pyruvate.

When O2 is present and the electron transport chain is operating, pyruvate is next oxidized to acetyl-CoA, the substrate for the TCA cycle. This reaction yields 2 NADHs because 2 pyruvates arise from a glucose; therefore 6 more ATPs are formed. Oxidation of each acetyl-CoA in the TCA cycle will yield 1 GTP (or ATP), 3 NADHs, and a single FADH2 for a total of 2 GTPs (ATPs), 6 NADHs, and 2 FADH2s from two

acetyl-CoA molecules. As table 9.2 shows, this amounts to 24 ATPs when NADH and FADH2 from the cycle are oxidized in the electron transport chain. Thus the aerobic oxidation of glucose to 6 CO2 molecules supplies a maximum of 38 ATPs. In fact, the P/O ratios are more likely about 2.5 for NADH and 1.5 for FADH2. Thus the total ATP aerobic yield from glucose may be closer to 30 ATPs rather than 38.

Anaerobic Respiration

Electrons derived from sugars and other organic molecules are usually donated either to endogenous organic electron acceptors or to molecular O2 by way of an electron transport chain. However, many bacteria have electron transport chains that can operate with exogenous electron acceptors other than O2. This energy-yielding process is called anaerobic respiration. The major electron acceptors are nitrate, sulfate, and CO2, but metals and a few organic molecules can also be reduced. Some bacteria can use nitrate as the electron acceptor at the end of their electron transport chain and still produce ATP. Often this process is called **dissimilatory nitrate reduction.** Nitrate may be reduced to nitrite by nitrate reductase, which replaces cytochrome oxidase.

$$NO_3^- + 2e^- + 2H^+ \longrightarrow NO_2^- + H_2O$$

However, reduction of nitrate to nitrite is not a particularly effective way of making ATP, because a large amount of nitrate is required for growth (a nitrate molecule will accept only two electrons). The nitrite formed is also quite toxic. Therefore nitrate often is further reduced all the way to nitrogen gas, a process known as **denitrification.** Each nitrate will then accept five electrons, and the product will be nontoxic.

$$2NO_3^- + 10e^- + 12H^+ \longrightarrow N_2 + 6H_2O$$

There is considerable evidence that denitrification is a multistep process with four enzymes participating: nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase.

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

Interestingly, one of the intermediates is nitric oxide (NO). In mammals this molecule acts as a neurotransmitter, helps regulate blood pressure, and is used by macrophages to destroy bacteria and tumor cells. Two types of bacterial nitrite reductases

catalyze the formation of NO in bacteria. One contains cytochromes *c* and *d*1 (e.g., *Paracoccus* and *Pseudomonas aeruginosa*), and the other is a copper protein (e.g., *Alcaligenes*). Nitrite reductase seems to be periplasmic in gram-negative bacteria. Nitric oxide reductase catalyzes the formation of nitrous oxide from NO and is a membrane-bound cytochrome *bc* complex. Example of denitrification is gram-negative soil bacterium *Paracoccus denitrificans,* which reduces nitrate to N2 anaerobically. Its chain contains membrane-bound nitrate reductase and nitric oxide reductase, whereas nitrite reductase and nitrous oxide reductase are periplasmic. The four enzymes use electrons from coenzyme Q and *c*-type cytochromes to reduce nitrate and generate PMF. Denitrification is carried out by some members of the genera *Pseudomonas, Paracoccus,* and *Bacillus.* They use this route as an alternative to normal aerobic respiration and may be considered facultative anaerobes. If O2 is present, these bacteria use aerobic respiration (the synthesis of nitrate reductase is repressed by O2). Denitrification in anaerobic soil results in the loss of soil nitrogen and adversely affects soil fertility. Two other major groups of bacteria employing anaerobic respiration are obligate anaerobes. Those using CO2 or carbonate as a terminal electron acceptor are called methanogens because they reduce CO2 to methane. Sulfate also can act as the

final acceptor in bacteria such as *Desulfovibrio.* It is reduced to sulfide (S_2 or H_2S), and eight electrons are accepted.

$$SO_4^{2-} + 8e^- + 8H^+ \longrightarrow S^{2-} + 4H_2O$$

Anaerobic respiration is not as efficient in ATP synthesis as aerobic respiration that is, not as much ATP is produced by oxidative phosphorylation with nitrate, sulfate, or CO2 as the terminal acceptors. Reduction in ATP yield arises from the fact that these alternate electron acceptors have less positive reduction potentials than O2. The reduction potential difference between a donor like NADH and nitrate is smaller than the difference between NADH and O2. Because energy yield is directly related to the magnitude of the reduction potential difference, less energy is available to make ATP in anaerobic respiration. Nevertheless, anaerobic respiration is useful because it is more efficient than fermentation and allows ATP synthesis by electron transport and oxidative phosphorylation in the absence of O2. Anaerobic respiration is very prevalent in oxygen-depleted soils and sediments. Often one will see a succession of microorganisms in an environment when several electron acceptors are present. For example, if O2, nitrate, manganese ion, ferric ion, sulfate, and CO_2 are available in a particular environment, a predictable sequence of oxidant use takes place when an oxidizable substrate is available

to the microbial population. Oxygen is employed as an electron acceptor first because it inhibits nitrate use by microorganisms capable of respiration with either O2 or nitrate. While O2 is available, sulfate reducers and methanogens are inhibited because these groups are obligate anaerobes.

Once the O2 and nitrate are exhausted and fermentation products, including hydrogen, have accumulated, competition for use of other oxidants begins. Manganese and iron will be used first, followed by competition between sulfate reducers and

methanogens. This competition is influenced by the greater energy yield obtained with sulfate as an electron acceptor. Differences in enzymatic affinity for hydrogen, an important substrate used by both groups, also are important. The sulfate reducer *Desulfovibrio* grows rapidly and uses the available hydrogen at a faster rate than *Methanobacterium.* When the sulfate is exhausted, *Desulfovibrio* no longer oxidizes hydrogen, and the hydrogen concentration rises. The methanogens finally dominate the habitat and reduce CO2 to methane.

POSSIBLE QUESTION

UNIT 3

2 marks

1. Define Metabolism. Give example.
2. Difference between aerobic and anaerobic respiration?
3. What is oxidation? Give example.
4. Substrate level phosphorylation?
5. Define HMP Pathway?
6. Calculate the ATP generation for one molecule of glucose in Glycolysis cycle.
7. Explain about ATP generation?
8. What is reduction? Give example.
9. Define the oxidative phosphorylation?
10. Total yield in ATP by glycolysis pathway?

8 marks

1. Explain in detail about Aerobic fermentation
2. Brief notes on Pentose phosphate Pathway?
3. Give the clear sketch of Krebs cycle?
4. Detail account on Entner- Doudoroff Pathway?
5. Substrate and oxidative phosphorylation?
6. Electron transport chain in prokaryotes?
7. Aerobic respiration in eukaryotes?
8. Explain about ATP generation?

9. Write in detail about the electron transport chain.
10. Write a detail note on anaerobic respiration

Unit III Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
_____ is absent in membranes of prokaryotes	Sterols	Murein	NAM	NAG	Sterols
_____ is the first substrate in glycolytic cycle	Glucose	Fructose	Ribose	Erythrose	Glucose
_____ may be considered as an alternate pathway to HMP	Embden-Meyerhof pathway	Entner-Doudoroff pathway	TCA cycle	Glyoxalate cycle	Entner-Doudoroff pathway
_____ is non-heme containing iron sulfur protein	Ferredoxin	Quinone	Flavoprotein	Isoprenoid	Ferredoxin
_____ is an essential lipid carrier molecule involved in cell wall synthesis in both Gram positive & negative bacteria.	Bactoprenol	Dolichol	Sterol	Lipol	Bactoprenol
_____ is required for ATP generation by chemiosmotic synthesis	Proton motive force	Electrostatic force	Electric force	Chemical force	Proton motive force
_____ are integral components of all membranes,	Phospholipids	Glycolipids	Neutral lipids	Lipoproteins	Phospholipids

common to both prokaryotes and eukaryotes.					
_____ is a major intermediate in most of the common carbohydrate degradation pathways	Glyceraldehyde-3-phosphate	Glyceraldehyde-6-phosphate	Glycerol-3-phosphate	Glycerol-6-phosphate	Glyceraldehyde-3-phosphate
_____ is a storage substance formed from repeating units of β-hydroxybutyric acid.	Poly β-hydroxybutyrate	Poly α-butyrate	Poly γ-valerate	Poly β-valerate	Poly β-hydroxybutyrate
_____ is the connecting point between pyruvate and TCA cycle	Acetyl CoA	Acetyl CoB	Methyl CoA	Formyl CoA	Acetyl CoA
_____ is the major pathway for glucose conversion to pyruvate in microbes	EM pathway	HMP	TCA cycle	ED pathway	EM pathway
_____ mediated the final steps in mitochondrial electron transport.	Cytochrome complex	Cytochrome oxidase complex	Ubiquinone complex	Ubiquinone oxidase complex	Cytochrome oxidase comple
A major way in which depleted biosynthetic intrmediates are	Glyoxalate cycle	TCA cycle	Glycolysis	ED pathway	Glyoxalate cycle

replenished					
A storage substance not found in eukaryotes	Poly β-hydroxybutyrate	Carboxysomes	Mesosomes	Liposomes	Poly β-hydroxybutyrate
Aerobic respiration produces _____ ATP molecules per glucose molecule	2	4	6	8	2
Amount of Energy released in aerobic respiration is _____ kJ/mol of glucose.	2900	9200	2090	9020	2900
ATP molecules produced per hexose molecule by substrate phosphorylation	2	4	6	8	6
Bacterial photosynthesis has _____ photosystem	Single	Double	Triple	No	Single
Byproducts of anaerobic respiration	Lactic acid/ethanol	Water/CO_2	Acetate/N_2	Maleate/O_2	Lactic acid/ethanol
Citric acid cycle is also known as	Krebs cycle	TCA cycle	Glyoxalate cycle	Both a & b	Both a & b
Common byproduct of aerobic respiration	Water	Acetate	Lactate	Maleate	Water
Cyclic electron	Anoxygenic	Oxygenic	Oxidative	Reductive	Anoxygenic

transport is			hexose pathway		
During non-cyclic photophosphorylation, deficit of electrons is replenished by taking electrons from	Water	CO_2	Lactic acid	Ethanol	Water
EM pathway is	Amphibolic	Amphiphilic	Amphipathic	Aromatic	Amphibolic
EM pathway, in consort with TCA cycle, produces _____ ATP (net) molecules	32	34	36	38	38
Embden-Meyerhof-Parnas is also called as	Glycolysis	Glyconeogenesis	Citric acid cycle	HMP shunt	Glycolysis
End product of glycolysis is	Pyruvate	Glutamate	Lactate	Acetate	Pyruvate
Fatty acid synthesis require	Biotin	Avidin	Protein	Glucose	Biotin
Gateway compound to TCA cycle is	Pyruvate	Glutamate	Lactate	Acetate	Pyruvate
HMS pathway is also known as	Oxidative pentose pathway	Reductive pentose pathway	Oxidative hexose pathway	Reductive hexose pathway	Oxidative pentose pathway
Important enzyme involved in energy generation by chemiosmosis	ATP synthase	ADP synthase	NAD synthase	FAD synthase	ATP synthase

In chemotrophic electron transport, the first molecule in electron transport chain is	NAD/NADP	FAD/FADH	ATP/ADP	$FMN/FMNH_2$	NAD/NADP
In cyclic electron transport, there is	Gain of electrons	Loss of electrons	No net electron loss	No electrons involved	No net electron loss
In ED pathway, net ATP formed per glucose molecule is	1	2	3	4	1
In eukaryotes, electron transport takes place in _____	Mitochondrion	Golgi apparatus	Endoplasmic reticulum	Nucleus	Mitochondrion
In microbes ADP and inorganic phosphate combines to form	ATP	FAD	NADP	NADPH	ATP
In microbes, _____ play a critical role in membrane formation and function.	Lipids	Minerals	Vitamins	Amino acids	Lipids
In neutral lipids, organic lipids are esterified to	Glycerol	Glyceraldehyde	Glycerin	Glyceride	Glycerol
In photosynthetic prokaryotes, _____ type electron transport is	Cyclic	Non-cyclic	Aromatic	Aliphatic	Cyclic

seen					
Major source of reducing power for biosynthesis	NADP	NADPH	ADP	ATP	NADPH
Microorganism which use organic acid as electron donors.	*Bacillus sp*.	*Rhodococcus sp*.	*Pseudomonas sp*.	*Spirillum*	*Rhodococcus sp*.
Number of ATP molecules produced per turn in TCA cycle	12	14	16	18	12
Number of critical biosynthetic intermediates supplied by glycolytic pathway	3	4	5	6	6
Number of net ATP molecules produced in TCA cycle	12	24	36	40	24
Oxidative phosphorylation occurs in	Membranes	Cytoplasm	Periplasm	Nucleoid	Membranes
Phycoilisomes are found in	Cyanobacteria	Bacteria	Plants	Animals	Cyanobacteria
Prokaryotic electron transport occurs in	Membranes	Periplasm	Nucleus	Golgi apparatus	Membranes
Reduced form of flavin mononucleotide	FMNH	$FMNH^+$	$FMHN^-$	FMNH2	FMNH2
Substrate level	Cytoplasm	Periplasm	Ribosome	Mesosome	Cytoplasm

phosphorylation occurs in					
Sugar containing lipids are called	Glycolipids	Neutral lipids	Lipoproteins	Phospholipids	Glycolipids
TCA cycle is	Catabolic	Anabolic	Both anabolic & catabolic	Amphipathic	Both anabolic & catabolic
TCA cycle participates in both	Energy generation and biosynthesis	Energy reduction and catalysis	Energy oxidation and desynthesis	Energy degardation and synthesis	Energy generation and biosynthesis
TCA cycle turns _____ for each glucose molecule	Once	Twice	Thrice	Never	Twice
Terminal electron acceptor in anaerobic respiration	Sulfate	Nitrate	Fumarate	All of the above	All of the above
The Amount of Energy released in anaerobic respiration is _____ kJ/mol of glucose.	120	210	102	201	120
The driving force behind ATP generation is	Difference in proton concentration	Positive proton concentration	Negative proton concentration	No proton concentration	Difference in proton concentration
The two light harvesting centers of purple bacteria are	P_{850} & P_{875}	P_{950} & P_{975}	P_{760} & P_{800}	P_{800} & P_{975}	P_{850} & P_{875}
Togather, electron transport, FAD, NAD	32	34	36	38	34

& NADP reduction yields _____ ATP molecules per hexose molecule					
Total ATP yield by electron transport per turn of TCA cycle is	11	12	21	22	11
Which acts as a "pore" through which protons may flow through the layer, for ATP generation	F_0 portion	F_1 portion	F_0F_1 portion	F_0F_1-ATPase complex	F_0 portion
Which cycle is called bypass cycle?	Glycolysis	Glyoxalate cycle	ED pathway	TCA cycle	Glyoxalate cycle

Unit IV–

Catabolism of Carbohydrates and Intracellular Reserve Polymers

Microorganisms can catabolize many carbohydrates besides glucose.These carbohydrates may come either from outside the cell or from internal sources. Often the initial steps in the degradation of external carbohydrate polymers differ from those employed with internal reserves.

Carbohydrates

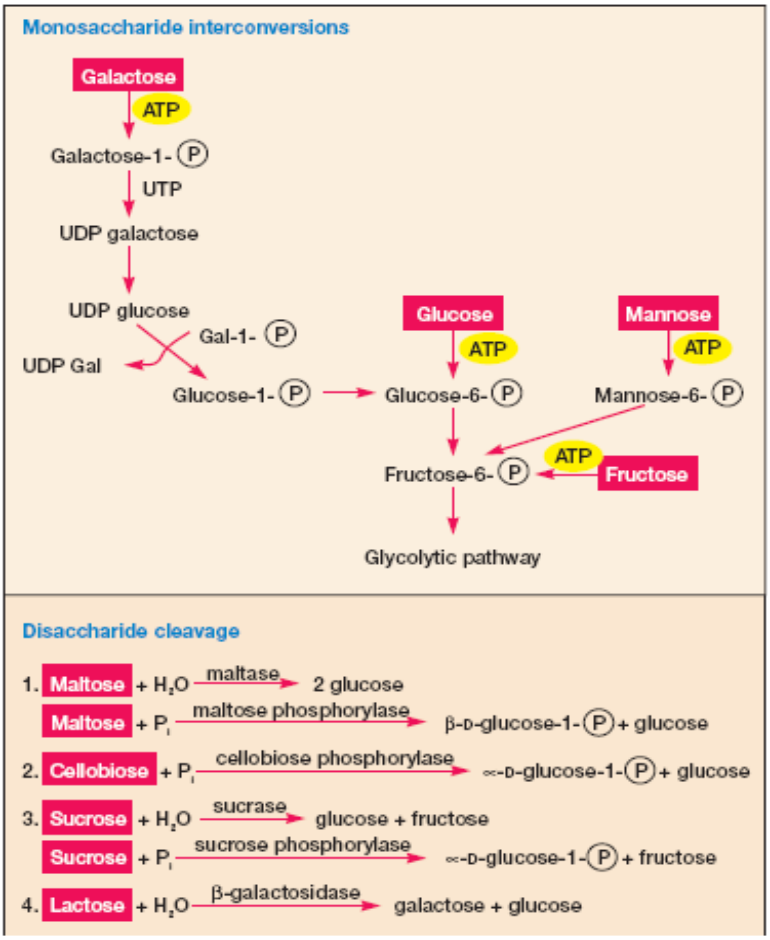


Figure Carbohydrate Catabolism.

Figure outlines some catabolic pathways for the monosaccharides (single sugars) glucose, fructose, mannose, and galactose. The first three are phosphorylated using ATP and easily enter the glycolytic pathway. In contrast, galactose must be converted to uridine diphosphate galactose after initial phosphorylation, then changed into glucose 6-phosphate in a three-step process. The common disaccharides are cleaved to monosaccharides by at least two mechanisms. Maltose, sucrose, and lactose can be directly hydrolyzed to their constituent sugars. Many disaccharides (e.g., maltose, cellobiose, and sucrose) are also split by a phosphate attack on the bond joining the two sugars, a process called phosphorolysis.

Polysaccharides, like disaccharides, are cleaved by both hydrolysis and phosphorolysis. Bacteria and fungi degrade external polysaccharides by secreting hydrolytic enzymes that cleave polysaccharides into smaller molecules, which can then be assimilated. Starch and glycogen are hydrolyzed by amylases to glucose, maltose, and other products. Cellulose is more difficult to digest; many fungi and a few bacteria (some gliding bacteria, clostridia, and actinomycetes) produce cellulases that hydrolyze cellulose to cellobiose and glucose. Some members of the bacterial genus *Cytophaga*, isolated from marine habitats, excrete an agarase that degrades agar. Many soil bacteria and bacterial plant pathogens degrade pectin, a polymer of galacturonic acid (a galactose derivative) that is an important constituent of plant cell walls and tissues. In the context of compounds that are recalcitrant or difficult to digest, it should be noted that microorganisms also can degrade xenobiotic compounds (foreign substances not formed by natural biosynthetic processes) such as pesticides and various aromatic compounds. They transform these molecules to normal metabolic intermediates by use of special enzymes and pathways, then continue catabolism in the usual way.

Lipid Catabolism

Microorganisms frequently use lipids as energy sources. Triglycerides or triacylglycerols, esters of glycerol and fatty acids (**figure**), are common energy sources and will serve as our examples.

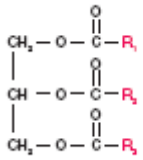


Figure A Triacylglycerol or Triglyceride. The R groups represent the fatty acid side chains.

They can be hydrolyzed to glycerol and fatty acids by microbial lipases. The glycerol is then phosphorylated, oxidized to dihydroxyacetone phosphate, and catabolized in the glycolytic pathway. Fatty acids from triacylglycerols and other lipids are often oxidized in the β-**oxidation pathway** after conversion to coenzyme A esters (**figure**).

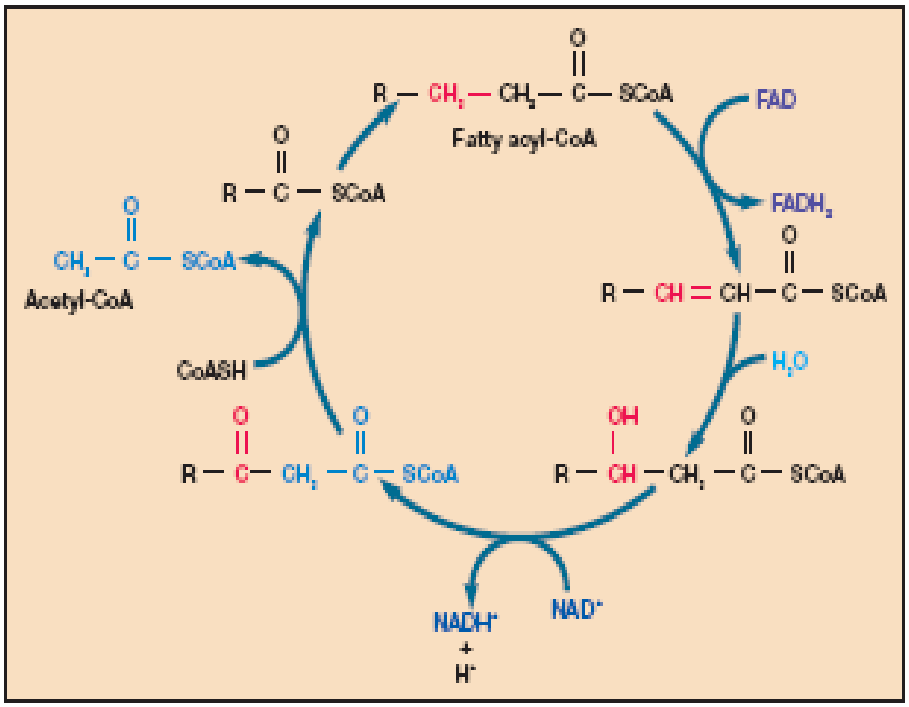


Figure: **Fatty Acid β-Oxidation.**

In this cyclic pathway fatty acids are degraded to acetyl-CoA, which can be fed into the TCA cycle or used in biosynthesis. One turn of the cycle produces acetyl-CoA, NADH, and FADH2; NADH and FADH2 can be oxidized by the electron transport chain to provide more ATP. The fatty acyl-CoA, shortened by two carbons, is ready for another

turn of the cycle. Lipid fatty acids are a rich source of energy for microbial growth. In a similar fashion some microorganisms grow well on petroleum hydrocarbons under aerobic conditions.

Fermentations

In the absence of aerobic or anaerobic respiration, NADH is not oxidized by the electron transport chain because no external electron acceptor is available. Yet NADH produced in the glycolytic pathway during the oxidation of glyceraldehyde 3-phosphate

to 1,3-bisphosphoglycerate must still be oxidized back to NAD+. If NAD+ is not regenerated, the oxidation of glyceraldehydes 3-phosphate will cease and glycolysis will stop. Many microorganisms solve this problem by slowing or stopping pyruvate dehydrogenase activity and using pyruvate or one of its derivatives as an electron and hydrogen acceptor for the reoxidation of NADH in a fermentation process (**figure**).

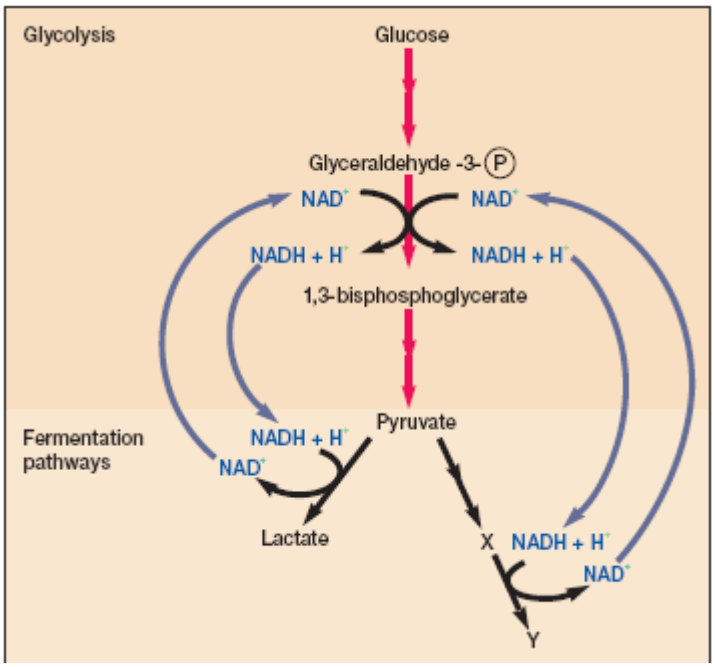


Figure Reoxidation of NADH During Fermentation.

This may lead to the production of more ATP. The process is so effective that some chemoorganoheterotrophs do not carry out respiration even when oxygen or another exogenous acceptor is available. There are many kinds of fermentations, and they often are characteristic of particular microbial groups (**figure**). Two unifying themes should be kept in mind when microbial fermentations are examined: (1) NADH is oxidized to NAD_, and (2) the electron acceptor is often either pyruvate or a pyruvate

derivative. In fermentation the substrate is partially oxidized, ATP is formed by substrate-level phosphorylation only, and oxygen is not needed.

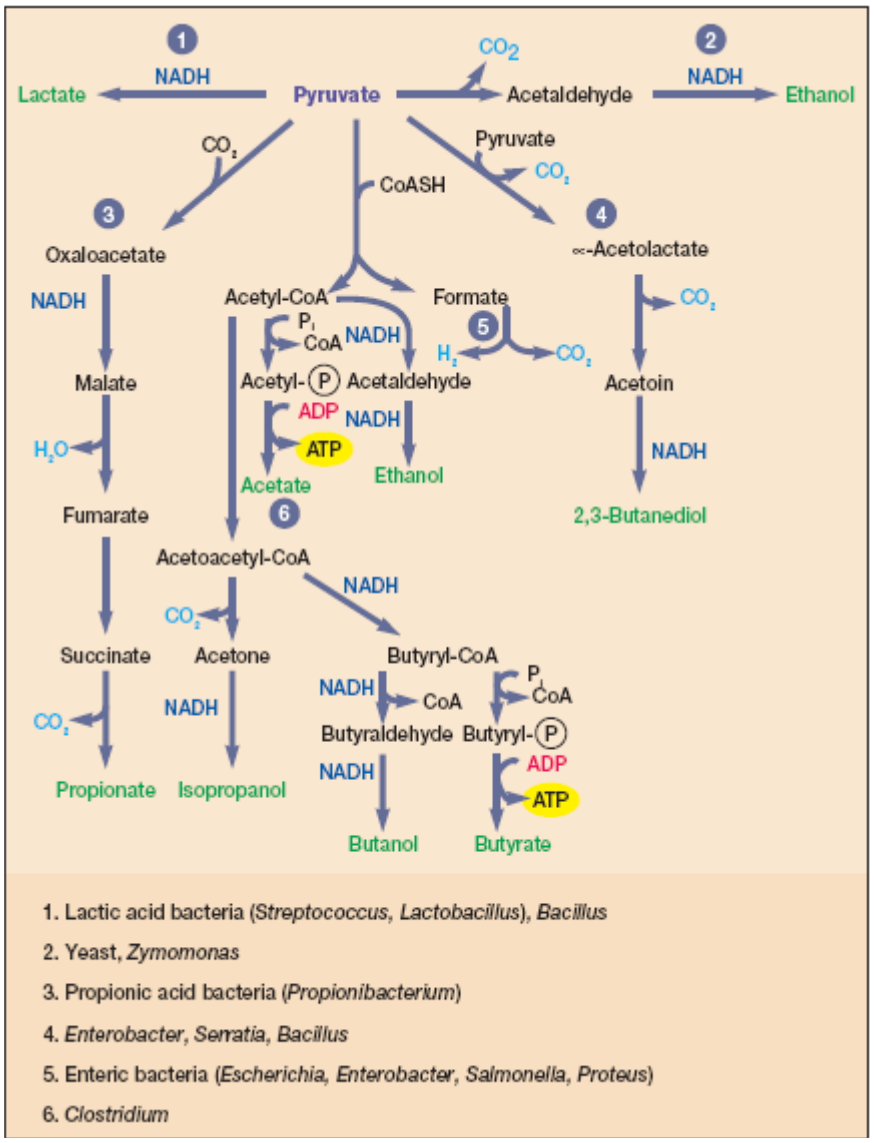


Figure Some Common Microbial Fermentations.

Many fungi and some bacteria, algae, and protozoa ferment sugars to ethanol and CO2 in a process called **alcoholic fermentation.** Pyruvate is decarboxylated to

acetaldehyde, which is then reduced to ethanol by alcohol dehydrogenase with NADH as the electron donor. **Lactic acid fermentation,** the reduction of pyruvate to lactate, is even more common. It is present in bacteria (lactic acid bacteria, *Bacillus*), algae (*Chlorella*), some water molds, protozoa, and even in animal skeletal muscle. Lactic acid fermenters can be separated into two groups. **Homolactic fermenters** use the glycolytic

pathway and directly reduce almost all their pyruvate to lactate with the enzyme lactate dehydrogenase. **Heterolactic fermenters** form substantial amounts of products other than lactate; many produce lactate, ethanol, and CO2 by way of the phosphoketolase pathway. Alcoholic and lactic acid fermentations are quite useful. Alcoholic fermentation by yeasts produces alcoholic beverages; CO2 from this fermentation causes bread to rise. Lactic acid fermentation can spoil foods, but also is used to make yogurt, sauerkraut, and pickles Many bacteria, especially members of the family *Enterobacteriaceae,* can metabolize pyruvate to formic acid and other products in a process sometimes called the formic acid fermentation. Formic acid may be converted to H2 and CO2 by formic hydrogenlyase (a combination of at least two enzymes).

$$HCOOH \rightarrow CO_2 + H_2$$

There are two types of formic acid fermentation. **Mixed acid fermentation** results in the excretion of ethanol and a complex mixture of acids, particularly acetic, lactic, succinic, and formic acids. If formic hydrogenlyase is present, the formic acid will be degraded to H2 and CO2. This pattern is seen in *Escherichia, Salmonella, Proteus,* and other genera. The second type, **butanediol fermentation,** is characteristic of *Enterobacter, Serratia, Erwinia,* and some species of *Bacillus*. Pyruvate is converted to acetoin, which is then reduced to 2,3-butanediol with NADH. A large amount of ethanol

is also produced, together with smaller amounts of the acids found in mixed acid fermentation. Formic acid fermentations are very useful in identification of members of the *Enterobacteriaceae.*

Butanediol fermenters can be distinguished from mixed acid fermenters in three ways.

1. The Voges-Proskauer test is a colorimetric procedure that detects the acetoin precursor of butanediol and is positive with butanediol fermenters but not with mixed acid fermenters. The Voges-Proskauer test is used by both the Enterotube II and API 20E microbial identification systems to identify enteric bacteria.

2. Mixed acid fermenters produce four times more acidic products than neutral ones, whereas butanediol fermenters form mainly neutral products. Thus mixed acid fermenters acidify incubation media to a much greater extent. This is the basis of the methyl red test. The test is positive only for mixed acid fermentation because the pH drops below 4.4 and the color of the indicator changes from yellow to red.

3 . CO_2 and H_2 arise in equal amounts from formic hydrogenlyase activity during mixed acid fermentation. Butanediol fermenters produce excess CO_2 and the CO_2/H_2 ratio is closer to 5:1. Formic acid fermenters sometimes generate ATP while reoxidizing

NADH. They use acetyl-CoA to synthesize acetyl phosphate, which then donates its phosphate to ADP.

$$\text{Acetyl-CoA} + P_i \longrightarrow \text{CoASH} + \text{acetyl-P}$$
$$\text{Acetyl-P} + \text{ADP} \longrightarrow \text{acetate} + \text{ATP}$$

Microorganisms carry out fermentations other than those already mentioned. Protozoa and fungi often ferment sugars to lactate, ethanol, glycerol, succinate, formate, acetate, butanediol, and additional products. Substances other than sugars also are fermented by microorganisms. For example, some members of the genus *Clostridium*

prefer to ferment mixtures of amino acids. Proteolytic clostridia such as the pathogens *C. sporogenes* and *C. botulinum* will carry out the **Stickland reaction** in which one amino acid is oxidized and a second amino acid acts as the electron acceptor.

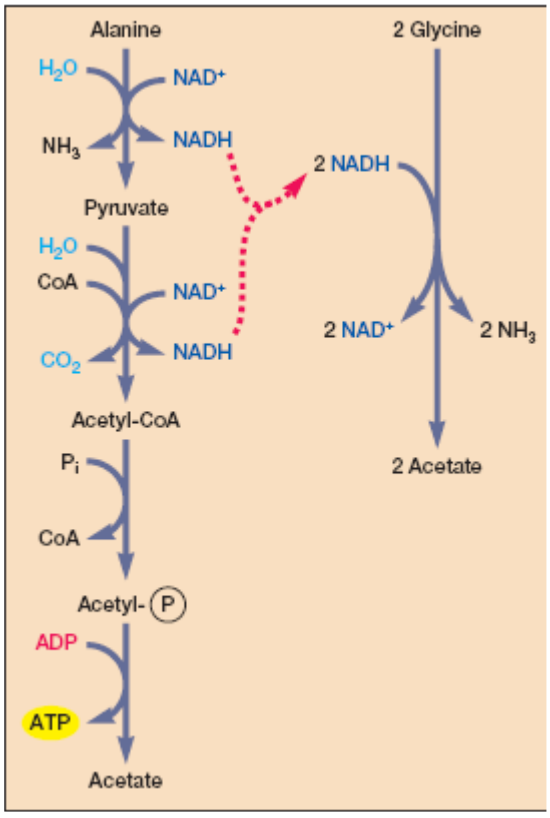


Figure The Stickland Reaction. Alanine is oxidized to acetate and glycine is used to reoxidize the NADH generated during alanine degradation. The fermentation also produces some ATP.

Figure shows the way in which alanine is oxidized and glycine reduced to produce acetate, CO2, and NH3. Some ATP is formed from acetyl phosphate by substrate-level phosphorylation, and the fermentation is quite useful for growing in anaerobic, protein-rich environments. The Stickland reaction is used to oxidize several amino acids: alanine, leucine, isoleucine, valine, phenylalanine, tryptophan, and

histidine. Bacteria also ferment amino acids (e.g., alanine, glycine, glutamate, threonine, and arginine) by other mechanisms. In addition to sugars and amino acids, organic acids such as acetate, lactate, propionate, and citrate are fermented. Some of these fermentations are of great practical importance. For example, citrate can be converted to diacetyl and give flavor to fermented milk

Metabolism: The Use of Energy in Biosynthesis

Biosynthesis of carbohydrates

Heterotrophic organisms are unable to incorporate inorganic carbon as CO2 or HCO_3^- into hexose sugars, most commonly via the Calvin cycle, and must convert a range of organic compounds into glucose by a series of reactions called *gluconeogenesis*.

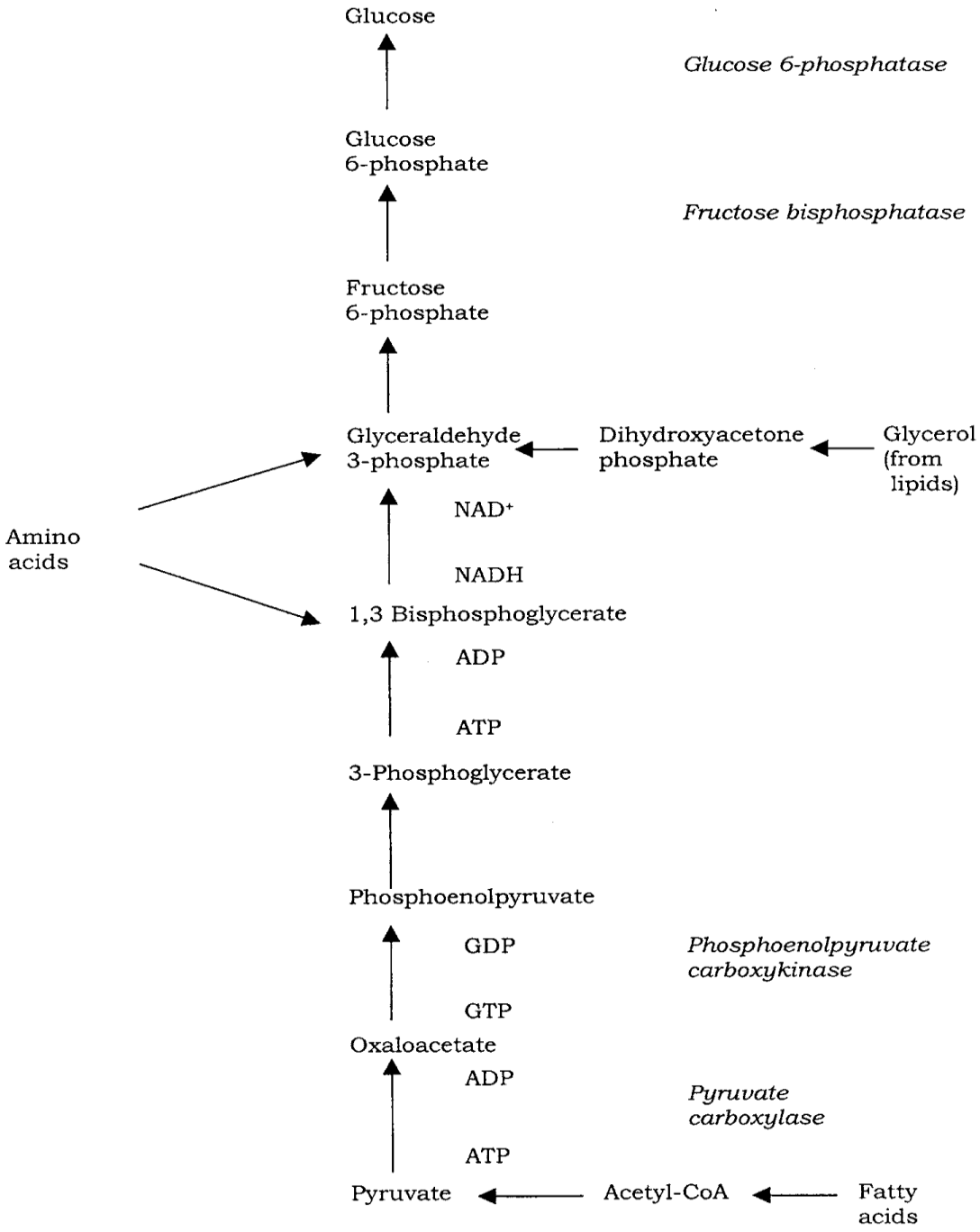

Glucose
Glucose 6-phosphatase
Glucose 6-phosphate
Fructose bisphosphatase
Fructose 6-phosphate
Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate
Glycerol (from lipids)
NAD+
Amino acids
NADH
1,3 Bisphosphoglycerate
ADP
ATP
3-Phosphoglycerate
Phosphoenolpyruvate
GDP
Phosphoenolpyruvate carboxykinase
GTP
Oxaloacetate
ADP
Pyruvate carboxylase
ATP
Pyruvate
Acetyl-CoA
Fatty acids

Figure Gluconeogenesis. Non-carbohydrate precursors can feed into a pathway that converts pyruvate to glucose in a series of reactions that are mostly the reverse of glycolysis.

Many compounds such as lactate or certain amino acids can be converted to pyruvate directly or via other members of the TCA cycle, and thence to glucose. To all intents and purposes, gluconeogenesis reverses the steps of glycolysis, although not all the enzymes involved are exactly the same. This is because three of the reactions are essentially irreversible, so different enzymes must be used to overcome this.

Once glucose or fructose has been produced, it can be converted to other hexose sugars by simple rearrangement reactions. Building up these sugars into bigger carbohydrates (polysaccharides) requires them to be in an energised form: this usually takes the form of either an ADP or UDP-sugar, and necessitates an input of energy. Pentose sugars such as ribose are important in the synthesis of nucleotides for nucleic acids and coenzymes

Biosynthesis of fatty acid

Fatty acids are synthesised by a stepwise process that involves the addition of two carbon units to form a chain, most commonly of 16–18 carbons. The starting point of fatty acid metabolism is the two-carbon compound The basic building blocks in the synthesis of fatty acids are acetyl-CoA (two-carbon) and malonyl-CoA (three-carbon). We have encountered acetyl-CoA before, when discussing the TCA cycle; malonyl-CoA is formed by the carboxylation of acetyl-CoA. Acetyl–CoA + CO2 → Malonyl–CoA 2 − carbon 3 − carbon Carbon dioxide is essential for this step, but is not incorporated into the fatty acid as it is removed in a subsequent decarboxylation step. In order to take part in the biosynthesis of fatty acids, both molecules have their coenzyme A element replaced by an *acyl carrier protein (ACP).* In a condensation reaction, one carbon is lost as CO2 and one of the ACPs is released. The resulting four-carbon molecule is reduced, with the involvement of two NADPH molecules, to *butyryl-ACP*.

This is then extended two carbon atoms at a time by a series of further condensations with malonyl-ACP.

Thus, extending a fatty acid chain by two carbons involves the expenditure of one

ATP and two NADPH molecules. The overall equation for the synthesis of a 16-carbon fatty acid such as palmitic acid can be represented:

8 Acetyl-CoA +7 ATP + 14 NADPH + 6H+ −⟶ Palmitate + 14 NADP+ + 8 CoA +

6 H2O + 7 ADP + 7 Pi

Once formed, fatty acids may be incorporated into phospholipids, the major form of lipid found in microbial cells. Recall from Chapter 2 that a phospholipid molecule has

three parts: fatty acid, glycerol and phosphate. These last two are provided in the form of glycerol phosphate, which derives from the dihydroxyacetone phosphate of glycolysis. Glycerol phosphate replaces the ACP of two fatty acid-ACP conjugates to yield phosphatidic acid, an important precursor for a variety of membrane lipids. The energy for this reaction is provided, unusually, not by ATP but by CTP (cytidine triphosphate).

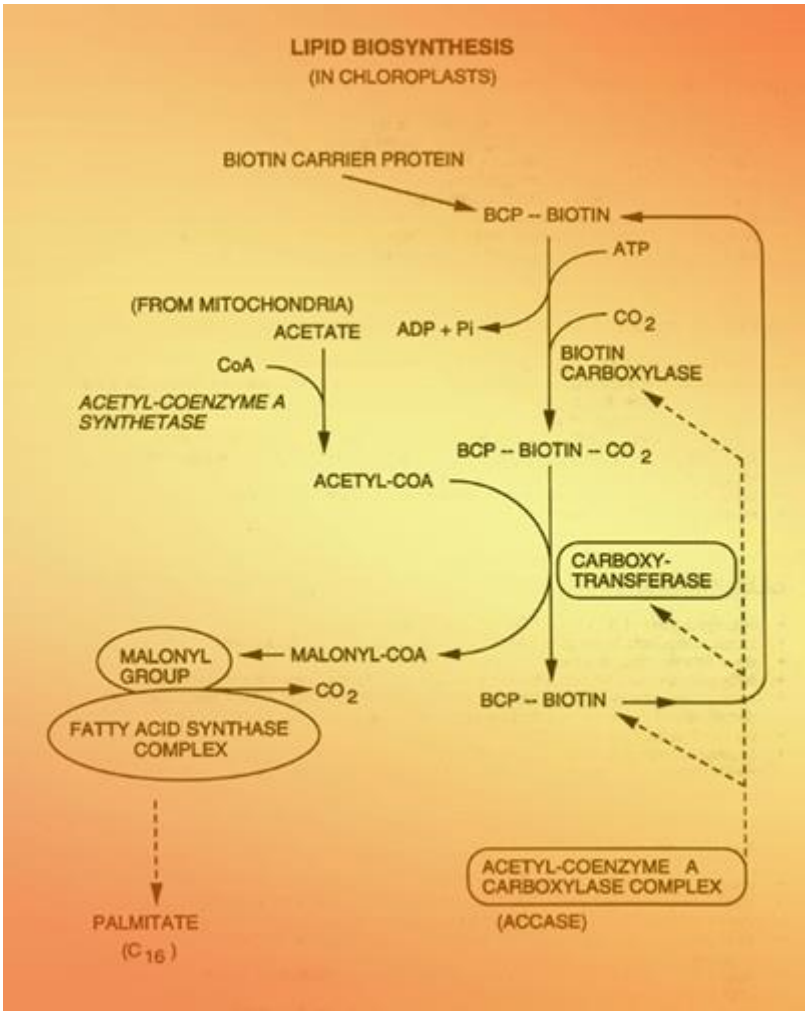


Figure Fatty acid biosynthesis. Acetyl- and malonyl-ACPs condense with the loss of CO2 to give a four-carbon molecule butyryl-ACP. The addition of further two-carbon acetyl groups is achieved by re-entering the pathway and reacting with further molecules of malonyl-ACP

The Synthesis of Purines, Pyrimidines, and Nucleotides

Purine and pyrimidine biosynthesis is critical for all cells because these molecules are used in the synthesis of ATP, several cofactors, ribonucleic acid (RNA),

deoxyribonucleic acid (DNA), and other important cell components. Nearly all microorganisms can synthesize their own purines and pyrimidines as these are so crucial to cell function.

Purines and **pyrimidines** are cyclic nitrogenous bases with several double bonds and pronounced aromatic properties. Purine consist of two joined rings, whereas pyrimidines have only one. The purines **adenine** and **guanine** and the pyrimidines **uracil, cytosine,** and **thymine** are commonly found in microorganisms. A purine or pyrimidine base joined with a pentose sugar, either ribose or deoxyribose, is a **nucleoside.** A **nucleotide** is a nucleoside with one or more phosphate groups attached to the sugar.

Purine Biosynthesis

The biosynthetic pathway for purines is a complex, 11-step sequence in which seven different molecules contribute parts to the final purine skeleton.

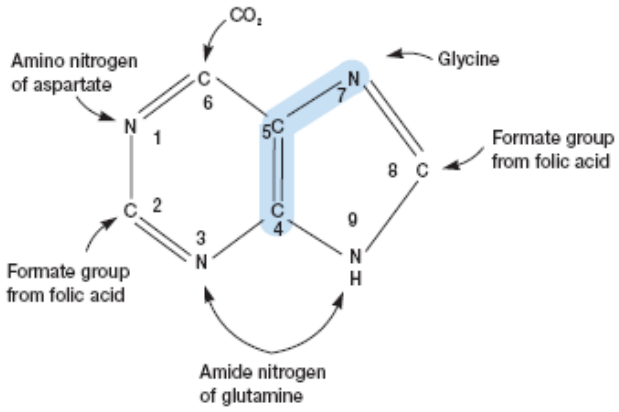


Figure Purine Biosynthesis. The sources of purine skeleton nitrogen and carbon are indicated. The contribution of glycine is shaded.

Because the pathway begins with ribose 5-phosphate and the purine skeleton is constructed on this sugar, the first purine product of the pathway is the nucleotide inosinic acid, not a free purine base. The cofactor folic acid is very important in purine biosynthesis. Folic acid derivatives contribute carbons two and eight to the purine skeleton. In fact, the drug sulfonamide inhibits bacterial growth by blocking folic acid synthesis. This interferes with purine biosynthesis and other processes that require folic acid. Once inosinic acid has been formed, relatively short pathways synthesize adenosine monophosphate and guanosine monophosphate (**figure**) and produce nucleoside diphosphates and triphosphates by phosphate transfers from ATP.

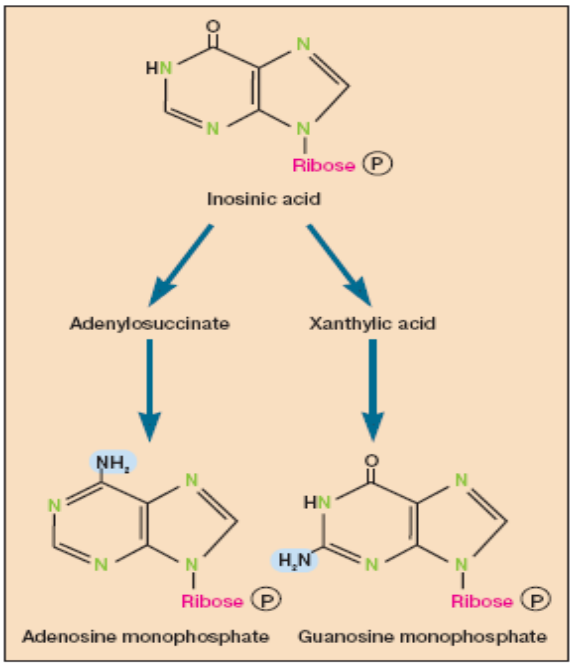


Figure Synthesis of Adenosine Monophosphate and Guanosine Monophosphate. The shaded groups are the ones differing from those in inosinic acid.

DNA contains deoxyribonucleotides (the ribose lacks a hydroxyl group on carbon two) instead of the ribonucleotides found in RNA. Deoxyribonucleotides arise

from the reduction of nucleoside diphosphates or nucleoside triphosphates by two different routes. Some microorganisms reduce the triphosphates with a system requiring vitamin B12 as a cofactor. Others, such as *E. coli*, reduce the ribose in nucleoside diphosphates. Both systems employ a small sulfur-containing protein called thioredoxin as their reducing agent.

Pyrimidine Biosynthesis

Pyrimidine biosynthesis begins with aspartic acid and carbamoyl phosphate, a high-energy molecule synthesized from CO2 and ammonia (**figure**).

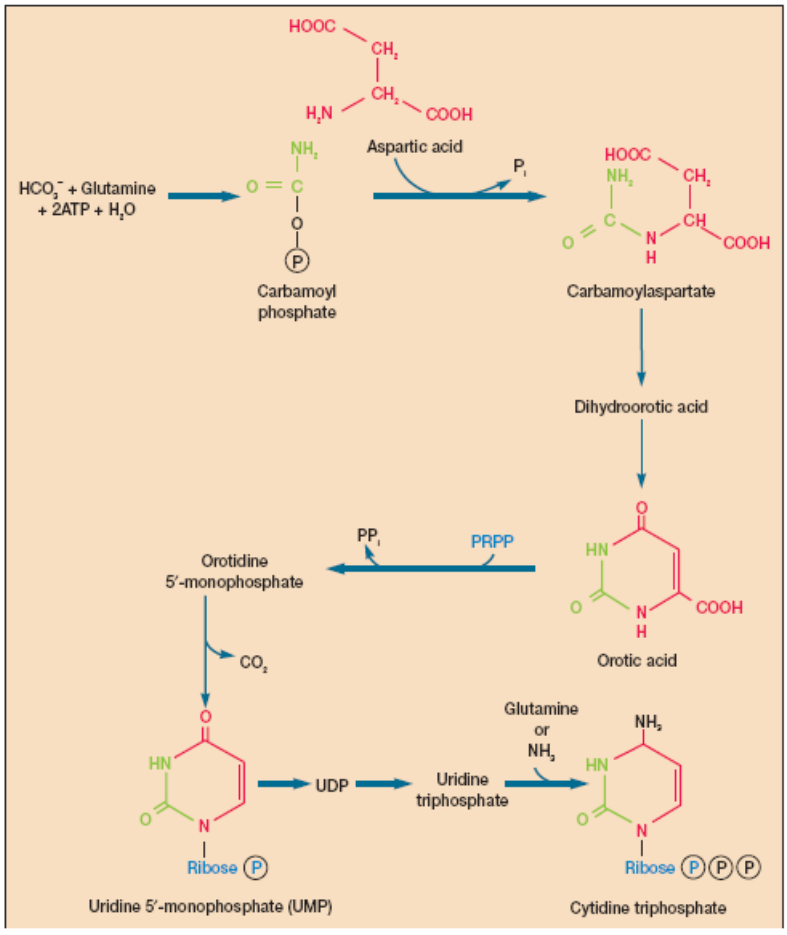


Figure Pyrimidine Synthesis. PRPP stands for 5-phosphoribose 1-pyrophosphoric acid, which provides the ribose 5-phosphate chain.

Aspartate carbamoyltransferase catalyzes the condensation of these two substrates to form carbamoylaspartate, which is then converted to the initial pyrimidine product, orotic acid. After synthesis of the pyrimidine skeleton, a nucleotide is produced by the ribose 5-phosphate addition using the highenergy intermediate 5-phosphoribosyl 1-pyrophosphate. Thus construction of the pyrimidine ring is completed before ribose is added, in contrast with purine ring synthesis, which begins with ribose 5-phosphate. Decarboxylation of orotidine monophosphate yields uridine monophosphate and eventually uridine triphosphate and cytidine triphosphate. The third common pyrimidine is thymine, a constituent of DNA. The ribose in pyrimidine nucleotides is reduced in the same way as it is in purine nucleotides. Then deoxyuridine monophosphate is methylated with a folic acid derivative to form deoxythymidine monophosphate (**figure**).

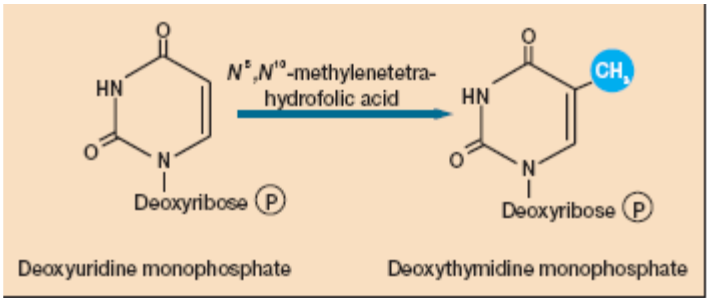


Figure Deoxythymidine Monophosphate Synthesis. Deoxythymidine differs from deoxyuridine in having the shaded methyl group.

Biosynthesis of amino acids

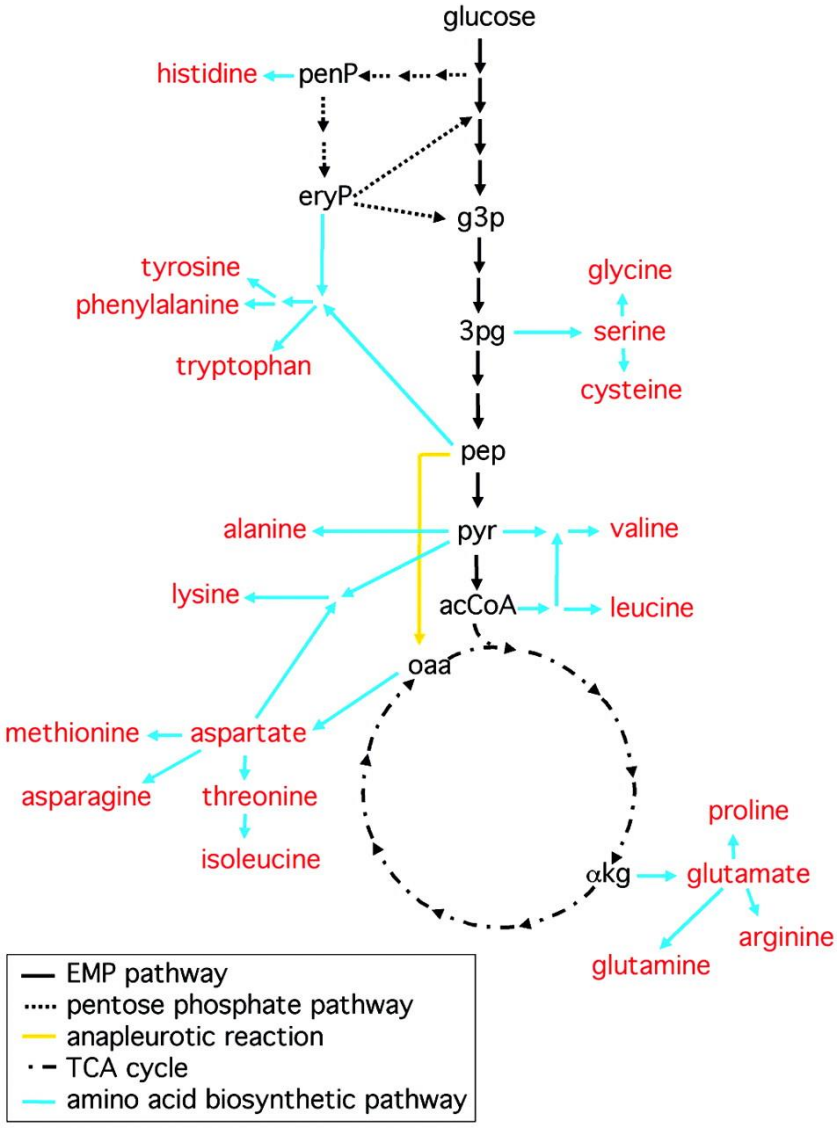
A very limited number of microorganisms are able to utilise molecular nitrogen from the atmosphere by incorporating it initially into ammonia and subsequently into organic compounds. Most organisms, however, need to have their nitrogen supplied as nitrate, nitrite, or ammonia itself. Ammonia can be incorporated into organic nitrogen compounds in several ways, including glutamate formation from α-ketoglutarate (see TCA cycle, above):

α-ketoglutarate + NH4+ + NADPH* + H+ $\longrightarrow$ glutamate + NADP+ + H2O

(*Some species utilise NADH as their electron donor)

The amino group can subsequently be transferred from the glutamate to make other amino acids by *transamination* reactions involving other keto acids: e.g. glutamate + oxaloacetate $\longrightarrow$ aspartate +α-ketoglutarate Glutamate plays a central role in the biosynthesis of other amino acids, as it usually donates the primary amino group of each: amino acids are broken down, they are likewise broken down into a handful of metabolic

intermediates, which then feed into the TCA cycle.



The regulation of metabolism

Microorganisms, like the rest of us, live in a changing world, and their needs do not always remain the same. It would be highly inefficient and (frequently wasteful) if all

their metabolic reactions were going on with equal intensity all the time, regardless of whether they were needed. Over evolutionary time, regulation systems

have developed, so that metabolism is tailored to the prevailing conditions. Essentially, this regulation involves controlling the activity of enzymes which direct the many biochemical reactions occurring in each cell. This can be done by: directly affecting enzyme activity, or indirectly, at the genetic level, by controlling the level at which enzymes are synthesised. Direct control of enzymatic activity occurs by the mechanism of *feedback inhibition*, whereby the final product of a metabolic pathway acts as an inhibitor to the enzyme that catalyses an early step (usually the first) in the pathway. It thus prevents more of its own formation. When the concentration of the product subsequently falls below a certain level, it is no longer inhibitory, and biosynthesis resumes.Regulation of metabolic pathways can also be achieved by controlling whether or not an enzyme is synthesised in the first place, and if so, the rate at which it is produced. This is done at the DNA level, by one of two mechanisms, *induction* and *repression*, which respectively 'switch on' and 'switch off' the machinery of protein synthesis.

Archael lipid biosynthesis

Introduction

In 1977, Carl Woese suggested a new taxonomy for living systems that uprooted the prokaryote-eukaryote view of the world and proposed a structure based on 3 domains, Eubacteria, Eukarya and a new group that he identified as the Archaea. The Archaea comprise a diverse group of organisms that thrive in extreme environments of high temperatures, high salt concentrations, and acidic hot springs. They also include the methanogens that produce methane gas from carbon dioxide. In the past two decades, biologists have learned that the Archaea are more pervasive than initially realized and they have been found in deep sea thermal vents, oil reservoirs, and refuse piles including

intermediate and even low temperature environments. Their ability to thrive at conditions that would be lethal to other organisms has attracted the interest of the biotechnical community and stimulated investigations into their accommodation and survival strategies. This paper examines one of the most distinctive features of the Archaea, their cell membranes, and explores the structure, biosynthesis, function and potential applications of the archaeal systems.

Archaeal Membranes

Lipids of all organisms are characterized by diversity, and the lipid pattern within a cell may vary qualitatively and quantitatively, depending on how the organism was grown or at what stage of the growth the lipid analysis has been carried out. However, eukaryotic membrane lipid structures typically consist of two hydrophobic linear hydrocarbon chains attached via ester linkage to a glycerophosphate "backbone" that is frequently attached to additional polar groups to form glycolipids or even more complex surface structures. The lipid structures of the Archaea are distinctively different, with regard to the following three features:

1. the hydrocarbon "tails" are linked to the glycerol backbone with an ether linkage rather than an ester linkage,
2. the hydrocarbon tails are derived from isoprenoid units rather than fatty acid alkyls,
3. the chirality of the glycerol is sn-glycerol-1-phosphate (stereospecific numbering), rather than sn-glycerol-3-phosphate found in the other two domains.

The first feature listed above (ether linkage) is characteristic of virtually all archaea, but it is not exclusive. Ether glycerophospholipids are found in eukarya and eubacteria, often in combination with ester linkages (e.g., plasmalogens, platelet activating factor). But, ester linkages are not found in archaea. The second feature

(isoprenoid hydrocarbons) again is not exclusive and some bacteria have isoprenoid chains. However, the third feature is exclusive, archaea are distinctively different from the other two domains with regard to the chirality of the glycerol backbone. When ether linkages appear in bacteria, they typically form in the bacterial stereostructure rather than the archaeal stereostructure. Thus, the hallmark feature of Archaea is the unique ether-linked isoprenoid stereospecific membrane lipid. The total lipid fraction of a typical archaea cell comprises about 2 – 6% of the cell dry weight, and includes two components, polar lipids and neutral lipids. The neutral lipid fraction is typically around 10 to 20 % and often includes isoprenoid-derived chains of lengths ranging up to squalene and quinones. The quinones typically play an important role in electron transport, oxidative phosphorylation and active transport. The polar lipid fraction may be analyzed directly, or subjected to acid hydrolysis to separate the core lipid components from the broad spectrum of head groups that are included.

Archaeal Lipid Biosynthesis Pathways

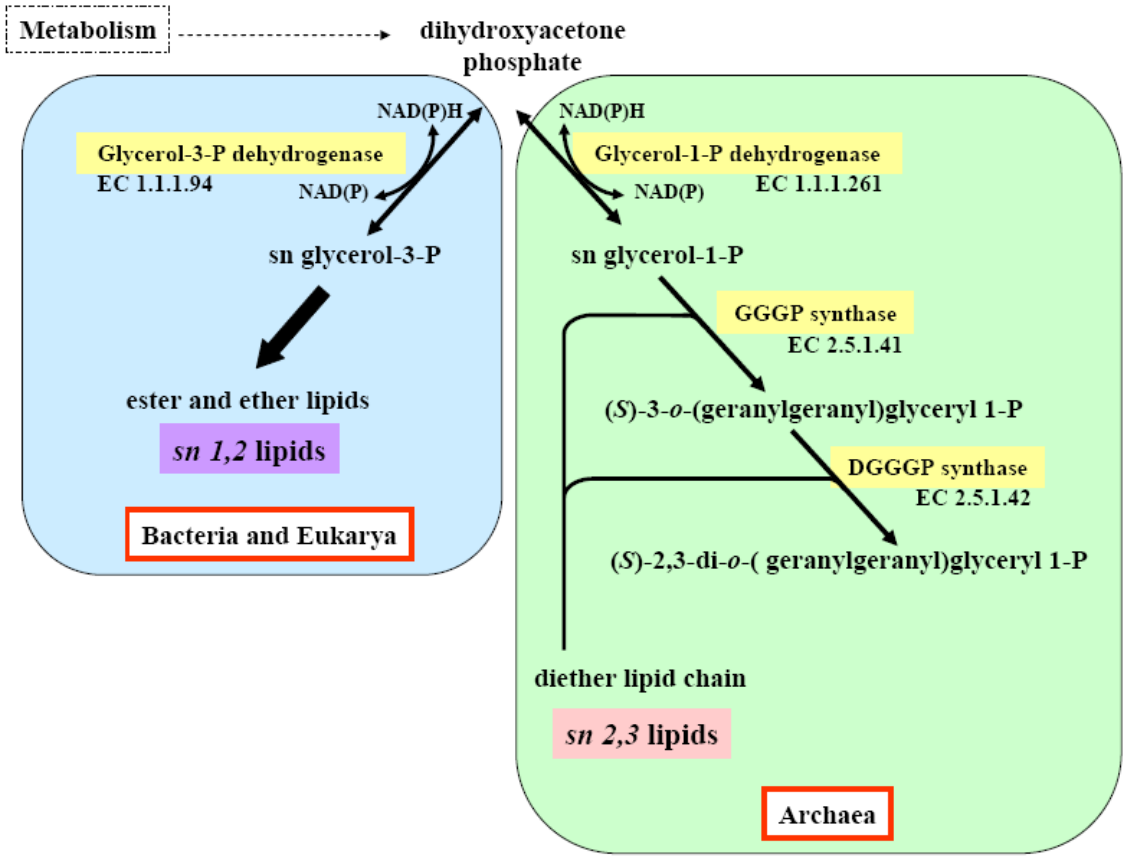
The biosynthesis pathway for the archaeal lipids is a complex multienzyme, membranebound system that was extensively reported in the 1970's. The alkyl chains in the trademark core lipids are produced via the mevalonate pathway used by bacteria and eukaryotes for isoprenoid synthesis (Kates 1992). 14C radioisotopic studies of 1-C acetate and 2-C mevalonate show a very low level of incorporation into the glycerol moiety of archaeol. But there is a much higher level of incorporation in the isoprenoid moiety, suggesting a conventional isoprenoidic pathway involving the incorporation of mevalonate (DeRosa 1986). The malonyl-CoA pathway for fatty acid biosynthesis is operative in the Archaea, but at very low levels and only trace quantities of fatty acids have been detected. The fatty acid biosynthesis and the isoprenoid biosynthesis pathways are similar in the initial stages, but then diverge. It does not appear that environmental conditions alone are the determining factor on the choice of pathway since thermophilic eubacteria, that inhabit similar environments to archaea, synthesize

conventional fatty acid ester lipid membrane systems. The similarities in the early stages of the isoprenoid pathway suggest an early evolutionary divergence from a common ancestral path.

The biosynthetic pathway begins with the formation of mevalonate from acetyl-CoA. Two acetyl-CoA molecules undergo Claisen condensation, catalyzed by ketothiolase, to form acetoacetyl-CoA. Another Claisen condensation between this product and another acetyl-CoA is catalyzed by HMG-CoA synthase to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). Finally, HMG-CoA reductase catalyzes two NADPH-dependent reductions to convert the thioester (HMG-CoA) to a primary alcohol, 3R-mevalonate. Mevalonate then undergoes a series of 2 ATP driven reactions by mevalonate kinase and phosphomevalonate kinase to produce 5-pyrophosphomevalonate. Next this product is catalyzed by pyrophosphomevalonate decarboxylase, using another ATP to form isopentenyl pyrophosphate (also P and CO2). Isopentenyl pyrophosphate isomerase then rearranges this molecule to dimethylallyl pyrophosphate. The isopentenyl-PP will then react (condensation) with the dimethylallyl-PP to form geranyl-PP. Eventually farnesyl- PP, geranylgeranyl-PP, and even longer diphosphates can be formed. In each case, the units are combining in a head-to-tail fashion and releasing pyrophosphate. Head-to-tail is the common condensation. Tail-to-tail is the exception and results in the release of 2 pyrophosphate molecules. It appears that the final reduction of geranylgeranyl-PP to the phytanyl group takes place only after linkage to the glycerol backbone. While the enzyme responsible for the formation of the lipid backbone structure (sn-glycerol-3-phosphate or G-3-P) was identified over three decades ago, the corresponding archaeal enzyme has been more elusive. Finally, in 1995, Nishihara and Koga found the responsible enzyme, NAD(P)- linked G-1-P dehydrogenase, in the methanogenic archaeon *Methanobacterium thermoautotrophicum*. The enzyme forms G-3-P from dihydroxyacetonephosphate (DHAP). The metabolic pathway and enzymes catalyzing

CO2 assimilation and conversion to DHAP have also been detected in *M. thermoautotrophicum*. The biosynthesis of the nonitol moiety appearing in some of the tetraethers found in *S.sulfataricus* indicates that the nonitol is formed first by aldolic condensation between dihydroxyacetone and fructose. This yields a 2-keto derivative that is reduced and then alkylated to add the isoprenoidic chains eventually leading to the tetraether. The biosynthesis of the tetraether polar lipids occurs by head-to-head condensation of diether polar lipids. Experiments using labeled phosphorous confirmed this hypothesis.

The cyclopentane rings originate via C-C coupling between the methyls at C17 and C18 and methylenes at C6 and C10. NMR data indicates that the rings are 1,3 trans substituted, but the mutual stereochemistry of the vicinal rings is not known. Archaebacteria have the capability to biohydrogenate the isoprenoids to produce more saturated lipid chains, which does not occur frequently in other biological systems. This occurs after the core structures have been formed, linked and cyclopentanes incorporated.



Synthesis of Peptidoglycan

Bacteria is composed of a rigid, tight-knit molecular complex called peptidoglycan. Peptidoglycan, also known as murein, is a vast polymer consisting of interlocking chains of identical peptidoglycan monomers. It functions to prevent bacterial osmotic lysis.

In order for bacteria to divide by binary fission and increase their size following division, links in the peptidoglycan must be broken, new peptidoglycan monomers must be inserted, and the peptide cross links must be resealed.

1. A group of bacterial enzymes called autolysins break the glycosidic bonds between the peptidoglycan monomers at the point of growth along the existing peptidoglycan. They also break the peptide cross-bridges that link the rows of sugars together. In this way, new peptidoglycan monomers can be inserted and enable bacterial growth.

2. Peptidoglycan monomers are synthesized in the cytosol of the bacterium where they attach to a membrane carrier molecule called bactoprenol. The bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and helps insert them into the growing peptidoglycan chains.

a. First, N-acetylglucosamine (NAG) links up with uridine diphosphate (UDP) to form UDP-NAG. Some of the NAG is enzymatically converted to N-acetylmuramic acid (NAM) forming UDP-NAM.

b. Five amino acids are sequentially added to the UDP- NAM forming a pentapeptide. The last two are D-alanine molecules enzymatically produced from L-alanine, the usual form of the amino acid.

c. The NAM-pentapeptide is attached to the bactoprenol carrier molecule in the cytoplasmic membrane, the energy being supplied by one of the high-energy phosphate groups of the UDP.

d. The NAG is attached to the NAM-pentapeptide on the bactoprenol to complete the peptidoglycan monomer.

Bactoprenols then insert the peptidoglycan monomers into the breaks in the peptidoglycan at the growing point of the cell wall.

3. Transglycosylase enzymes catalize the formation of glycosidic bonds between the NAM and NAG of the peptidoglycan momomers and the NAG and NAM of the existing peptidoglycan.

4. Finally, transpeptidase enzymes reform the peptide cross-links between the rows and layers of peptidoglycan to make the wall strong.

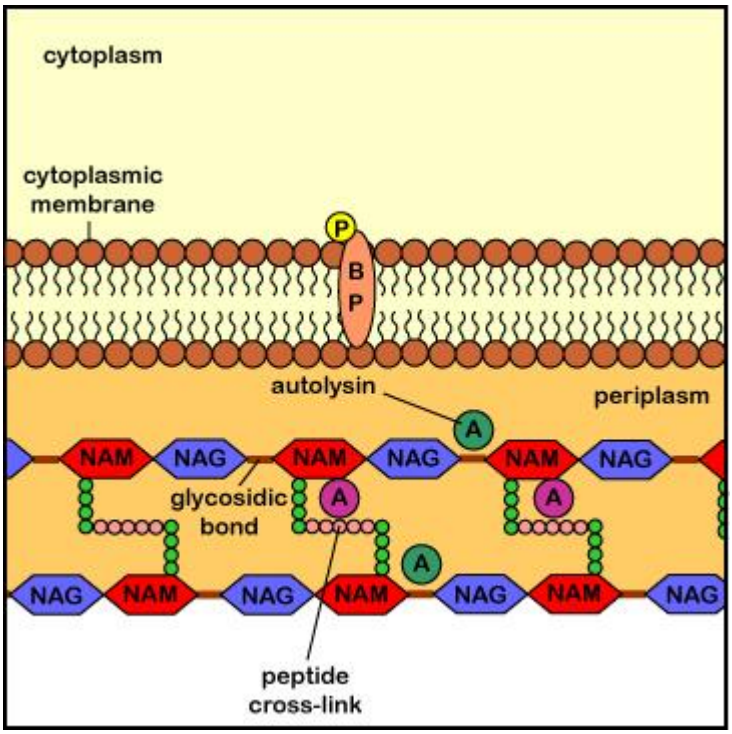
In order for bacteria to increase their size following binary fission, links in the peptidoglycan must be broken, new peptidoglycan monomers must be inserted, and the peptide cross links must be resealed.

The following sequence of events occur:

1. Bacterial enzymes called **autolysins:**

a) **Break the glycosidic bonds** between the peptidoglycan monomers at the point of growth along the existing peptidoglycan

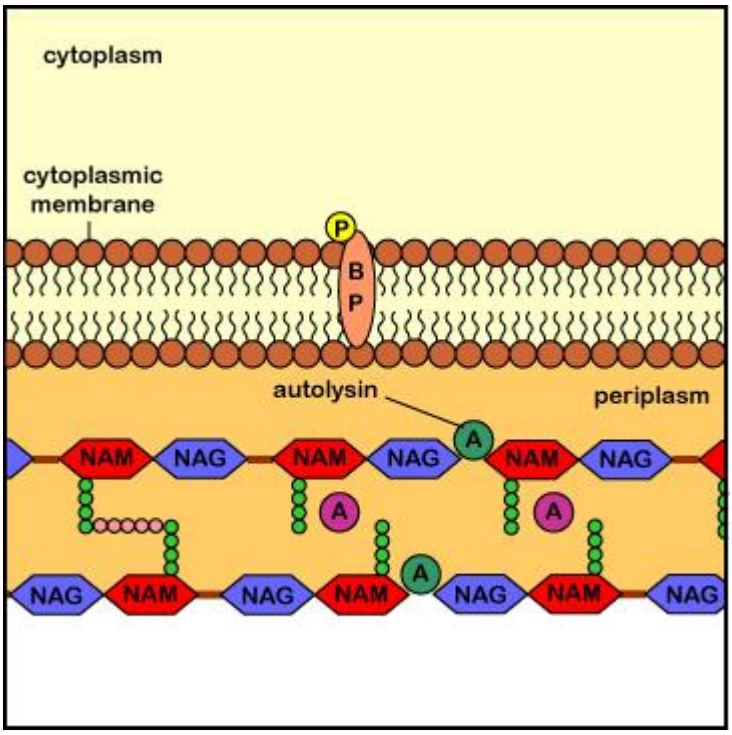
Fig. : Function of Autolysins in Peptidoglycan Synthesis, Step 1



A group of bacterial enzymes called autolysins break the glycosidic bonds between the peptidoglycan monomers at the point of growth along the existing peptidoglycan. They also break the peptide cross-bridges that link the rows of sugars

together. In this way, new peptidoglycan monomers can be inserted and enable bacterial growth.

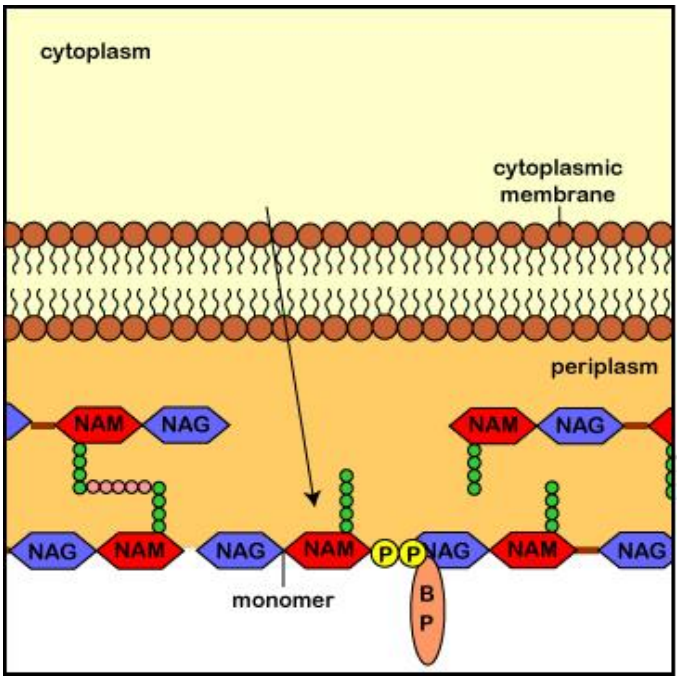
Fig.: Function of Autolysins in Peptidoglycan Synthesis, Step 2



b) **Break the peptide cross-bridges** that link the rows of sugars together

2. The **bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane** and **interacts with transglycosidases to insert the monomers into existing peptidoglycan**

Fig.: Synthesis of Peptidoglycan Monomers and Action of Bactoprenol, Step 3



Peptidoglycan monomers are synthesized in the cytosol of the bacterium where they attach to a membrane carrier molecule called bactoprenol. The bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and helps insert them into the growing peptidoglycan chains. After the bactoprenol inserts the peptidoglycan monomer it is transporting, it loses a phosphate group on its way back to the cytoplasmic membrane to be recycled and pick up another monomer.

Fig.: Synthesis of Peptidoglycan Monomers and Action of Bactoprenol, Step 4

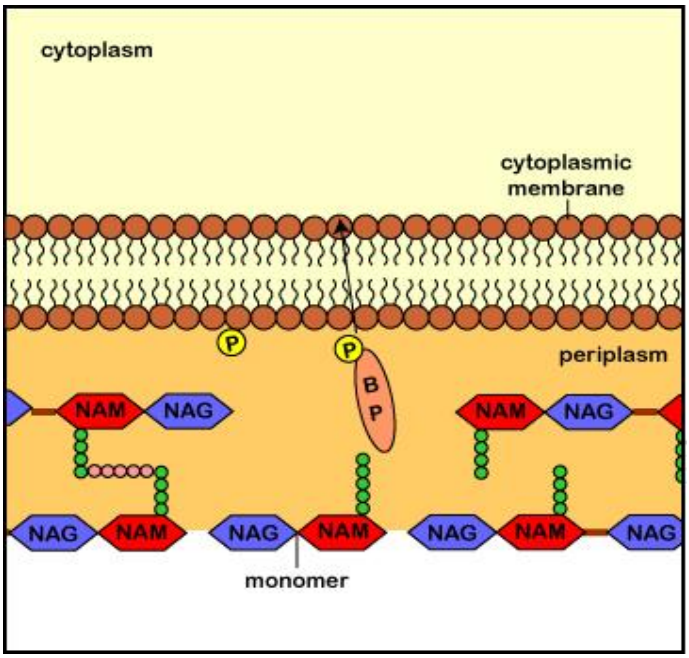
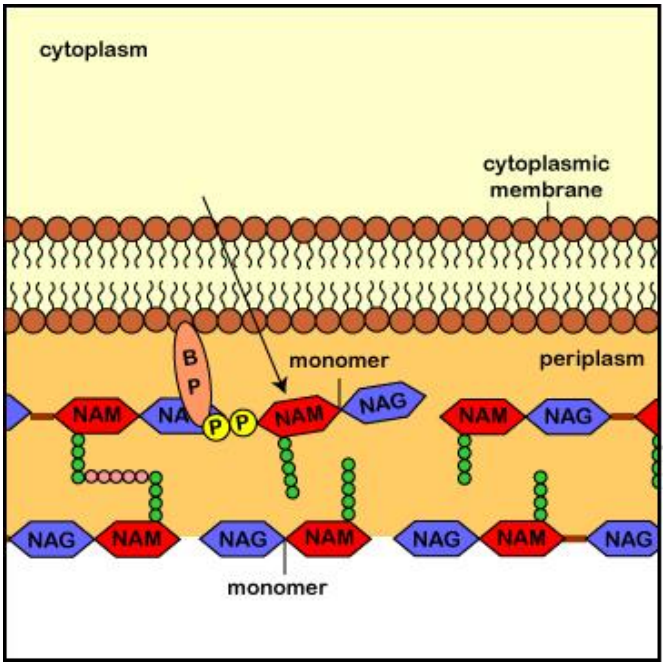
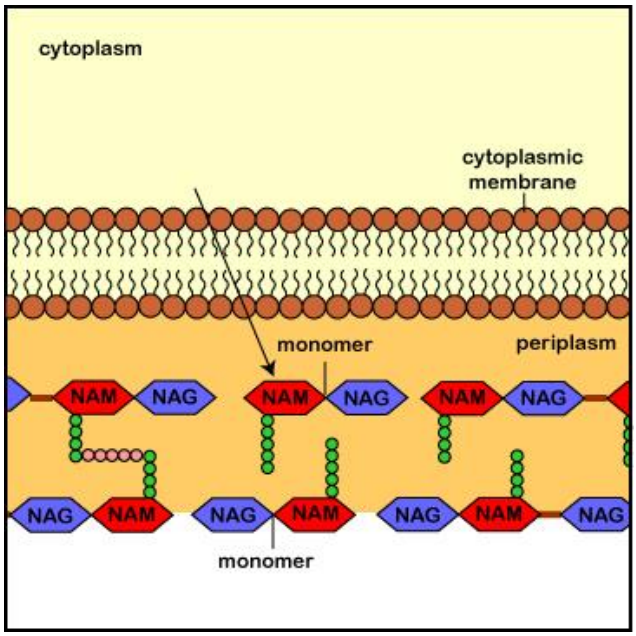


Fig.: Synthesis of Peptidoglycan Monomers and Action of Bactoprenol,



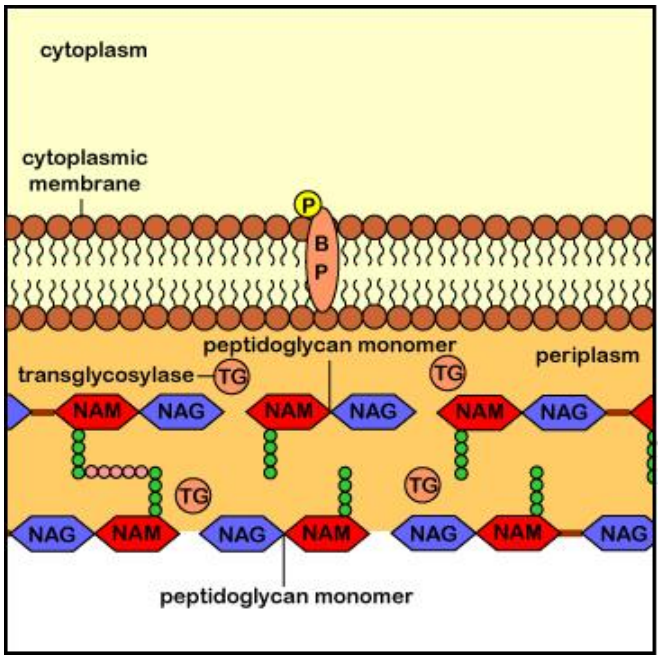
Step5

Fig.: Synthesis of Peptidoglycan Monomersand Action of Bactoprenol, Step 6



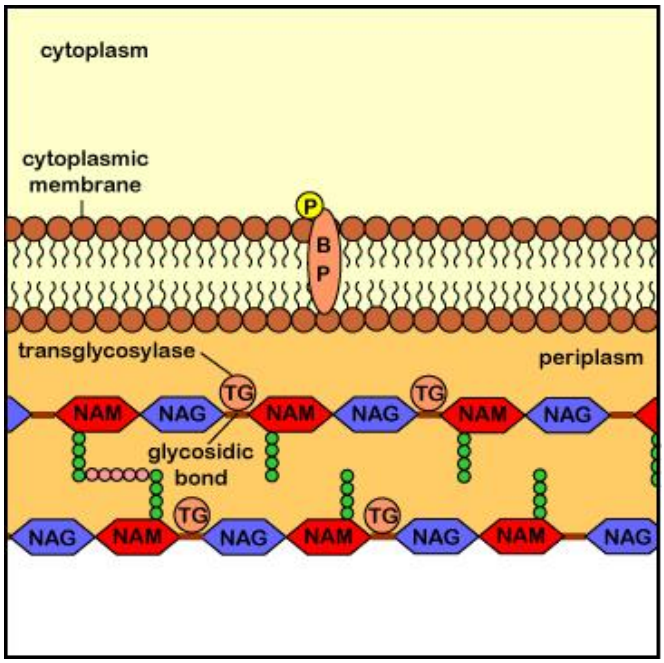
3. **Transglycosylase** (transglycosidase) **enzymes insert and link new peptidoglycan monomers into the breaks in the peptidoglycan**

Fig. : Action of Transglycosylase in Peptidoglycan Synthesis, Step 1



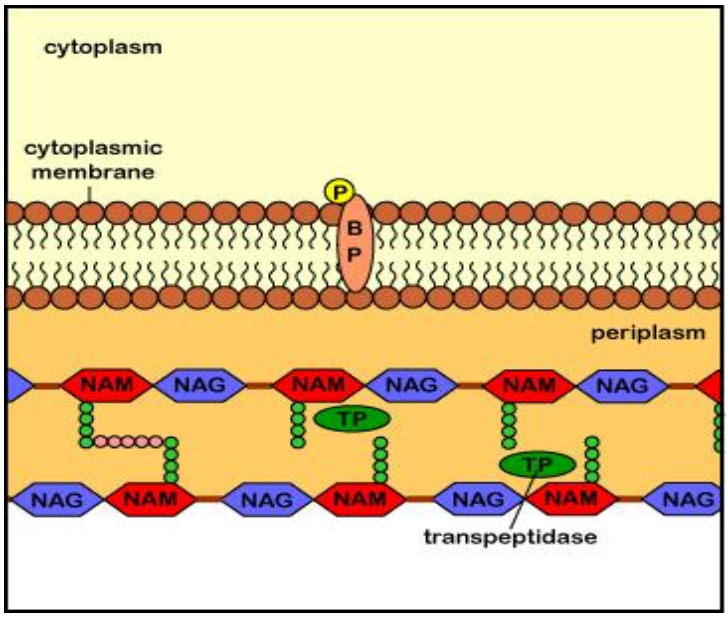
Transglycosylase enzymes catalize the formation of glycosidic bonds between the NAM and NAG of the peptidoglycan momomers and the NAG and NAM of the existing peptidoglycan.

Fig. : Action of Transglycosylase in Peptidoglycan Synthesis, Step 2



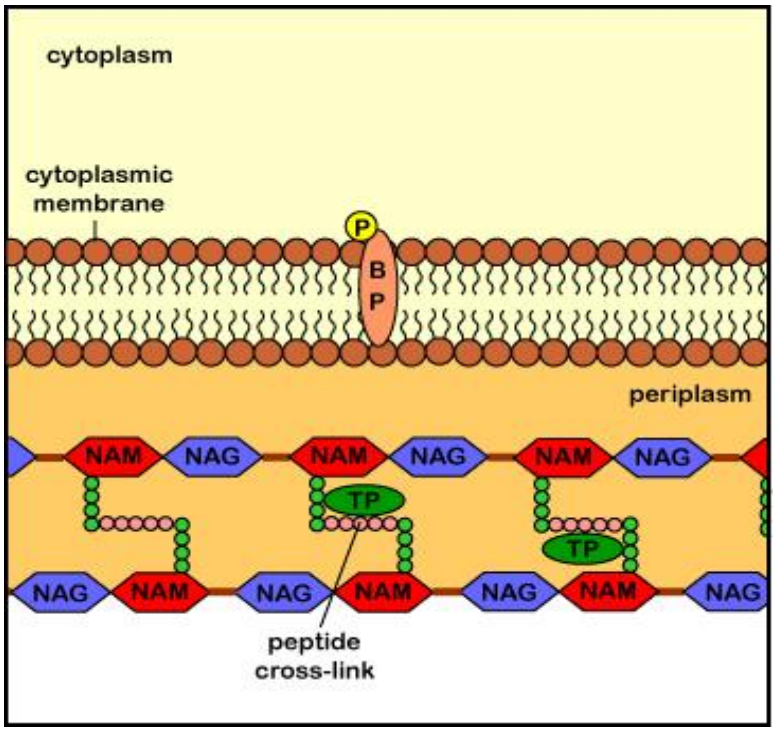
4. Finally, **transpeptidase enzymes reform the peptide cross-links** between the rows and layers of peptidoglycan to make the wall strong

Fig. 7: Action of Transpeptidase in Peptidoglycan Synthesis, Step 1



Finally, transpeptidase enzymes reform the peptide cross-links between the rows and layers of peptidoglycan to make the wall strong.

Fig.: Action of Transpeptidase in Peptidoglycan Synthesis, Step 2



Mechanisms of Bacterial Pathogenicity

Introduction

A **pathogen** is a microorganism that is able to cause disease in a plant, animal or insect. **Pathogenicity** is the ability to produce disease in a host organism. Microbes express their pathogenicity by means of their **virulence**, a term which refers to the degree of pathogenicity of the microbe. Hence, the **determinants of virulence** of a pathogen are any of its genetic or biochemical or structural features that enable it to produce disease in a host. The relationship between a host and a pathogen is dynamic, since each modifies the activities and functions of the other. The outcome of such a relationship depends on the virulence of the pathogen and the relative degree of resistance or susceptibility of the host, due mainly to the effectiveness of the host defense mechanisms.

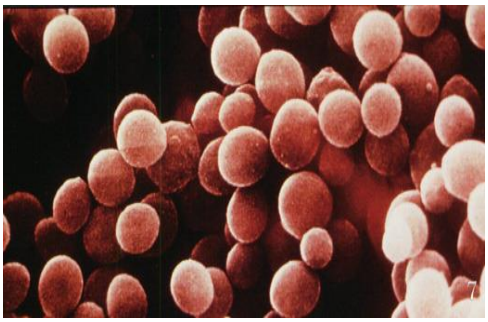
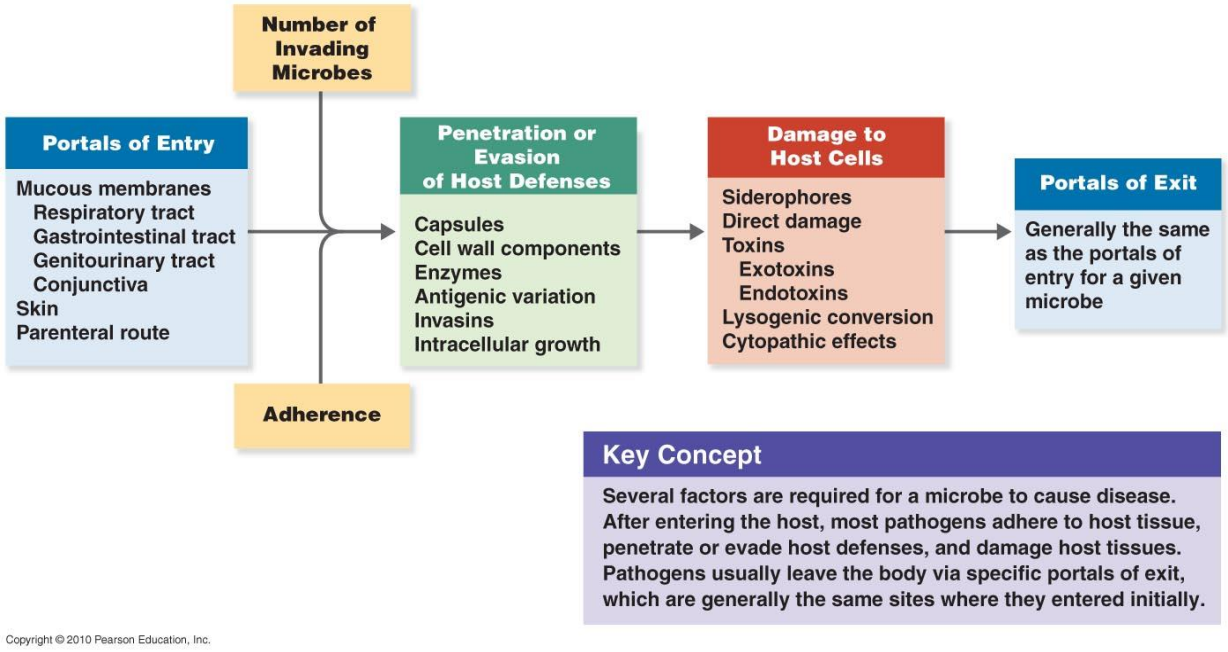


Fig: *Staphylococcus aureus*, arguably the most prevalent pathogen of humans, may cause up to one third of all bacterial diseases ranging from boils and pimples to food poisoning, to septicemia and toxic shock. Electron micrograph from Visuals Unlimited, with permission.



The Underlying Mechanisms of Bacterial Pathogenicity

Two broad qualities of pathogenic bacteria underlie the means by which they cause disease:

Invasiveness is the ability to invade tissues. It encompasses mechanisms for **colonization** (adherence and initial multiplication), **production of extracellular**

substances which facilitate invasion (**invasins**) and **ability to bypass or overcome host defense mechanisms**.

2. Toxigenesis is the ability to produce toxins. Bacteria may produce two types of toxins called **exotoxins** and **endotoxins**. **Exotoxins** are released from bacterial cells and may act at tissue sites removed from the site of bacterial growth. **Endotoxins** are cell-associated substance. (In a classic sense, the term **endotoxin** refers to the lipopolysaccharide component of the outer membrane of Gram-negative bacteria). However, endotoxins may be released from growing bacterial cells and cells that are lysed as a result of effective host defense (e.g. lysozyme) or the activities of certain antibiotics (e.g. penicillins and cephalosporins). Hence, bacterial toxins, both soluble and cell-associated, may be transported by blood and lymph and cause cytotoxic effects at tissue sites remote from the original point of invasion or growth. Some bacterial toxins may also act at the site of colonization and play a role in invasion.

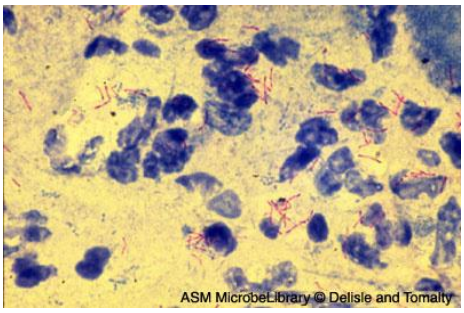


Fig: Acid-fast stain of *Mycobacterium tuberculosis,* the agent of tuberculosis (TB). The bacteria are the small pink-staining rods. More than one-third of the world population is infected. The organism has caused more human deaths than any other bacterium in the history of mankind. Although its ability to produce disease is multifactorial, it is not completely understood. American Society of Microbiology, with permission.

COLONIZATION

The first stage of microbial infection is **colonization**: the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment. Sites of entry in human hosts include the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva. Organisms that infect these regions have usually developed tissue adherence mechanisms and some ability to overcome or withstand the constant pressure of the host defenses at the surface.

Bacterial Adherence to Mucosal Surfaces. In its simplest form, bacterial adherence or attachment to a eucaryotic cell or tissue surface requires the participation of two factors: a **receptor** and an **ligand**. The receptors so far defined are usually

specific carbohydrate or peptide residues on the eucaryotic cell surface. The bacterial ligand, called an **adhesin**, is typically a macromolecular component of the bacterial cell surface which interacts with the host cell receptor. Adhesins and receptors usually interact in a complementary and specific fashion. Table 1 is a list of terms that are used in medical microbiology to refer to microbial adherence to surfaces or tissues.

TABLE TERMS USED TO DESCRIBE ADHERENCE FACTORS IN HOST-PARASITE INTERACTIONS

ADHERENCE FACTOR	DESCRIPTION
Adhesin	A surface structure or macromolecule that binds a bacterium to a specific surface
Receptor	A complementary macromolecular binding site on a (eucaryotic) surface that binds specific adhesins or ligands
Lectin	Any protein that binds to a carbohydrate
Ligand	A surface molecule that exhibits specific binding to a receptor molecule on another surface
Mucous	The mucopolysaccharide layer of glucosaminoglycans covering animal cell mucosal surfaces
Fimbriae	Filamentous proteins on the surface of bacterial cells that may behave as adhesins for specific adherence
Common pili	Same as fimbriae
Sex pilus	A specialized pilus that binds mating procaryotes together for the purpose of DNA transfer
Type 1 fimbriae	Fimbriae in *Enterobacteriaceae* which bind

	specifically to mannose terminated glycoproteins on eucaryotic cell surfaces
Type 4 pili	Pili in certain Gram-positive and Gram-negative bacteria. In *Pseudomonas*, thought to play a role in adherence and biofilm formation
S-layer	Proteins that form the outermost cell envelope component of a broad spectrum of bacteria, enabling them to adhere to host cell membranes and environmental surfaces in order to colonize.
Glycocalyx	A layer of exopolysaccharide fibers on the surface of bacterial cells which may be involved in adherence to a surface. Sometimes a general term for a capsule.
Capsule	A detectable layer of polysaccharide (rarely polypeptide) on the surface of a bacterial cell which may mediate specific or nonspecific attachment
Lipopolysaccharide (LPS)	A distinct cell wall component of the outer membrane of Gram-negative bacteria with the potential structural diversity to mediate specific adherence. Probably functions as an adhesin
Teichoic acids and lipoteichoic acids (LTA)	Cell wall components of Gram-positive bacteria that may be involved in nonspecific or specific adherence

Specific Adherence of Bacteria to Cell and Tissue Surfaces

Several types of observations provide indirect evidence for **specificity of adherence** of bacteria to host cells or tissues:

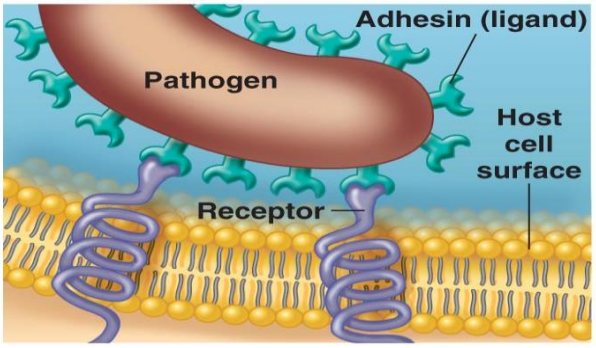
1. **Tissue tropism**: particular bacteria are known to have an apparent preference for certain tissues over others, e.g. *S. mutans* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue; the reverse is true for *S. salivarius* which is attached in high numbers to epithelial cells of the tongue but is absent in dental plaque.

2. **Species specificity**: certain pathogenic bacteria infect only certain species of animals, e.g. *N. gonorrhoeae* infections are limited to humans; Enteropathogenic *E. coli* K-88 infections are limited to pigs; *E. coli* CFA I and CFA II infect humans; *E. coli* K-99 strain infects calves.; Group A streptococcal infections occur only in humans.

3. **Genetic specificity within a species**: certain strains or races within a species are genetically immune to a pathogen , e.g. Certain pigs are not susceptible to *E. coli* K-88 infections; Susceptibility to *Plasmodium vivax* infection (malaria) is dependent on the presence of the Duffy antigens on the host's redblood cells.

Although other explanations are possible, the above observations might be explained by the existence of specific interactions between microorganisms and eucaryotic tissue surfaces which allow microorganisms to become established on the surface.

Mechanisms of Adherence to Cell or Tissue Surfaces



(a) Surface molecules on a pathogen, called adhesins or ligands, bind specifically to complementary surface receptors on cells of certain host tissues.

The mechanisms for adherence may involve two steps:

1. **nonspecific adherence: reversible attachment** of the bacterium to the eucaryotic surface (sometimes called "docking")

2. **specific adherence: reversible permanent attachment** of the microorganism to the surface (sometimes called "anchoring").

The usual situation is that reversible attachment precedes irreversible attachment but in some cases, the opposite situation occurs or specific adherence may never occur.

Nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eucaryotic cell surface. Possible interactions and forces involved are:

1. hydrophobic interactions
2. electrostatic attractions
3. atomic and molecular vibrations resulting from fluctuating dipoles of similar frequencies
4. Brownian movement
5. recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx (capsule)

Specific adherence involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface. Complementary receptor and adhesin molecules must be accessible and arranged in such a way that many bonds form over the area of contact between the two cells. Once the bonds are formed, attachment under physiological conditions becomes virtually irreversible.

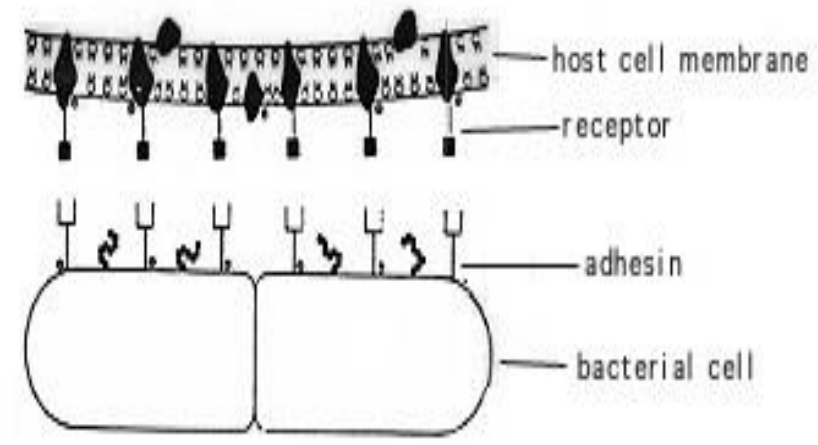


Fig: Specific adherence involves complementary chemical interactions between the host cell or tissue surface and the bacterial surface. In the language of medical microbiologist, a bacterial "adhesin" attaches covalently to a host "receptor" so that the

bacterium "docks" itself on the host surface. The adhesins of bacterial cells are chemical components of capsules, cell walls, pili or fimbriae. The host receptors are usually glycoproteins located on the cell membrane or tissue surface.

Several types of experiments provide direct evidence that receptor and/or adhesin molecules mediate specificity of adherence of bacteria to host cells or tissues. These include:

1. The bacteria will bind isolated receptors or receptor analogs.

2. The isolated adhesins or adhesin analogs will bind to the eucaryotic cell surface.

3. Adhesion (of the bacterium to the eucaryotic cell surface) is inhibited by:

a. isolated adhesin or receptor molecules

b. adhesin or receptor analogs

c. enzymes and chemicals that specifically destroy adhesins or receptors

d. antibodies specific to surface components (i.e., adhesins or receptors)

Some Specific Bacterial Adhesins and their Receptors

The adhesins of ***E. coli*** are their common pili or fimbriae. A single strain of *E. coli* is known to be able to express several distinct types of fimbriae encoded by distinct regions of the chromosome or plasmids. This genetic diversity permits an organism to adapt to its changing environment and exploit new opportunities presented by different host surfaces. Many of the adhesive fimbriae of *E. coli* have probably evolved from fimbrial ancestors resembling Type-I and Type IV fimbriae.

Type-I fimbriae enable *E. coli* to bind to D-mannose residues on eucaryotic cell surfaces. Type-I fimbriae are said to be "mannose-sensitive" since exogenous mannose blocks binding to receptors on red blood cells. Although the primary 17kDa fimbrial subunit is the major protein component of Type-1 fimbriae, the mannose-binding site is not located here, but resides in a minor protein (28-31kDa) located at the tips or inserted along the length of the fimbriae. By genetically varying the minor "tip protein" adhesin,

the organisms can gain ability to adhere to different receptors. For example, tip proteins on pyelonephritis-associated (pap) pili recognize a galactose-galactose disaccharide, while tip proteins on S-fimbriae recognize sialic acid.

Pseudomonas, Vibrio and ***Neisseria*** possess Type IV pili that contain protein subunit with a methylated amino acid, often phenylalanine, at or near its amino terminus. These "N-methylphenylalanine pili" have been established as virulence determinants in pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. These type of fimbriae occur in *Neisseria gonorrhoeae* and their receptor is thought to be an oligosaccharide. Type IV pili are the tcp (toxin coregulated pili) fimbriae used in attachment of Vibrio cholerae to the gastrointestinal epithelium.

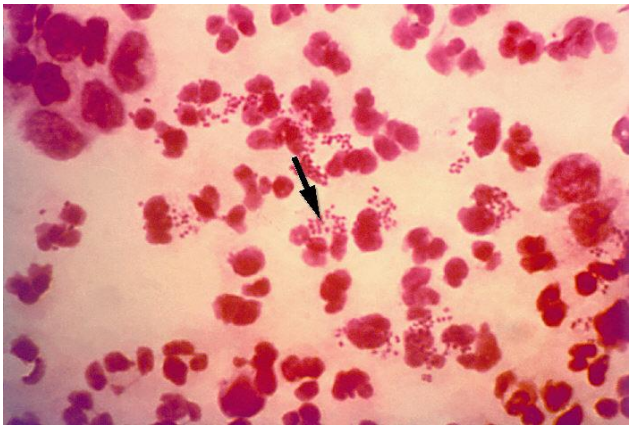


Fig: Gram stain of *Neisseria gonorrhoeae,* the agent of the STD gonorrhea. The bacteria are seen as pairs of cocci (diplococci) in association with host pmn's (polymorphonuclear leukocytes). Gonorrhea is the second most prevalent STD in the U.S. behind chlamydia. The bacterium has multiple determinants of virulence including the ability to attach to and enter host cells, resist phagocytic killing and produce endotoxins which eventually lead to an intense inflammatory response.

The adhesins of ***Streptococcus pyogenes*** are controversial. In 1972, Gibbons and his colleagues demonstrated that attachment of streptococci to the oral mucosa of mice is dependent on M protein. Olfek and Beachey argued that lipoteichoic acid (LTA), rather than M protein, was responsible for streptococcal adherence to buccal epithelial cells. In 1996, Hasty and Courtney proposed a two-step model of attachment that involved both M protein and teichoic acids. They suggested that LTA loosely tethers streptococci to epithelial cells, and then M protein secures a firmer, irreversible association. In 1992, protein F was discovered and found to be a fibronectin binding protein. More recently, in 1998, M proteins M1 and M3 were also found to bind to fibronectin. Apparently, *S. pyogenes* produces multiple adhesins with varied specificities.

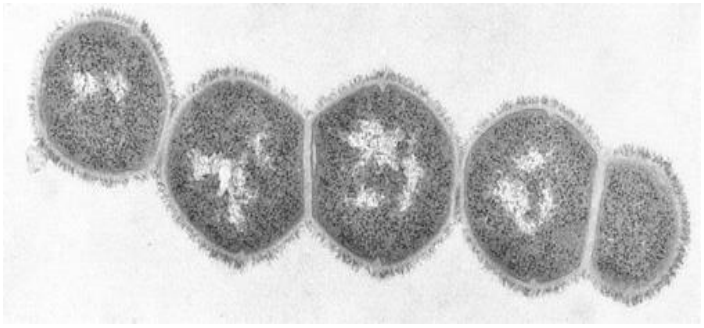


Fig: Electron micrograph of *Streptococcus pyogenes*. The cell surface fibrils, that consist primarily of M protein, are clearly evident. The M protein has several possible roles in virulence: it is involved in adherence, resistance to phagocytosis, and in antigenic variation of the pathogen.

Staphylococcus aureus also binds to the amino terminus of fibronectin by means of a fibronectin-binding protein which occurs on the bacterial surface. Apparently *S. aureus* and Group A streptococci use different mechanisms but adhere to the same receptor on epithelial surfaces.

Treponema pallidum has three related surface adhesins (P1, P2 and P3) which bind to a four-amino acid sequence (Arg-Gly-Asp-Ser) of the cell-binding domain of fibronectin. It is not clear if *T. pallidum* uses fibronectin to attach to host surfaces or coats itself with fibronectin to avoid host defenses (phagocytes and immune responses).

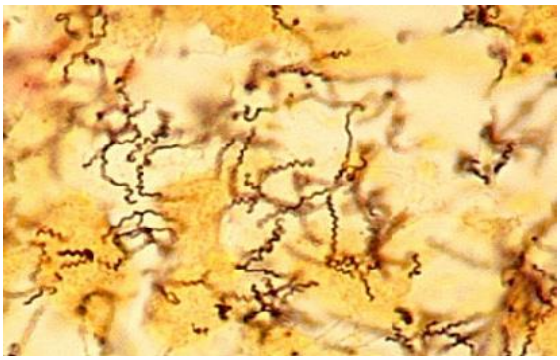


Fig: *Treponema pallidum*, the spirochete that causes syphilis. Silver stain.

TABLE 2. EXAMPLES OF SPECIFIC ATTACHMENTS OF BACTERIA TO HOST CELL OR TISSUE SURFACES

Bacterium	Adhesin	Receptor	Attachment site	Disease
Streptoc	Protein	Amino	Phar	Sore

occus pyogenes	F	terminus of fibronectin	yngeal epithelium	throat
Streptoccus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptoccus salivarius	Lipoteichoic acid	Unknown	Buccal epithelium of tongue	None
Streptoccus pneumoniae	Cell-bound protein	N-acetylhexos-amine-galactose disaccharide	Mucosal epithelium	pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	Type IV pili (N-methylphenyl-alanine pili)	Glucosamine-galactose carbohydrate	Urethral/ cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-I fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea
Uropathogenic *E. coli*	Type I fimbriae	Complex carbohydrate	Urethral epithelium	Urethritis

Uropathogenic *E. coli*	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenyl-alanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera
Treponema pallidum	Peptide in outer membrane	Surface protein (fibronectin)	Mucosal epithelium	Syphilis
Mycoplasma	Membrane protein	Sialic acid	Respiratory epithelium	Pneumonia
Chlamydia	Unknown	Sialic acid	Conjunctival or urethral epithelium	Conjunctivitis or urethritis

INVASION

The invasion of a host by a pathogen may be aided by the production of bacterial extracellular substances which act against the host by breaking down primary or secondary defenses of the body. Medical microbiologists have long referred to these substances as **invasins**. Most invasins are proteins (enzymes) that act locally to damage host cells and/or have the immediate effect of facilitating the growth and spread of the

pathogen. The damage to the host as a result of this invasive activity may become part of the pathology of an infectious disease.

The extracellular proteins produced by bacteria which promote their invasion are not clearly distinguished from some extracellular protein toxins ("exotoxins") which also damage the host. Invasins usually act at a short range (in the immediate vicinity of bacterial growth) and may not actually kill cells as part of their range of activity; exotoxins are often cytotoxic and may act at remote sites (removed from the site of bacterial growth). Also, exotoxins typically are more specific and more potent in their activity than invasins. Even so, some classic exotoxins (e.g. diphtheria toxin, anthrax toxin) may play some role in colonization or invasion in the early stages of an infection, and some invasins (e.g. staphylococcal leukocidin) have a relatively specific cytopathic effect.

A Survey of Bacterial Invasins

Spreading Factors

"Spreading Factors" is a descriptive term for a family of bacterial enzymes that affect the physical properties of tissue matrices and intercellular spaces, thereby promoting the spread of the pathogen.

Hyaluronidase. is the original spreading factor. It is produced by streptococci. staphylococci, and clostridia. The enzyme attacks the interstitial cement ("ground substance") of connective tissue by depolymerizing hyaluronic acid.

Collagenase is produced by *Clostridium histolyticum* and *Clostridium perfringens*. It breaks down collagen, the framework of muscles, which facilitates gas gangrene due to these organisms.

Neuraminidase is produced by intestinal pathogens such as *Vibrio cholerae* and *Shigella dysenteriae*. It degrades neuraminic acid (also called sialic acid), an intercellular cement of the epithelial cells of the intestinal mucosa.

Streptokinase and **staphylokinase** are produced by streptococci and staphylococci, respectively. Kinase enzymes convert inactive plasminogen to plasmin which digests fibrin and prevents clotting of the blood. The relative absence of fibrin in spreading bacterial lesions allows more rapid diffusion of the infectious bacteria.

Enzymes that Cause Hemolysis and/or Leucolysis

These enzymes usually act on the animal cell membrane by insertion into the membrane (forming a pore that results in cell lysis), or by enzymatic attack on phospholipids, which destabilizes the membrane. They may be referred to as **lecithinases** or **phospholipases**, and if they lyse red blood cells they are sometimes called **hemolysins**. **Leukocidins**, produced by staphylococci and **streptolysin** produced by **streptococci** specifically lyse phagocytes and their granules. These latter two enzymes are also considered to be bacterial exotoxins.

Phospholipases, produced by *Clostridium perfringens* (i.e., alpha toxin), hydrolyze phospholipids in cell membranes by removal of polar head groups.

Lecithinases, also produced by *Clostridium perfringens,* destroy lecithin (phosphatidylcholine) in cell membranes.

Hemolysins, notably produced by staphylococci (i.e., alpha toxin), streptococci (i.e., streptolysin) and various clostridia, may be channel-forming proteins or phospholipases or lecithinases that destroy red blood cells and other cells (i.e., phagocytes) by lysis.

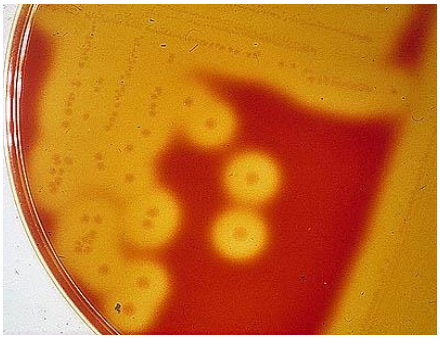


Fig: Beta-hemolytic *Streptococcus*. This is the characteristic appearance of a blood agar plate culture of the bacterium. Note the translucency around the bacterial colonies, representing hemolysis of the red cells in the culture medium due to production of a diffusible hemolysin (streptolysin).

Staphylococcal coagulase

Coagulase, formed by *Staphylococcus aureus*, is a cell-associated and diffusible enzyme that converts fibrinogen to fibrin which causes clotting. Coagulase activity is almost always associated with pathogenic *S. aureus* and almost never associated with nonpathogenic *S. epidermidis*, which has led to much speculation as to its role as a determinant of virulence. Possibly, cell bound coagulase could provide an antigenic

disguise if it clotted fibrin on the cell surface. Or a staphylococcal lesion encased in fibrin (e.g. a boil or pimple) could make the bacterial cells resistant to phagocytes or tissue bactericides or even drugs which might be unable to diffuse to their bacterial target.

Extracellular Digestive Enzymes

Heterotrophic bacteria, in general, produce a wide variety of extracellular enzymes including **proteases**, **lipases**, **glycohydrolases**, **nucleases**, etc., which are not clearly shown to have a direct role in invasion or pathogenesis. These enzymes presumably have other functions related to bacterial nutrition or metabolism, but may aid in invasion either directly or indirectly.

Toxins With Short-Range Effects Related to Invasion

Bacterial protein toxins which have adenylate cyclase activity, are thought to have immediate effects on host cells that promote bacterial invasion. One component of the anthrax toxin (**EF** or **Edema Factor**) is an **adenylate cyclase** that acts on nearby cells to cause increased levels of cyclic AMP and disruption of cell permeability. One of the toxins of *Bordetella pertussis*, the agent of whooping cough, has a similar effect. These toxins may contribute to invasion through their effects on macrophages or lymphocytes in the vicinity which are playing an essential role to contain the infection. For example, since they use ATP as a substrate, they may deplete phagocyte reserves of energy needed for ingestion. Edema is seen as a pathology because the increase in cAMP in affected cells disrupts equilibrium.

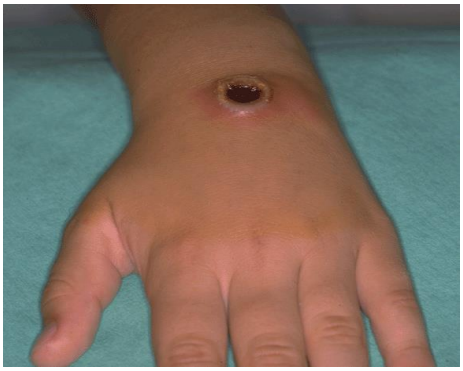


Fig: Gelatinous edema seen in a cutaneous anthrax lesion.

The following table summarizes the activities of many bacterial proteins that are noted for their contribution to bacterial invasion of tissues.

TABLE 3. SOME EXTRACELLULAR BACTERIAL PROTEINS THAT ARE CONSIDERED INVASINS

Invasin	Bacteria Involved	Activity
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic of connective tissue
Collagenase	*Clostridium* species	Dissolves collagen framework of muscles
Neuraminidase	*Vibrio cholerae* and *Shigella dysenteriae*	Degrades neuraminic acid of intestinal mucosa
Coagulase	*Staphylococcus aureus*	Converts fibrinogen to fibrin which causes clotting
Kinases	Staphylococci and streptococci	Converts plasminogen to plasmin which digests fibrin
Leukocidin	*Staphylococcus aureus*	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Streptolysin	*Streptococcus pyogenes*	Repels phagocytes and disrupts phagocyte membrane and causes discharge of lysosomal granules
Hemolysins	Streptococci, staphylococci and clostridia	Phospholipases or lecithinases that destroy red blood cells (and other cells) by lysis

Lecithinases	*Clostridium perfringens*	Destroy lecithin in cell membranes
Phospholipases	*Clostridium perfringens*	Destroy phospholipids in cell membrane
Anthrax EF	*Bacillus anthracis*	One component (EF) is an adenylate cyclase which causes increased levels of intracellular cyclic AMP
Pertussis AC	*Bordetella pertussis*	One toxin component is an adenylate cyclase that acts locally producing an increase in intracellular cyclic AMP

EVASION OF HOST DEFENSES

Some pathogenic bacteria are inherently able to resist the bactericidal components of host tissues. For example, the poly-D-glutamate capsule of *Bacillus anthracis* protects the organisms against cell lysis by cationic proteins in sera or in phagocytes. The outer membrane of Gram-negative bacteria is a formidable permeability barrier that is not easily penetrated by hydrophobic compounds such as bile salts which are harmful to the bacteria. Pathogenic mycobacteria have a waxy cell wall that resists attack or digestion by most tissue bactericides. And intact lipopolysaccharides (LPS) of Gram-negative pathogens may protect the cells from complement-mediated lysis or the action of lysozyme.

Most successful pathogens, however, possess additional structural or biochemical features which allow them to resist the main lines of host internal defense against them, i.e., the phagocytic and immune responses of the host.

Overcoming Host Phagocytic Defenses

Microorganisms invading tissues are first and foremost exposed to phagocytes. Bacteria that readily attract phagocytes, and that are easily ingested and killed, are generally unsuccessful as parasites. In contrast, most bacteria that are successful as parasites interfere to some extent with the activities of phagocytes or in some way avoid their attention.

Microbial strategies to avoid phagocytic killing are numerous and diverse, but are usually aimed at blocking one or of more steps in the phagocytic process. Recall the steps in phagocytosis:

1. Contact between phagocyte and microbial cell

2. Engulfment

3. Phagosome formation

4. Phagosome-lysosome fusion

5. Killing and digestion

Avoiding Contact with Phagocytes

Bacteria can avoid the attention of phagocytes in a number of ways.

1. Invade or remain confined in regions inaccessible to phagocytes. Certain internal tissues (e.g. the lumen of glands) and surface tissues (e.g. the skin) are not patrolled by phagocytes.

2. Avoid provoking an overwhelming inflammatory response. Some pathogens induce minimal or no inflammation required to focus the phagocytic defenses.

3. Inhibit phagocyte chemotaxis. e.g. Streptococcal streptolysin (which also kills phagocytes) suppresses neutrophil chemotaxis, even in very low concentrations. Fractions of *Mycobacterium tuberculosis* are known to inhibit leukocyte migration. *Clostridium ø* toxin inhibits neutrophil chemotaxis.

4. Hide the antigenic surface of the bacterial cell. Some pathogens can cover the surface of the bacterial cell with a component which is seen as "self" by the host phagocytes and immune system. Phagocytes cannot recognize bacteria upon contact and the possibility of opsonization by antibodies to enhance phagocytosis is minimized. For example, pathogenic *Staphylococcus aureus* produces cell-bound coagulase which clots fibrin on the bacterial surface. *Treponema pallidum* binds fibronectin to its surface. Group A streptococci are able to synthesize a capsule composed of hyaluronic acid.

Inhibition of Phagocytic Engulfment

Some bacteria employ strategies to **avoid engulfment (ingestion)** if phagocytes do make contact with them. Many important pathogenic bacteria bear on their surfaces substances that inhibit phagocytic adsorption or engulfment. Clearly it is the bacterial surface that matters. Resistance to phagocytic ingestion is usually due to a component of the bacterial cell wall, or fimbriae, or a capsule enclosing the bacterial wall. Classical examples of antiphagocytic substances on the bacterial surface include:

Polysaccharide capsules of *S. pneumoniae*, *Haemophilus influenzae*, *Treponema pallidum* and *Klebsiella pneumoniae*

M protein and fimbriae of Group A streptococci

Surface slime (polysaccharide) produced by *Pseudomonas aeruginosa*

O antigen associated with LPS of *E. coli*

K antigen of *E. coli* or the analogous Vi antigen of *Salmonella typhi*

Cell-bound or soluble Protein A produced by *Staphylococcus aureus*

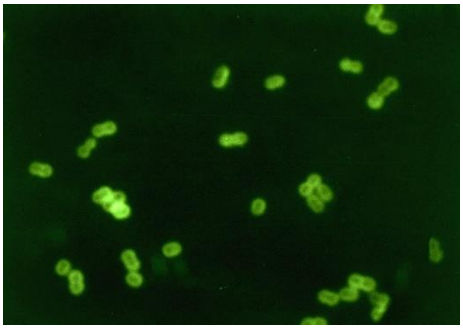


Fig: *Streptococcus pneumoniae*, FA stain showing its antphagocytic capsule (CDC). *S. pneumoniae* cells that possess a capsule are virulent; nonencapsulated strains are avirulent. Although *S. pneumoniae* strains possess a variety of determinants of virulence, this illustrates the essential role of their capsule in ability to resist phagocytosis by alveolar macrophages in order to initiate disease.

Survival Inside of Phagocytes

Some bacteria survive inside of phagocytic cells, in either neutrophils or macrophages. Bacteria that can resist killing and survive or multiply inside of phagocytes are considered intracellular parasites. The environment of the phagocyte may be a protective one, protecting the bacteria during the early stages of infection or until they develop a full complement of virulence factors. The intracellular environment

guards the bacteria against the activities of extracellular bactericides, antibodies, drugs, etc.

Most intracellular parasites have special (genetically-encoded) mechanisms to get themselves into their host cell as well as special mechanisms to survive once they are inside. Intracellular parasites usually survive by virtue of mechanisms which interfere with the bactericidal activities of the host cell. Some of these bacterial mechanisms include:

1. **Inhibition of phagosome-lysosome fusion**. The bacteria survive inside of phagosomes because they prevent the discharge of lysosomal contents into the phagosome environment. Specifically phagolysosome formation is inhibited in the phagocyte. This is the strategy employed by *Salmonella*, *M. tuberculosis*, *Legionella* and the *Chlamydiae*.

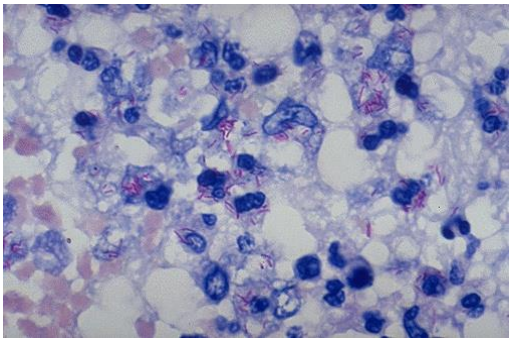


Fig: **Intracellular *Mycobacterium tuberculosis* in lung. Ziehl-Neelsen acid fast stain**

2. **Survival inside the phagolysosome**. With some intracellular parasites, phagosome-lysosome fusion occurs but the bacteria are resistant to inhibition and killing by the lysosomal constituents. Also, some extracellular pathogens can resist killing in phagocytes utilizing similar resistance mechanisms. Little is known of how bacteria can resist phagocytic killing within the phagocytic vacuole, but it may be due to the surface components of the bacteria or due to extracellular substances that they produce which interfere with the mechanisms of phagocytic killing. *Bacillus anthracis*, *Mycobacterium tuberculosis* and *Staphylococcus aureus* all possess mechanisms to survive intracellular killing in macrophages.

3. **Escape from the phagosome**. Early escape from the phagosome vacuole is essential for growth and virulence of some intracellular pathogens. This is a very clever strategy employed by the Rickettsias which produce a phospholipase enzyme that lyses the phagosome membrane within thirty seconds of after ingestion.

Products of Bacteria that Kill or Damage Phagocytes

One obvious strategy in defense against phagocytosis is direct attack by the bacteria upon the professional phagocytes. Any of the substances that pathogens produce that cause damage to phagocytes have been referred to as "aggressins". Most of these are actually extracellular enzymes or toxins that kill phagocytes. Phagocytes may be killed by a pathogen before or after ingestion.

Killing phagocytes before ingestion. Many Gram-positive pathogens, particularly the pyogenic cocci, secrete extracellular enzymes which kill phagocytes. Many of these enzymes are called "hemolysins" because their activity in the presence of red blood cells results in the lysis of the rbcs.

Pathogenic streptococci produce streptolysin. Streptolysin O binds to cholesterol in membranes. The effect on neutrophils is to cause lysosomal granules to explode, releasing their contents into the cell cytoplasm.

Pathogenic staphylococci produce leukocidin, which also acts on the neutrophil membrane and causes discharge of lysosomal granules.

Other examples of bacterial extracellular proteins that inhibit phagocytosis include the Exotoxin A of *Pseudomonas aeruginosa* which kills macrophages, and the bacterial exotoxins that are adenylate cyclases (e.g. anthrax toxin EF and pertussis AC) which decrease phagocytic activity.

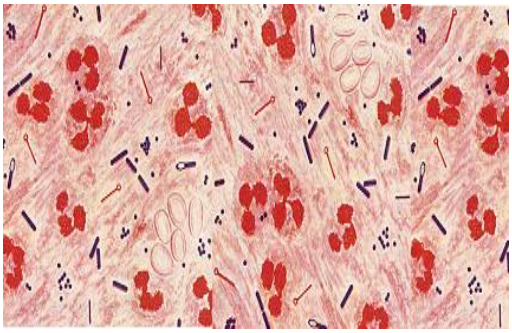


Fig: Gram stain of a pustular exudate from a mixed bacterial infection. Pus is the usual outcome of the battle between phagocytes and bacterial strategies to kill them.

Killing phagocytes after ingestion. Some bacteria exert their toxic action on the phagocyte after ingestion has taken place. They may grow in the phagosome and release substances which can pass through the phagosome membrane and cause discharge of lysosomal granules, or they may grow in the phagolysosome and release toxic substances which pass through the phagolysosome membrane to other target sites in the cell. Many bacteria which are the intracellular parasites of macrophages (e.g. Mycobacteria, *Brucella*, *Listeria*) usually destroy macrophages in the end, but the mechanisms are not understood.

Evading Complement

Antibodies that are bound to bacterial surfaces will activate complement by the classical pathway and bacterial polysaccharides activate complement by the alternative pathway. Bacteria in serum and other tissues, especially Gram-negative bacteria, need protection from the antimicrobial effects of complement before and during an immunological response.

One role of **capsules** in bacterial virulence is to protect the bacteria from complement activation and the ensuing inflammatory response. Polysaccharide capsules can hide bacterial components such as LPS or peptidoglycan which can induce the alternate complement pathway. Some bacterial capsules are able to inhibit formation of the C3b complex on their surfaces, thus avoiding C3b opsonization and subsequent formation of C5b and the membrane attack complex (MAC) on the bacterial cell surface. Capsules that contain sialic acid (a common component of host cell glycoproteins), such as found in *Neisseria meningitidis*, have this effect.

One of the principal targets of complement on Gram-negative bacteria is LPS. It serves as the attachment site for C3b and triggers the alternative pathway of activation. It also binds C5b.

LPS can be modified by pathogens in two ways that affects its interaction with complement. First, by attachment of sialic acid residues to the LPS O antigen, a bacterium can prevent the formation of C3 convertase just as capsules that contain sialic acid can do so. Both *Neisseria meningitidis* and *Haemophilus influenzae*, which cause bacterial meningitis, are able to covalently attach sialic acid residues to their O antigens resulting in resistance to MAC. Second, LPS with long, intact O antigen side-chains can prevent effective MAC killing. Apparently the MAC complex is held too far from the vulnerable outer membrane to be effective.

Bacteria that are not killed and lysed in serum by the complement MAC are said to be **serum resistant**. As might be expected many of the Gram-negative bacteria that cause systemic infections, (bacteremia or septicemia) are serum resistant. Gram-positive bacteria are naturally serum-resistant since their cells are not enclosed in an outer membrane.

Other ways that pathogens are able to inhibit the activity of complement is to destroy one or more of the components of complement. *Pseudomonas aeruginosa* produces an extracellular **elastase** enzyme that inactivates components of complement.

Avoiding Host Immunological Responses

On epithelial surfaces the main antibacterial immune defense of the host is the protection afforded by secretory antibody (IgA). Once the epithelial surfaces have been penetrated, however, the major host defenses of inflammation, complement, phagocytosis, Antibody-mediated Immunity (AMI), and Cell-mediated Immunity (CMI) are encountered. If there is a way for a pathogen to successfully bypass or overcome these host defenses, then some bacterial pathogen has probably discovered it. Bacteria evolve very rapidly in relation to their host, so that most of the feasible anti-host strategies are likely to have been tried out and exploited. Ability to defeat the immune defenses may play a major role in the virulence of a bacterium and in the pathology of disease. Several strategic bacterial defenses are described below.

Immunological Tolerance to a Bacterial Antigen

Tolerance is a property of the host in which there is an immunologically-specific reduction in the immune response to a given Ag. Tolerance to a bacterial Ag does not involve a general failure in the immune response but a particular deficiency in relation to the specific antigen(s) of a given bacterium. If there is a depressed immune response to relevant antigens of a parasite, the process of infection is facilitated. Tolerance can involve either AMI or CMI or both arms of the immunological response.

Tolerance to an Ag can arise in a number of ways, but three are possibly relevant to bacterial infections.

1. **Fetal exposure to Ag**

2. **High persistent doses of circulating Ag**

3. **Molecular mimicry**. If a bacterial Ag is very similar to normal host "antigens", the immune responses to this Ag may be weak giving a degree of tolerance. Resemblance between bacterial Ag and host Ag is referred to as molecular mimicry. In this case the antigenic determinants of the bacterium are so closely related chemically to host "self" components that the immunological cells cannot distinguish between the two and an immune response cannot be raised. Some bacterial capsules are composed of polysaccharides (hyaluronic acid, sialic acid) so similar to host tissue polysaccharides that they are not immunogenic.

Antigenic Disguise

Bacteria may be able to coat themselves with host proteins (fibrin, fibronectin, antibody molecules) or with host polysaccharides (sialic acid, hyaluronic acid) so that they are able to hide their own antigenic surface components from the immunological system.

Immunosuppression

Some pathogens (mainly viruses and protozoa, rarely bacteria) cause immunosuppression in the infected host. This means that the host shows depressed immune responses to antigens in general, including those of the infecting pathogen. Suppressed immune responses are occasionally observed during chronic bacterial infections such as leprosy and tuberculosis.

Persistence of a Pathogen at Bodily Sites Inaccessible to the Immune Response

Some pathogens can avoid exposing themselves to immune forces.

Intracellular pathogens can evade host immune responses as long as they stay inside of infected cells and they do not allow microbial Ag to form on the cell surface. Macrophages support the growth of the bacteria and at the same time give them protection from immune responses.

Some pathogens persist on the luminal surfaces of the GI tract, oral cavity and the urinary tract, or the lumen of the salivary gland, mammary gland or the kidney tubule.

Induction Ineffective Antibody

Many types of antibody are formed against a given Ag, and some bacterial components may display various antigenic determinants. Antibodies tend to range in their capacity to react with Ag (the ability of specific Ab to bind to an Ag is called **avidity**). If Abs formed against a bacterial Ag are of low avidity, or if they are directed against unimportant antigenic determinants, they may have only weak antibacterial action. Such "ineffective" (non-neutralizing) Abs might even aid a pathogen by combining with a surface Ag and blocking the attachment of any functional Abs that might be present.

Antibodies Absorbed by Soluble Bacterial Antigens

Some bacteria can liberate antigenic surface components in a soluble form into the tissue fluids. These soluble antigens are able to combine with and "neutralize" antibodies before they reach the bacterial cells. For example, small amounts of endotoxin (LPS) may be released into surrounding fluids by Gram-negative bacteria.

Antigenic Variation

One way bacteria can avoid forces of the immune response is by periodically changing antigens, i.e., undergoing antigenic variation. Some bacteria avoid the host antibody response by changing from one type of fimbriae to another, by switching fimbrial tips. This makes the original AMI response obsolete by using new fimbriae that do not bind the previous antibodies. Pathogenic bacteria can vary (change) other surface proteins that are the targets of antibodies. Antigenic variation is prevalent among pathogenic viruses as well.

Changing antigens during the course of an infection

Antigens may vary or change within the host during the course of an infection, or alternatively antigens may vary among multiple strains (antigenic types) of a parasite in the population. Antigenic variation is an important mechanism used by pathogenic microorganisms for escaping the neutralizing activities of antibodies. Antigenic variation usually results from site-specific inversions or gene conversions or gene rearrangements in the DNA of the microorganisms.

Changing antigens between infections

Many pathogenic bacteria exist in nature as multiple antigenic types or serotypes, meaning that they are variant strains of the same pathogenic species. For example, there are multiple serotypes of *Salmonella typhimurium* based on differences in cell wall (O) antigens or flagellar (H) antigens. There are 80 different antigenic types of *Streptococcus pyogenes* based on M-proteins on the cell surface. There are over one hundred strains of *Streptococcus pneumoniae* depending on their capsular polysaccharide antigens. Based on minor differences in surface structure chemistry there are multiple serotypes of *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhoeae* and an assortment of other bacterial pathogens.

TOXIGENESIS

Two types of bacterial toxins

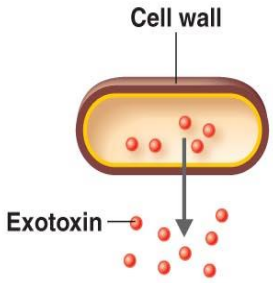
At a chemical level there are two types of bacterial toxins:

lipopolysaccharides, which are associated with the cell walls of Gram-negative bacteria.

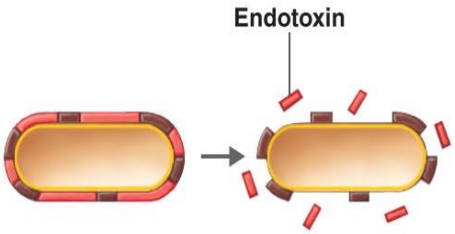
proteins, which may be released into the extracellular environment of pathogenic bacteria.

The lipopolysaccharide (LPS) component of the Gram-negative bacterial outer membrane bears the name endotoxin because of its association with the cell wall of bacteria.

Most of the protein toxins are thought of as exotoxins, since they are "released" from the bacteria and act on host cells at a distance.



(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.



(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.

Key Concept

Toxins are of two general types: exotoxins and endotoxins.

BACTERIAL PROTEIN TOXINS

The protein toxins are typically soluble proteins secreted by living bacteria during exponential growth. The production of protein toxins is generally specific to a particular

bacterial species (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin (or range of toxins) while nonvirulent strains do not, such that the toxin is the major determinant of virulence. Both Gram-positive and Gram-negative bacteria produce soluble protein toxins. Bacterial protein toxins are the most potent poisons known and may show activity at very high dilutions.

The protein **toxins resemble enzymes** in a number of ways. Like enzymes, bacterial exotoxins:

are **proteins**

are **denatured by heat**, acid, proteolytic enzymes

have a **high biological activity** (most act catalytically)

exhibit **specificity** of action

As enzymes attack specific substrates, so bacterial protein toxins are **highly specific** in the substrate utilized and in their mode of action. The substrate (in the host) may be a component of tissue cells, organs, or body fluid. Usually the site of damage caused by the toxin indicates the location of the substrate for that toxin. Terms such as "enterotoxin", "neurotoxin", "leukocidin" or "hemolysin" are sometimes used to indicate the target site of some well-defined protein toxins.

Certain protein toxins have very specific **cytotoxic activity** (i.e., they attack specific cells, for example, tetanus or botulinum toxins), but some (as produced by staphylococci, streptococci, clostridia, etc.) have fairly broad cytotoxic activity and cause nonspecific death of tissues (necrosis). Toxins that are phospholipases may be relatively nonspecific in their cytotoxicity because they cleave phospholipids which are components of host cell membranes resulting in the death of the cell by leakage of cellular contents. This is also true of pore-forming "hemolysins" and "leukocidins".

A few protein toxins obviously bring about the death of the host and are known as "lethal toxins", and even though the tissues affected and the target sites may be known, the precise mechanism by which death occurs is not understood (e.g. anthrax toxin).

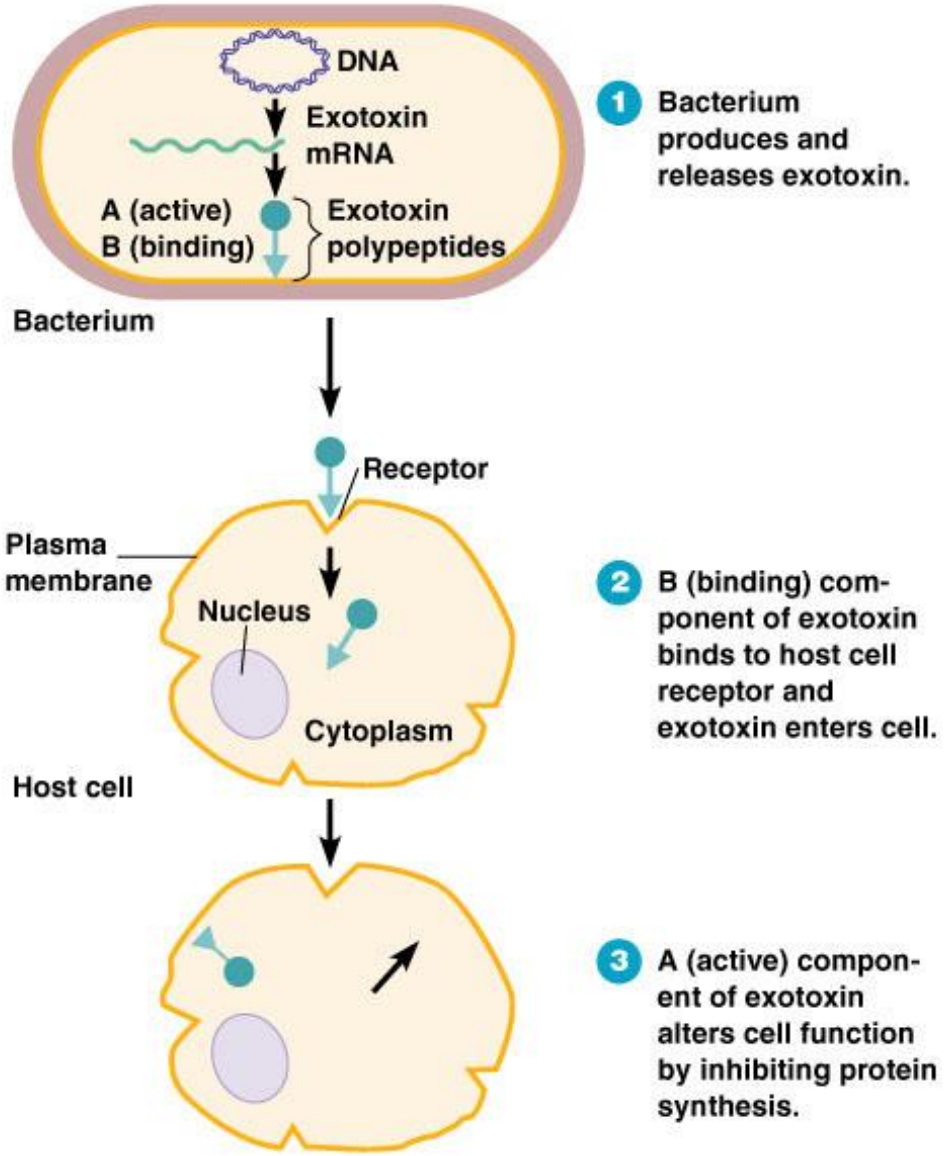
As "foreign" substances to the host, most of the protein toxins are **strongly antigenic**. In vivo, **specific antibody (antitoxin) neutralizes the toxicity** of these bacterial proteins. However, in vitro, specific antitoxin may not fully inhibit their

enzymatic activity. This suggests that the antigenic determinant of the toxin is distinct from the active (enzymatic) portion of the protein molecule. The degree of neutralization of the enzymatic site may depend on the distance from the antigenic site on the molecule. However, since the toxin is fully neutralized in vivo, this suggests that other (host) factors must play a role.

Protein toxins are inherently unstable: in time they lose their toxic properties but retain their antigenic ones. This was first discovered by Ehrlich and he coined the term toxoid for this product. **Toxoids** are detoxified toxins which retain their antigenicity and their immunizing capacity. The formation of toxoids can be accelerated by treating toxins with a variety of reagents including formalin, iodine, pepsin, ascorbic acid, ketones, etc. The mixture is maintained at 37^{o} at pH range 6 to 9 for several weeks. The resulting toxoids can be use for artificial immunization against diseases caused by pathogens where the primary determinant of bacterial virulence is toxin production. Toxoids are the immunizing agents against diphtheria and tetanus that are part of the DPT vaccine.

A + B Subunit Arrangement of Protein Toxins

Many protein toxins, notably those that act intracellularly (with regard to host cells), consist of two components: one component (subunit A) is responsible for the enzymatic activity of the toxin; the other component (subunit B) is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native toxin. Isolated A subunits are enzymatically active and but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native A+B toxin), but they are nontoxic. There are a variety of ways that toxin subunits may be synthesized and arranged: **A-B** or **A-5B** indicates that subunits synthesized separately and associated by noncovalent bonds; **A/B** denotes subunit domains of a single protein that may be separated by proteolytic cleavage; **A + B** indicates separate protein subunits that interact at the target cell surface; **5B** indicates that the binding domain is composed of 5 identical subunits.


DNA
Exotoxin mRNA
A (active)
B (binding)
Exotoxin polypeptides
Bacterium
1 Bacterium produces and releases exotoxin.
Receptor
Plasma membrane
Nucleus
Cytoplasm
Host cell
2 B (binding) component of exotoxin binds to host cell receptor and exotoxin enters cell.
3 A (active) component of exotoxin alters cell function by inhibiting protein synthesis.

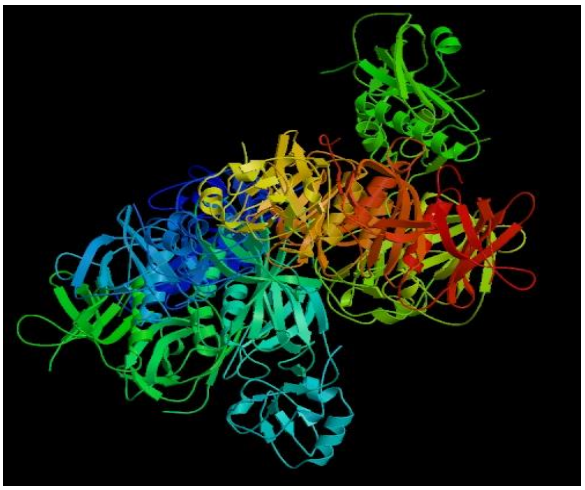


Fig: Tertiary structure of the pertussis toxin produced by *Bordetella pertussis*. Pertussis toxin is a member of the A-B bacterial toxin superfamily. It is a hexameric protein comprising five distinct subunits, designated S1-S5. S2, S3, S4 and S5 comprise the B oligomer, responsible for binding the toxin to the cell surface. Each subunit is translated separately with an amino-terminal signal sequence which is cleaved during transport to the periplasm. S2 and S3 function as adhesins, S2 binds specifically to a glycolipid called lactosylceramide, which is found primarily on the ciliated epithelial cells. S3 binds to a glycoprotein found mainly on phagocytic cells.

Attachment and Entry of Toxins

There are at least two mechanisms of toxin entry into target cells. In one mechanism called **direct entry**, the B subunit of the native toxin (A+B) binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm. In an alternative mechanism, the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of **receptor-mediated endocytosis** (**RME**). The toxin is internalized in the cell in a membrane-enclosed vesicle called an endosome. H^+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. Somehow, the B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface. In both cases, a large protein molecule must insert into and cross a membrane lipid bilayer. This activity is reflected in the ability of most A/B native toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways.

Other Considerations

In keeping with the observation that genetic information for functions not involved in viability of bacteria is frequently located extrachromosomally, the genes encoding toxin production are generally located on plasmids or in lysogenic bacteriophages. Thus the processes of genetic exchange in bacteria, notably conjugation and transduction, can mobilize these genetic elements between strains of bacteria, and therefore may play a role in determining the pathogenic potential of a bacterium.

Why certain bacteria produce such potent toxins is mysterious and is analogous to asking why an organism should produce an antibiotic. The production of a toxin may play a role in adapting a bacterium to a particular niche, but it is not essential to the viability of the organism. Many toxigenic bacteria are free-living in Nature and in associations with humans in a form which is phenotypically identical to the toxigenic strain but lacking the ability to produce the toxin.

There is conclusive evidence for the pathogenic role of diphtheria, tetanus and botulinum toxins, various enterotoxins, staphylococcal toxic shock syndrome toxin, and streptococcal erythrogenic toxin. And there is clear evidence for the pathological involvement of pertussis toxin, anthrax toxin, shiga toxin and the necrotizing toxins of clostridia in host-parasite relationships.

* The "pyrogenic exotoxins" produced by *Staphylococcus aureus* and *Streptococcus pyogenes* have been designated as superantigens. They represent a family of molecules with the ability to elicit massive activation of the immune system. These proteins share the ability to stimulate T cell proliferation by interaction with Class II MHC molecules on APCs and specific V beta chains of the T cell receptor. The important feature of this interaction is the resultant production of IL-1, TNF, and other lymphokines which appear to be the principal mediators of disease processes associated with these toxins.

Table 4. SOURCES AND ACTIVITIES OF BACTERIAL TOXINS

NAME OF TOXIN	BACTERIUM INVOLVED	ACTIVITY
Anthrax toxin (EF)	*Bacillus anthracis*	Edema Factor (EF) is an adenylate cyclase that causes increased levels in intracellular cyclic AMP in phagocytes

		and formation of ion-permeable pores in membranes (hemolysis)
Adenylate cyclase toxin	*Bordetella pertussis*	Acts locally to increase levels of cyclic AMP in phagocytes and formation of ion-permeable pores in membranes (hemolysis)
Cholera enterotoxin	*Vibrio cholerae*	ADP ribosylation of G proteins stimulates adenylate cyclase and increases cAMP in cells of the GI tract, causing secretion of water and electrolytes
E. coli LT toxin	*Escherichia coli*	Similar to cholera toxin
Shiga toxin	*Shigella dysenteriae*	Enzymatically cleaves rRNA resulting in inhibition of protein synthesis in susceptible cells
Botulinum toxin	*Clostridium botulinum*	Zn^{++} dependent protease that inhibits neurotransmission at neuromuscular synapses resulting in flaccid paralysis
Tetanus toxin	*Clostridium tetani*	Zn^{++} dependent protease that inhibits neurotransmission at inhibitory synapses resulting in spastic paralysis
Diphtheria toxin	*Corynebacterium diphtheriae*	ADP ribosylation of elongation factor 2 leads to inhibition of protein synthesis in target cells
Pertussis	*Bordetella*	ADP ribosylation of G proteins

toxin	*pertussis*	blocks inhibition of adenylate cyclase in susceptible cells
Staphylococcus enterotoxins*	*Staphylococcus aureus*	Massive activation of the immune system, including lymphocytes and macrophages, leads to emesis (vomiting)
Toxic shock syndrome toxin (TSST-1)*	*Staphylococcus aureus*	Acts on the vascular system causing inflammation, fever and shock
Pyrogenic exotoxins (SPE) e.g. Erythrogenic toxin (scarlet fever toxin)*	*Streptococcus pyogenes*	Causes localized erythematous reactions

ENDOTOXINS

Endotoxins are part of the outer cell wall of bacteria. Endotoxins are invariably associated with Gram-negative bacteria as constituents of the outer membrane of the cell wall. Although the term **endotoxin** is occasionally used to refer to any "cell-associated" bacterial toxin, it should be reserved for the lipopolysaccharide complex associated with the outer envelope of Gram-negative bacteria such as *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus*, and other leading pathogens. Lipopolysaccharide (LPS) participates in a number of outer membrane functions that are essential for bacterial growth and survival, especially within the context of a host-parasite interaction.

The biological activity of endotoxin is associated with the **lipopolysaccharide** (**LPS**). Toxicity is associated with the lipid component (**Lipid A**) and immunogenicity (antigenicity) is associated with the polysaccharide components. The cell wall antigens (**O antigens**) of Gram-negative bacteria are components of LPS. LPS activates complement by the alternative (properdin) pathway and may be a part of the pathology of most Gram-negative bacterial infections.

For the most part, endotoxins remain associated with the cell wall until disintegration of the bacteria. In vivo, this results from autolysis, external lysis, and phagocytic digestion of bacterial cells. It is known, however, that small amounts of endotoxin may be released in a soluble form, especially by young cultures.

Compared to the classic exotoxins of bacteria, endotoxins are less potent and less specific in their action, since they do not act enzymatically. Endotoxins are heat stable (boiling for 30 minutes does not destabilize endotoxin), but certain powerful oxidizing agents such as , superoxide, peroxide and hypochlorite degrade them. Endotoxins, although strongly antigenic, cannot be converted to toxoids. A comparison of the properties of bacterial endotoxins compared to classic exotoxins is shown in Table 5.

Table 5. CHARACTERISTICS OF BACTERIAL ENDOTOXINS AND EXOTOXINS

PROPERTY	ENDOTOXIN	EXOTOXIN
CHEMICAL NATURE	Lipopolysaccharide (mw = 10kDa)	Protein (mw = 50-1000kDa)
RELATIONSHIP TO CELL	Part of outer membrane	Extracellular, diffusible
DENATURED BY BOILING	No	Usually
ANTIGENIC	Yes	Yes
FORM TOXOID	No	Yes
POTENCY	Relatively low (>100ug)	Relatively high (1 ug)
SPECIFICITY	Low degree	High degree

ENZYMATIC ACTIVITY	No	Usually
PYROGENICITY	Yes	Occasionally

Lipopolysaccharides are complex amphiphilic molecules with a mw of about 10kDa, that vary widely in chemical composition both between and among bacterial species. In a basic ground plan common to all endotoxins, LPS consists of three components or regions:

(1) Lipid A---- (2) Core polysaccharide---- (3) O polysaccharide

Lipid A is the lipid component of LPS. It contains the hydrophobic, membrane-anchoring region of LPS. Lipid A consists of a phosphorylated N-acetylglucosamine (NAG) dimer with 6 or 7 fatty acids (FA) attached. Usually 6 FA are found. All FA in Lipid A are saturated. Some FA are attached directly to the NAG dimer and others are esterified to the 3-hydroxy fatty acids that are characteristically present. The structure of Lipid A is highly conserved among Gram-negative bacteria. Among ***Enterobacteriaceae*** Lipid A is virtually constant.

The **Core (R) polysaccharide** is attached to the 6 position of one NAG. The R antigen consists of a short chain of sugars. For example: KDO - Hep - Hep - Glu - Gal - Glu - GluNAc.
Two unusual sugars are usually present, heptose and 2-keto-3-deoxyoctonoic acid (KDO), in the core polysaccharide. KDO is unique and invariably present in LPS and so has been an indicator in assays for LPS (endotoxin).

With minor variations, the core polysaccharide is common to all members of a bacterial genus (e.g. *Salmonella*), but it is structurally distinct in other genera of Gram-negative bacteria. *Salmonella*, *Shigella* and *Escherichia* have similar but not identical cores.

The **O polysaccharide** (also referred to as the **O antigen** or **O side chain**) is attached to the core polysaccharide. It consists of repeating oligosaccharide subunits made up of 3-5 sugars. The individual chains vary in length ranging up to 40 repeat units. The O polysaccharide is much longer than the core polysaccharide and it maintains the hydrophilic domain of the LPS molecule. Often, a unique group of sugars, called **dideoxyhexoses**, occurs in the O polysaccharide.

A major antigenic determinant (antibody-combining site) of the Gram-negative cell wall resides in the O polysaccharide. Great variation occurs in the composition of the sugars in the O side chain between species and even strains of Gram-negative bacteria.

LPS and virulence of Gram-negative bacteria

Endotoxins are toxic to most mammals. They are strong antigens but they seldom elicit immune responses which give full protection to the animal against secondary challenge with the endotoxin. They cannot be toxoided. Endotoxins released from multiplying or disintegrating bacteria significantly contribute to the symptoms of Gram-negative bacteremia and septicemia, and therefore represent important pathogenic factors in Gram-negative infections. Regardless of the bacterial source, all endotoxins produce the same range of biological effects in the animal host. The injection of living or killed Gram-negative cells, or purified LPS, into experimental animals causes a wide spectrum of nonspecific **pathophysiological reactions related to inflammation** such as:

fever

changes in white blood cell counts

disseminated intravascular coagulation

tumor necrosis

hypotension

shock

lethality

The sequence of events follows a regular pattern: 1. latent period; 2. physiological distress (fever, diarrhea, prostration, shock); 3. death. How soon death occurs varies on the dose of the endotoxin, route of administration, and species of animal. Animals vary in their susceptibility to endotoxin.

The role of Lipid A

The physiological activities of endotoxins are mediated mainly by the Lipid A component of LPS. Lipid A is the toxic component of LPS, as evidence by the fact that

injection of purified Lipid A into an experimental animal will elicit the same response as intact LPS. The primary structure of Lipid A has been elucidated, and Lipid A has been chemically synthesized. Its biological activity appears to depend on a peculiar conformation that is determined by the glucosamine disaccharide, the PO_4 groups, the acyl chains, and also the KDO-containing inner core. Lipid A is known to react at the surfaces of macrophages causing them to release cytokines that mediate the pathophysiological response to endotoxin.

The role of the O polysaccharide

Although nontoxic, the polysaccharide side chain (O antigen) of LPS may act as a determinant of virulence in Gram-negative bacteria. The O polysaccharide is responsible for the property of "smoothness" of bacterial cells, which may contribute to their resistance to phagocytic engulfment. The O polysaccharide is hydrophilic and may allow diffusion or delivery of the toxic lipid in the hydrophilic (in vivo) environment. The long side chains of LPS afforded by the O polysaccharide may prevent host complement from depositing on the bacterial cell surface which would bring about bacterial cell lysis. The O polysaccharide may supply a bacterium with its specific ligands (adhesins) for colonization which is essential for expression of virulence. Lastly, the O-polysaccharide is antigenic, and the usual basis for antigenic variation in Gram-negative bacteria rests in differences in their O polysaccharides.

PathogenicityIslands

Pathogenicity Islands (PAI) are a distinct class of genomic islands which are acquired by horizontal gene transfer. They are incorporated in the genome of pathogenic bacteria but are usually absent from non-pathogenic organisms of the same or closely related species. They usually occupy relatively large genomic regions ranging from 10-200 kb and encode genes which contribute to virulence of the pathogen. Typical examples are adhesins, toxins, iron uptake systems, invasins, etc.

One species of bacteria may have more than one pathogenicity island. For example, in *Salmonella*, five pathogenicity islands have been identified. They are found mainly in Gram-negative bacteria, but have been shown in a few Gram-positives. They are found in pathogens that undergo gene transfer by plasmid, phage, or a conjugative transposon and are typically transferred through mechanisms of horizontal gene transfer (HGT).

Pathogenicity islands may be located on the bacterial chromosome or may be a part of a plasmid. They are high in Guanine + Cytosine content. They are flanked by direct repeats i.e., the sequence of bases at two ends are the same. They are associated with

tRNA genes, which target sites for the integration of DNA. They have characteristics of transposons in that they carry functional genes, e.g. integrase, transposase, or part of insertion sequences, and may move from one tRNA locus to another on the chromosome or plasmid.

Pathogenicity islands play a pivotal role in the virulence of bacterial pathogens of humans and are also essential for virulence in pathogens of animals and plants. The availability of a large number of genome sequences of pathogenic bacteria and their nonpathogenic relatives has allowed the identification of novel pathogen-specific genomic islands. PAI apparently have been acquired during the speciation of pathogens from their nonpathogenic or environmental ancestors. The acquisition of PAI not only is an ancient evolutionary event that led to the appearance of bacterial pathogens on a timescale of millions of years but also may represent a mechanism that contributes to the appearance of new pathogens within a human life span. The acquisition of knowledge about PAI, their structure, their mobility, and the pathogenicity factors they encode not only is helpful in gaining a better understanding of bacterial evolution and interactions of pathogens with eucaryotic host cells but also may have important practical implications such as providing delivery systems for vaccination and tools for the development of new strategies for therapy of bacterial infections.

PAIs represent distinct genetic elements encoding virulence factors of pathogenic bacteria, but they belong to a more general class of **genomic islands**, which are common genetic elements sharing a set of unifying features. Genomic islands have been acquired by horizontal gene transfer. In recent years many different genomic islands have been discovered in a variety of pathogenic as well as non-pathogenic bacteria. Because they promote genetic variability, genomic islands play an important role in microbial evolution.

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POSSIBLE QUESTION

UNIT 4

2 marks

1. Explain biosynthesis of cell membrane in bacteria.
2. What are secondary metabolites? Give example.
3. Write note on Archael lipids
4. Write about the organelles involved in cell wall biosynthesis?
5. Define secondary metabolites and write about the impotance.
6. What is Lipid catabolism?
7. Write all natural amino acids with their three letter abbreviation
8. Define mycotoxins. Give example.
9. What do you know about fatty acid and their role in metabolism of bacteria
10. Write the importance of phospholipid

8 marks

1. Explain biosynthesis of cell membrane
2. Explain biosynthesis of cell wall in Gram positive bacteria.
3. Give a detailed account on structure of a Gram negative cell wall.
4. How are nucleotides synthesized in bacteria
5. Illuminate the structural details of phospholipids and their significance
6. What are secondary metabolites? Give example
7. What are toxins? Give an account on bacterial toxins
8. What do you know about fatty acid and their role in metabolism of bacteria?

9. Give a note on Phospholipids and add note on the function.

Outline the biosynthesis of proteins in Prokaryotes

Unit IV Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
? - Guanosine - Guanine	GAP	GMP	GPA	GPM	GMP
_____ serves as starting point for synthesis of purines and pyrimidines	PRPP	Vitamin B_{12}	Carotene	Nucleic acid	PRPP
_____ acts as precursor for synthesis of purine ring	Ribose-5-phosphate	Ribose-5-lactate	Ribose-5-succinate	Ribose-5-maleate	Ribose-5-phosphate
_____ acts as template for protein synthesis	mRNA	tRNA	rRNA	sRNA	mRNA
_____ is a major precursor of fatty acid synthesis	Acetyl-CoA	Butyl-CoA	Methyl-CoA	Fumaryl-CoA	Acetyl-CoA
_____ is an inhibitor of Gram positive cell wall synthesis	Rifampicin	Chloramphenicol	Penicillin	Streptomycin	Penicillin
_____ is the first amino acid during translation of proteins	Threonine	Leucine	Methionine	Valine	Methionine
_____ are nucleic acid derivatives	Mono enzymes	Di enzymes	Holo enzymes	Co-enzymes	Co-enzymes
_____ components of chlorophylls and are	Plastid	Hexose	Tetrapyrrole	Acetate	Tetrapyrrole

involved in oxygen transport in animals					
_____ is a critical intermediate in the aromatic amino acid family	Acetic acid	Formic acid	Chorismic acid	Succinic acid	Chorismic acid
_____ transports peptidoglycan monomers across the cell membrane	Bactoprenol	Isoprenol	Lipoprenol	Glycoprenol	Bactoprenol
_____ intermediate is required for the biosynthesis of purines and pyrimidines	Pyrophosphate	Phospho ribosyl pyrophosphate	Ribosyl pyrophosphate	Phosphatase	Phospho ribosyl pyrophosphate
A characteristic 18-carbon, mono unsaturated compound of the anaerobic pathway in fatty acid synthesis	Acetic acid	Butyric acid	*cis*-vaccenic acid	Sulphuric acid	*cis*-vaccenic acid
A form of fatty acid seen in membranes of all living organisms	Valeric acid	Caspase	Choline	Phospholipids	Phospholipids
A major way in which depleted biosynthetic intrmediates are replenished	Glyoxalate cycle	TCA cycle	Glycolysis	ED pathway	Glyoxalate cycle
Aflatoxins are produced	*Aspergillus flavus*	*Penicillium*	*Rhizopus*	*Mucor*	*Aspergillus*

by		*notatum*	*stolonifer*	*racemosus*	*flavus*
Amino acids of glutamic acid family includes	Valine & Leucine	Proline & Arginine	Alanine & Threonine	Methionine & Aspartic acid	Proline & Arginine
AMP - Adenosine - ?	Adenine	APT	APD	Acetic acid	Adenine
AMP and GMP are synthesized from	Ionosine monophosphate	Iodine monophosphate	Ionosine	Monophosphate	Ionosine monophosphate
An extremophilic archaea	*Thermoplasma acidophilum*	*Lactobacillus acidophilus*	*Bacillus subtilis*	*Mycoplasma pneumoniae*	*Thermoplasma acidophilum*
An unsaturated, branched, linear lipids	Mevalonic acid	Isoprenoids	Dimethyl allyl	Isopentyl	Isoprenoids
Antibiotic is a	Primary metabolite	Secondary metabolite	Tertiary metabolite	Enzyme	Secondary metabolite
A-site is the ribosomal site most frequently occupied by the	Aminoacyl-rRNA	Aminoacyl-mRNA	Iminoacyl-tRNA	Aminoacyl-tRNA	Aminoacyl-tRNA
Aspartic acid family is also known as	Oxaloacetate family	Oxalate family	Acetate family	None	Oxaloacetate family
Bacteria and eukaryotes lack _____ that Archaea have in their membranes	S-glycerol	T-glycerol	L-glycerol	M-glycerol	L-glycerol
Basic units of Gram positive cell wall	AMN & AGN	MNA & GNA	MAN & MAG	NAM & NAG	NAM & NAG
Biosynthesis of nucleotides fall into two classes namely _____	*de novo* and *salvage* pathways	Glycolysis and TCA pathways	Krebs and Glyoxalate cycles	Glycolysis and Glyoxalate cycles	*de novo* pathways and *salvage* pathways

Cell membrane of Archaea contains	Murein	Isoprenoid chains	Peptidoglycan	Keratin	Isoprenoid chains
Central participant in amino acid synthesis process	Formaldehyde	acetyl-CoA	PEG	Sodium chloride	acetyl-CoA
Cholera toxin is composed of six protein subunits which is	1 A + 5 B subunits	2 A + 4 B subunits	3 A + 3 B subunits	4 A + 2 B subunits	1 A + 5 B subunits
Codon/Anticodon consists of _____ nucleotides	4	6	3	9	3
Components of energy resources such as ATP and GTP	Cytosine & Uracil	Thymine & cytosine	Adenosine & Guanosine	Adenine & Guanine	Adenosine & Guanosine
Cyclic AMP and cyclic GMP are involved in _____ both within and between cells.	Synthesis	Degradation	Cell cycle	Transmitting signals	Transmitting signals
Deoxyribonucleotides are synthesized by reduction of	CHO	Proteins	Ribonucleosides	Lipids	Ribonucleosides
During biosynthesis, glutamic acid arises from	α-ketoglutaric acid	Glucose	Acidic ketone	Basic benzene	α-ketoglutaric acid
During fatty acid synthesis, _____	NADP	NADPH	FMN	FMNH	NADPH

acts as reducing agent					
Edema factor and lethal factor is found in	Cholera toxin	Anthrax toxin	Mycotoxin	Botulinum toxin	Anthrax toxin
Estimated human median lethal dose (LD_{50}) of botulinum toxin is _____ , intravenously.	1.3 - 2.1 ng/kg	1.3 - 2.1 µg/kg	1.3 - 2.1 mg/kg	1.3 - 2.1 g/kg	1.3 - 2.1 ng/kg
Example of acidic amino acid	Valine	Aspartic acid	Tryptophan	Isoleucine	Aspartic acid
Fatty acid synthesis require	Biotin	Avidin	Protein	Glucose	Biotin
Histidine synthesis begins by reaction of _____ with ATP	RPPP	PPPR	PPPP	PRPP	PRPP
In fatty acid synthesis,the process of formation of even-chain acids begins with	Malonyl-Coa	Acetyl-CoA	Aspargyl-CoA	Butyl-CoA	Acetyl-CoA
In Gram negative bacteria, _____ forms aqueous channels across the outer membrane	Forins	Porins	Tripenes	Teichoic acid	Porins
In microbes, _____ play a critical role in membrane formation	Lipids	Minerals	Vitamins	Amino acids	Lipids

and function.					
In purine synthesis, the purine ring is built on _____ molecule	Pentose	Hexose	Disaccharide	Polysaccharide	Pentose
In RNA, thiamine is replaced by	Uracil	Adenine	Cytosine	Guanine	Uracil
Lysine is formed in prokaryotes and most algae by metabolism of	Semialdehyde	Aspartic-β-semialdehyde	Aspartic aldehyde	Aldehyde aspartate	Aspartic-β-semialdehyde
Most archaea are	Thermophiles	Halophiles	Alkalophiles	Extremophiles	Extremophiles
No codon specifies more than ___ amino acid	1	2	3	4	1
Non-coding regions are called as	Exons	Introns	Cistrons	Positrons	Exons
Number of natural amino acids	18	20	22	24	20
Precursor for the synthesis of methionine, isoleucine and threonine	Alpha serine	Beta serine	Homoserine	Zeta serine	Homoserine
Proteins are made up of monomeric units of	Amino acids	Imino acids	Glycogen	Hyaluronic acid	Amino acid
Ribose-5-phosphate used as a precursor for purine synthesis is normally derived from _____ pathway	Pentose phosphate	Glycolytic	Glyoxalate	TCA cycle	Pentose phosphate
Straight chain fatty acids	Saturated	Unsaturated	Both Saturated	None	Both Saturated

are			& Unsaturated		& Unsaturated
Sugar containing lipids are called	Glycolipids	Neutral lipids	Lipoproteins	Phospholipids	Glycolipids
Sulphur containing amino acid	Leucine	Valine	Cysteine	Proline	Cysteine
The cell wall of Archaea is made up of	PG layer	Pseudomurein	Lipoproteins	O-antigen	Pseudomurein
The dibasic amino acid found in cell wall of bacteria is	*meso*-diamoinopimelic acid	Dipicoloinic acid	Hydroxy ornithine	Cerulic acid	*meso*-diamoinopimelic acid
Which is not an aromatic amino acid	Phenyl alanine	Tyrosine	Tryptophan	Leucine	Leucine

Fermentation is a metabolic process that consumes sugar in the absence of oxygen. The products are organic acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. The science of fermentation is known as zymology.

In microorganisms, fermentation is the primary means of producing energy by the degradation of organic nutrients anaerobically. Humans have used fermentation to produce drinks and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt (see fermentation in food processing), as well as for producing alcoholic beverages such as wine (see fermentation in winemaking) and beer. Fermentation can even occur within the stomachs of animals, including humans.

Photosynthetic bacteria

Cyanobacteria also known as **Cyanophyta**, is a phylumof bacteria that obtain their energy through photosynthesis, and are the only photosynthetic prokaryotes able to produce oxygen. The name "cyanobacteria" comes from the color of the bacteria (Greek: κυανός (*kyanós*) = blue).[*citation needed*] Cyanobacteria (which are prokaryotes) used to be called "blue-green algae". They have been renamed 'cyanobacteria' in order to avoid the term "algae", which in modern usage is restricted to eukaryotes.[6]

Unlike heterotrophic prokaryotes, cyanobacteria have internal membranes. They are flattened sacs called thylakoids where photosynthesis is performed.

Phototrophic eukaryotes perform photosynthesis by plastids that have their ancestry in cyanobacteria, via a process called endosymbiosis. These endosymbiotic cyanobacteria in eukaryotes have evolved or differentiated into specialized organelles such as chloroplasts, etioplasts and leucoplasts.

By producing and releasing oxygen (as a byproduct of photosynthesis), cyanobacteria are thought to have converted the early oxygen-poor, reducing atmosphere, into an oxidizing one, causing the Great Oxygenation Event and the

"rusting of the Earth", which dramatically changed the composition of the Earth's life forms and led to the near-extinction of anaerobic organisms.

Purple sulfur bacteria

The **purple sulfur bacteria** are a group of Proteobacteria capable of photosynthesis, collectively referred to as purple bacteria. They are anaerobic or microaerophilic, and are often found in hot springs or stagnant water. Unlike plants, algae, andcyanobacteria, they do not use water as their reducing agent, so do not produceoxygen. Instead, they use hydrogen sulfide, which is oxidized to produce granules of elemental sulfur. This, in turn, may be oxidized to form sulfuric acid.

The purple sulfur bacteria are divided into two families, the Chromatiaceae and the Ectothiorhodospiraceae, which respectively produce internal and external sulfur granules, and show differences in the structure of their internal membranes. They make up the order Chromatiales, included in the gamma subdivision of the Proteobacteria. The genus *Halothiobacillus* is also included in the Chromatiales, in its own family, but it is not photosynthetic.

Purple sulfur bacteria are generally found in illuminated anoxic zones of lakes and other aquatic habitats where hydrogen sulfide accumulates and also in "sulfur springs" where geochemically or biologically produced hydrogen sulfide can trigger the formation of blooms of purple sulfur bacteria. Anoxic conditions are required for photosynthesis; these bacteria cannot thrive in oxygenated environments.[1]

The most favorable lakes for the development of purple sulfur bacteria are meromictic (permanently stratified) lakes. Meromictic lakes stratify because they have denser (usually saline) water in the bottom and less dense (usually fresh water) nearer the surface. If sufficient sulfate is present to support sulfate reduction, the sulfide, produced in the sediments, diffuses upward into the anoxic bottom waters, where purple sulfur bacteria can form dense cell masses, called blooms, usually in association with green phototrophic bacteria.

Purple sulfur bacteria are also a prominent component in intertidal microbial mats, such as the Sippewissett Microbial Mat, which have a dynamic environment due to the flow of the tides and incoming fresh water and gives them a similar favorable environment as meromictic lakes. Purple sulphur bacteria have bacteriopurpurin pigment. It uses inorganic sulphur substances as electron and H+ donors.

Green sulfur bacteria

The **green sulfur bacteria** are obligately a family of anaerobic photoautotrophic bacteria. Together with the non-photosynthetic **Ignavibacteriaceae**, they form the phylum **Chlorobi**. Most closely related to the distant Bacteroidetes, they are accordingly assigned their own phylum. Green sulfur bacteria are nonmotile (except *Chloroherpeton thalassium*, which mayglide). Photosynthesis is achieved using a Type 1 reaction centre usingbacteriochlorophyll (BChl) *a* and in chlorosomes which employ BChl *c*, *d*, or *e*; in addition chlorophyll *a* is also present.[2][1] They use sulfide ions, hydrogen or ferrous iron as an electron donor and the process is mediated by the type I reaction centreand Fenna-Matthews-Olson complex. Elemental sulfur deposited outside the cell may be further oxidized. By contrast, the photosynthesis in plants uses water as the electron donor and produces oxygen.

Chlorobium tepidum has emerged as a model organism for the group; although only 10 genomes have been sequenced, these are quite comprehensive of the family's biodiversity. Their 2-3 Mb genomes encode 1750-2800 genes, 1400-1500 of which are common to all strains. The apparent absence of two-component histidine-kinases and response regulators suggest limited phenotypic plasticity. Their small dependence on organic molecule transporters and transcription factors also indicate these organisms are adapted to a narrow range of energy-limited conditions, anecology shared with the simpler cyanobacteria, *Prochlorococcus* and*Synechococcus*.

A species of green sulfur bacteria has been found living near a black smoker off the coast of Mexico at a depth of 2,500 m in the Pacific Ocean. At this depth, the bacterium, designated GSB1, lives off the dim glow of the thermal vent since no sunlight can penetrate to that depth.

Green sulfur bacteria appear in Lake Matano, Indonesia, at a depth of about 110–120 m. The population may include the species *Chlorobium ferrooxidans*.

PHOTOSYNTHESIS

The ultimate source of all the chemical energy in cells comes from the sun. Because this source is directly available only to the cells of photosynthesizers, most organisms are either directly or indirectly dependent on photosynthesis. A few chemoautotrophs derive their energy and nutrients solely from inorganic substrates. The other major products of photosynthesis are organic carbon compounds, which are produced from carbon dioxide through a process called carbon fixation. On land, green plants are the primary photosynthesizers; and in aquatic ecosystems, where 80% to 90% of all photosynthesis occurs, algae, green and purple bacteria, and cyanobacteria fill this role. Other photosynthetic prokaryotes are green sulfur, purple sulfur, and purple nonsulfur bacteria. The summary equation for the main reactants and products of photosynthesis is

$$6CO_2 + 6H_2O \xrightarrow{\text{Light and pigment}} \underset{\text{Glucose}}{C_6H_{12}O_6} + 6O_2\uparrow$$

The anatomy of photosynthetic cells is adapted to trapping sunlight, and their physiology effectively uses this solar energy to produce high-energy glucose from low-energy CO 2 and water. Photosynthetic organisms achieve this remarkable feat through a series of reactions involving light, pigment, CO_2 , and water, which is used as a source for electrons. Photosynthesis proceeds in two phases: the **light-dependent reactions,** which proceed only in the presence of sunlight, and the **light-independent reactions,** which proceed regardless of the lighting conditions (light or dark) **(figure) .**

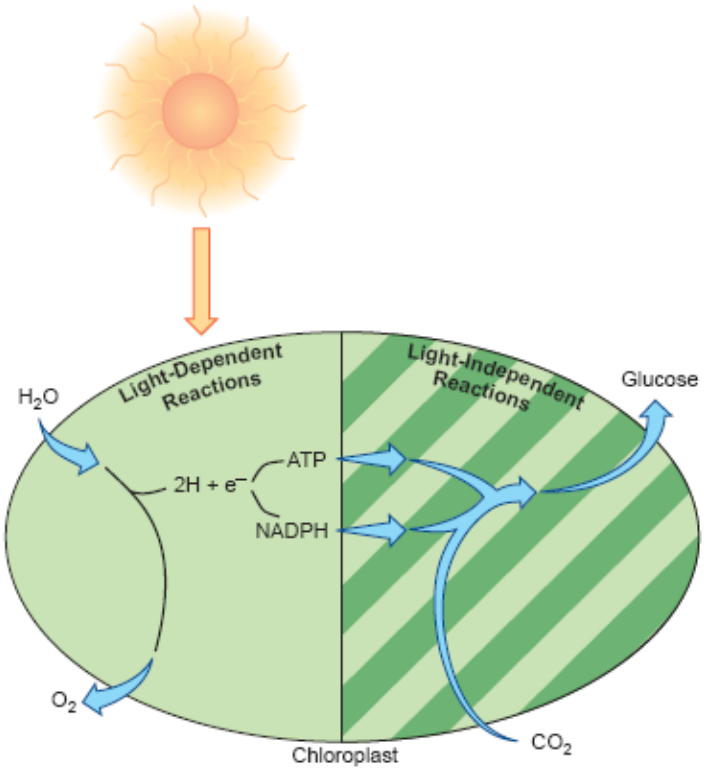
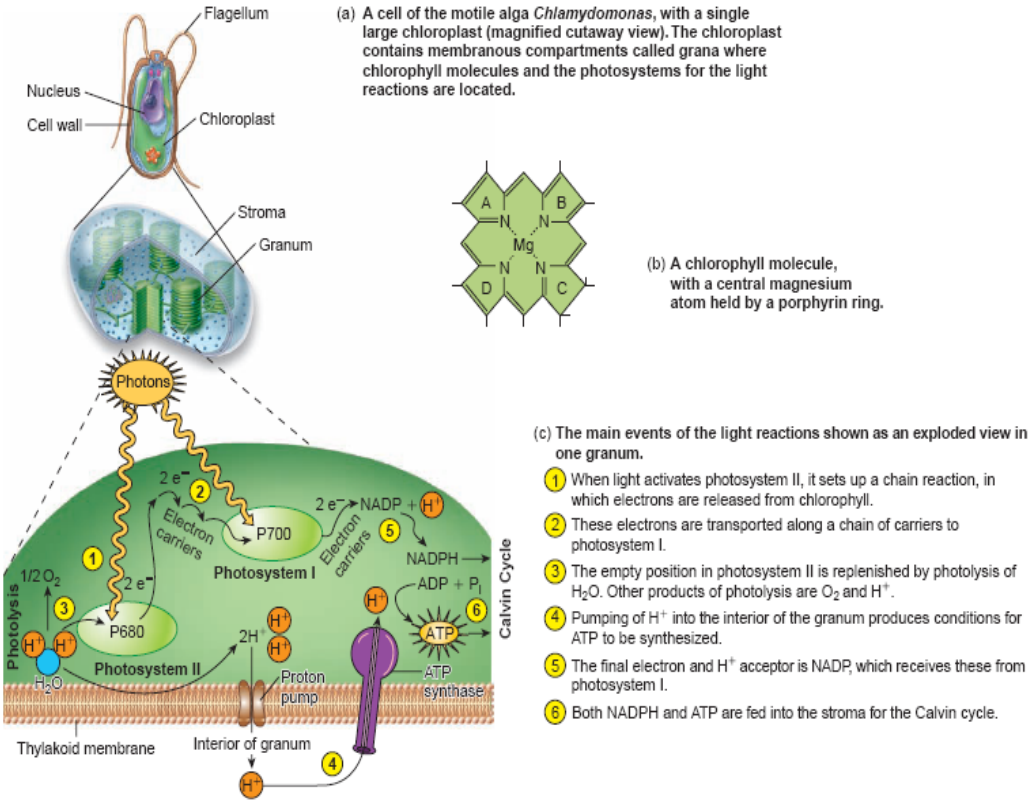


Figure Overview of photosynthesis.

The general reactions of photosynthesis, divided into two phases called light-dependent reactions and light-independent reactions. The dependent reactions require light to activate chlorophyll pigment and use the energy given off during activation to split an H2O molecule into oxygen and hydrogen, producing ATP and NADPH. The independent reactions, which occur either with or without light, utilize ATP and NADPH produced during the light reactions to fix CO2 into organic compounds such as glucose.



(a) A cell of the motile alga *Chlamydomonas*, with a single large chloroplast (magnified cutaway view). The chloroplast contains membranous compartments called grana where chlorophyll molecules and the photosystems for the light reactions are located.

(b) A chlorophyll molecule, with a central magnesium atom held by a porphyrin ring.

(c) The main events of the light reactions shown as an exploded view in one granum.

1. When light activates photosystem II, it sets up a chain reaction, in which electrons are released from chlorophyll.
2. These electrons are transported along a chain of carriers to photosystem I.
3. The empty position in photosystem II is replenished by photolysis of H_2O. Other products of photolysis are O_2 and H^+.
4. Pumping of H^+ into the interior of the granum produces conditions for ATP to be synthesized.
5. The final electron and H^+ acceptor is NADP, which receives these from photosystem I.
6. Both NADPH and ATP are fed into the stroma for the Calvin cycle.

Solar energy is delivered in discrete energy packets called **photons** (also called quanta) that travel as waves. The wavelengths of light operating in photosynthesis occur in the visible spectrum between 400 (violet) and 700 nanometers (red). As this light strikes photosynthetic pigments, some wavelengths are absorbed, some pass through, and some are reflected. The activity that has the greatest impact on photosynthesis is the absorbance of light by photosynthetic pigments. These include the **chlorophylls,** which are green; **carotenoids,** which are yellow, orange, or red; and **phycobilins,** which are red or blue-green. By far the most important of these pigments are the bacterial chlorophylls, which contain a *photocenter* that consists of a magnesium atom held in the center of a complex ringed molecule called a *porphyrin* **(figure *b*).**

Accessory photosynthetic pigments such as carotenes trap light energy and shuttle it to chlorophyll, thereby functioning like antennae. These light-dependent

reactions are catabolic (energyproducing) reactions, which pave the way for the next set of reactions, the light-independent reactions, which use the extracted energy for synthesis. During this phase, carbon atoms from CO_2 are fixed to the carbon backbones of organic molecules.

Light-Dependent Reactions

The same systems that carry the photosynthetic pigments are also the sites for the light reactions. They occur in the **thylakoid** membranes of compartments called grana (singular, granum) in chloroplasts and in specialized parts of the cell membranes in prokaryotes. These systems exist as two separate complexes called *photosystem I* (P700) and *photosystem II* (P680)**.** Both systems contain chlorophyll and they are simultaneously activated by light, but the reactions in photosystem II help drive photosystem I. Together the systems are activated by light, transport electrons, pump hydrogen ions, and form ATP and NADPH. When photons enter the photocenter of the P680 system (PS II), the magnesium atom in chlorophyll becomes excited and releases 2 electrons. The loss of electrons from the photocenter has two major effects:

1. It creates a vacancy in the chlorophyll molecule forceful enough to split an H_2O molecule into hydrogens (H_1) (electrons and hydrogen ions) and oxygen (O_2). This splitting of water, termed **photolysis,** is the ultimate source of the O_2 gas that is an important product of photosynthesis. The electrons released from the lysed water regenerate photosystem II for its next reaction with light.

2. Electrons generated by the first photoevent are immediately boosted through a series of carriers (cytochromes) to the P700 system. At this same time, hydrogen ions accumulate in the internal space of the thylakoid complex, thereby producing an electrochemical gradient. The P700 system (PS I) has been activated by light so that it is ready to accept electrons generated by the PS II. The electrons it receives are passed along a second transport chain to a complex that uses electrons and hydrogen ions to reduce NADP to NADPH.

A second energy reaction involves synthesis of ATP by a chemiosmotic mechanism. Channels in the thylakoids of the granum actively pump H_1 into the inner chamber, producing a charge gradient. ATP synthase located in this same thylakoid uses the energy from H 1 transport to phosphorylate ADP to ATP. Because it occurs in light, this process is termed **photophosphorylation.** Both NADPH and ATP are released into the stroma of the chloroplast, where they drive the reactions of the **Calvin cycle.**

The Light Reaction in Green and Purple Bacteria

Green and purple photosynthetic bacteria differ from cyanobacteria and eucaryotic photosynthesizers in several fundamental ways (**table**).

Table Properties of Microbial Photosynthetic Systems

Property	Eucaryotes	Cyanobacteria	Green and Purple Bacteria
Photosynthetic pigment	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll
Photosystem II	Present	Present	Absent
Photosynthetic electron donors	H_2O	H_2O	H_2, H_2S, S, organic matter
O_2 production pattern	Oxygenic	Oxygenic[a]	Anoxygenic
Primary products of energy conversion	ATP + NADPH	ATP + NADPH	ATP
Carbon source	CO_2	CO_2	Organic and/or CO_2

In particular, green and purple bacteria do not use water as an electron source or produce O2 photosynthetically, that is, they are **anoxygenic.** In contrast, cyanobacteria and eukaryotic photosynthesizers are almost always **oxygenic.** NADPH is not directly produced in the photosynthetic light reaction of purple bacteria.Green bacteria can reduce NAD+ directly during the light reaction. To synthesize NADH and NADPH, green and purple bacteria must use electron donors like hydrogen, hydrogen sulfide, elemental sulfur, and organic compounds that have more negative reduction potentials than water and are therefore easier to oxidize (better electron donors). Finally, green and purple bacteria possess slightly different photosynthetic pigments, **bacteriochlorophylls**, many with absorption maxima at longer wavelengths.

Bacteriochlorophylls *a* and *b* have maxima in ether at 775 and 790 nm, respectively. In vivo maxima are about 830 to 890 nm (bacteriochlorophyll *a*) and 1,020 to 1,040 nm (Bchl *b*). This shift of absorption maxima into the infrared region better adapts these bacteria to their ecological niches There are four groups of green and purple photosynthetic bacteria, each containing several genera: green sulfur bacteria (*Chlorobium*), green nonsulfur bacteria (*Chloroflexus*), purple sulfur bacteria (*Chromatium*), and purple nonsulfur bacteria (*Rhodospirillum, Rhodopseudomonas*).

Many differences found in green and purple bacteria are due to their lack of photosystem II; they cannot use water as an electron donor in noncyclic electron transport. Without photosystem II they cannot produce O2 from H2O photosynthetically and are restricted to cyclic photophosphorylation. Indeed, almost all purple and green sulfur bacteria are strict anaerobes. A tentative scheme for the photosynthetic electron transport chain of a purple nonsulfur bacterium is given in **figure.**

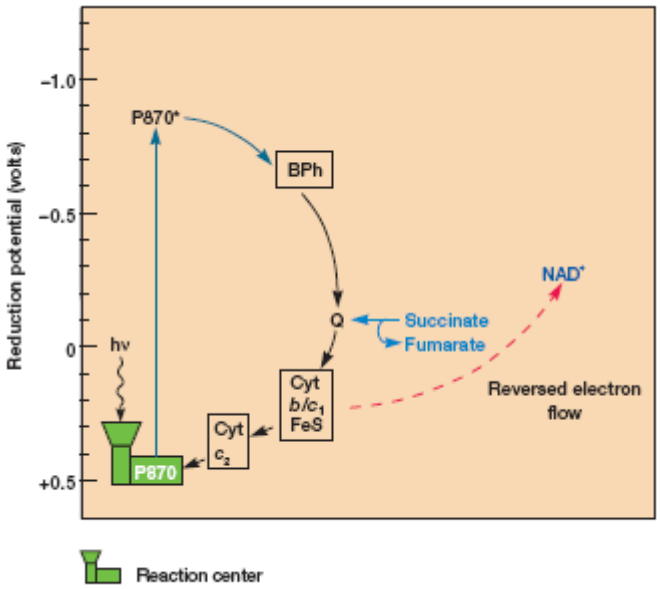


Figure Purple Nonsulfur Bacterial Photosynthesis. The photosynthetic electron transport system in the purple nonsulfur bacterium, *Rhodobacter sphaeroides.* This scheme is incomplete and tentative. Ubiquinone (Q) is very similar to coenzyme Q.

BPh stands for bacteriopheophytin. NAD_ and the electron source succinate are in color.

When the special reaction-center chlorophyll P870 is excited, it donates an electron to bacteriopheophytin. Electrons then flow to quinones and through an electron transport chain back to P870 while driving ATP synthesis. Note that although both green and purple bacteria lack two photosystems, the purple bacteria have a photosynthetic apparatus similar to photosystem II, whereas the green sulfur bacteria have a system similar to photosystem I. Green and purple bacteria face a further problem because they also require NADH or NADPH for CO2 incorporation. They may synthesize NADH in at least three ways. If they are growing in the presence of hydrogen gas, which has a reduction potential more negative than that of NAD+, the hydrogen can be used di- rectly to produce NADH. Like chemolithotrophs, many photosynthetic purple bacteria use proton motive force to reverse the flow of electrons in an electron transport chain and move them from inorganic or organic donors to NAD+ (**figures**).

Figure NAD Reduction in Green and Purple Bacteria. The use of reversed electron flow to reduce NAD+. The arrow in this diagram represents an electron transport chain that is being driven in reverse by proton motive force or ATP. That is, electrons are moving from donors with more positive reduction potentials to an acceptor (NAD+) with a more negative potential.

Green sulfur bacteria such as *Chlorobium* appear to carry out a simple form of noncyclic photosynthetic electron flow to reduce NAD+ (**figure**).

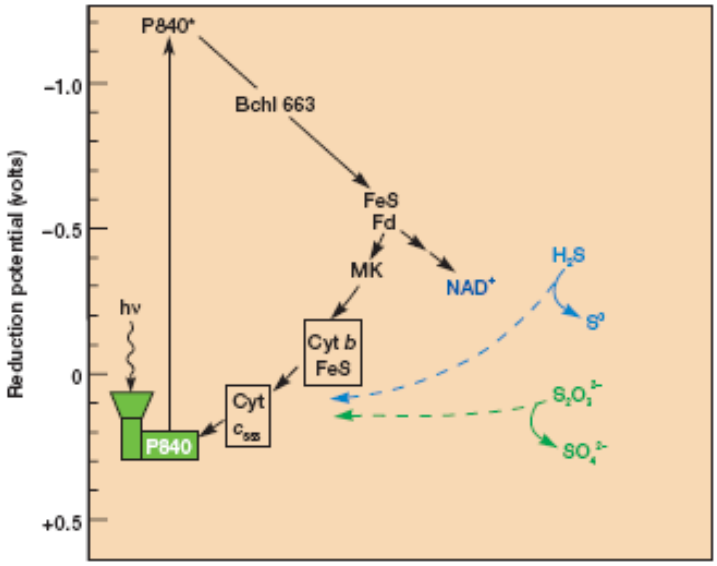


Figure Green Sulfur Bacterial Photosynthesis. The photosynthetic electron transport system in the green sulfur bacterium, *Chlorobium limicola*. Light energy is used to make ATP by cyclic photophosphorylation and move electrons from sulfur donors (green and blue) to NAD_ (red). The electron transport chain has a quinine called menaquinone (MK).

Light-Independent Reactions

The subsequent photosynthetic reactions that do not require light occur in the chloroplast stroma or the cytoplasm of cyanobacteria. These reactions use energy produced by the light phase to synthesize glucose by means of the Calvin cycle **(figure).**

The cycle begins at the point where CO 2 is combined with a doubly phosphorylated 5-carbon acceptor molecule called ribulose-1,5-bisphosphate **(RuBP) .** This process, called **carbon fixation,** generates a 6-carbon intermediate compound that immediately splits into two 3-carbon molecules of 3-phosphoglyceric acid (PGA). The

subsequent steps use the ATP and NADPH generated by the photosystems to form high-energy intermediates.

First, ATP adds a second phosphate to 3-PGA and produces 1,3-bisphosphoglyceric acid (BPG). Then, during the same step, NADPH contributes its hydrogen to BPG, and one high-energy phosphate is removed. These events give rise to glyceraldehyde-3- phosphate (PGAL). This molecule and its isomer dihydroxyacetone phosphate (DHAP) are key molecules in hexose synthesis leading to fructose and glucose.

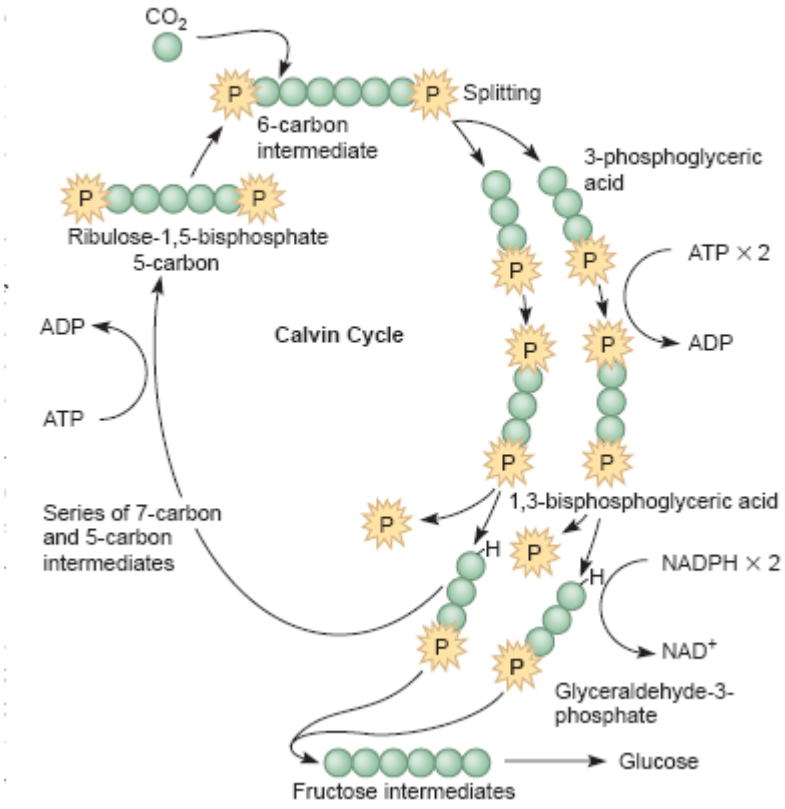


Figure The Calvin cycle.

The main events of the reactions in photosynthesis that do not require light. It is during this cycle that carbon is fixed into organic form using the energy (ATP and NADPH) released by the light reactions. The end product, glucose, can be stored as complex carbohydrates, or it can be used in various amphibolic pathways to produce other carbohydrate intermediates or amino acids.

Other Mechanisms of Photosynthesis

The **oxygenic,** or oxygen-releasing, photosynthesis that occurs in plants, algae, and cyanobacteria is the dominant type on the earth. Other photosynthesizers such as green and purple bacteria possess bacteriochlorophyll, which is more versatile in capturing light. They have only a cyclic photosystem I, which routes the electrons from the photocenter to the electron carriers and back to the photosystem again. This pathway generates a relatively small amount of ATP, and it may not produce NADPH. As photolithotrophs, these bacteria use H 2 , H 2 S, or elemental sulfur rather than H 2 O as a source of electrons and reducing power. As a consequence, they are **anoxygenic** (nonoxygen- producing), and many are strict anaerobes.

Photosynthetic Bacteria

There are three groups of photosynthetic bacteria: the purple bacteria, the green bacteria, and the cyanobacteria (**table**).

Table Characteristics of the Major Groups of Photosynthetic Bacteria

	Anoxygenic Photosynthetic Bacteria				*Oxygenic Photosynthetic Bacteria*
Characteristic	**Green Sulfur[a]**	**Green Nonsulfur[b]**	**Purple Sulfur**	**Purple Nonsulfur**	**Cyanobacteria**
Major photosynthetic pigments	Bacteriochlorophylls *a* plus *c*, *d*, or *e* (the major pigment)	Bacteriochlorophylls *a* and *c*	Bacteriochlorophyll *a* or *b*	Bacteriochlorophyll *a* or *b*	Chlorophyll *a* plus phycobiliproteins
Morphology of photosynthetic membranes	Photosynthetic system partly in chlorosomes that are independent of the plasma membrane	Chlorosomes present when grown anaerobically	Photosynthetic system contained in spherical or lamellar membrane complexes that are continuous with the plasma membrane	Photosynthetic system contained in spherical or lamellar membrane complexes that are continuous with the plasma membrane	Membranes lined with phycobilisomes
Photosynthetic electron donors	H_2, H_2S, S	Photoheterotrophic donors—a variety of sugars, amino acids, and organic acids; photoautotrophic donors—H_2S, H_2	H_2, H_2S, S	Usually organic molecules; sometimes reduced sulfur compounds or H_2	H_2O
Sulfur deposition	Outside of the cell		Inside the cell[c]	Outside of the cell	
Nature of photosynthesis	Anoxygenic	Anoxygenic	Anoxygenic	Anoxygenic	Oxygenic (sometimes facultatively anoxygenic)
General metabolic type	Obligately anaerobic photolithoautotrophs	Usually photoheterotrophic; sometimes photoautotrophic or chemoheterotrophic (when aerobic and in the dark)	Obligately anaerobic photolithoautotrophs	Usually anaerobic photoorganoheterotrophs; some facultative photolithoautotrophs (in dark, chemoorganoheterotrophs)	Aerobic photolithoautotrophs
Motility	Nonmotile; some have gas vesicles	Gliding	Motile with polar flagella; some are peritrichously flagellated	Motile with polar flagella or nonmotile; some have gas vesicles	Nonmotile or with gliding motility; some have gas vesicles
Percent G + C	48–58	53–55	45–70	61–72	35–71

The cyanobacteria differ most fundamentally from the green and purple photosynthetic bacteria in being able to carry out **oxygenic photosynthesis.** They use water as an electron donor and generate oxygen during photosynthesis. In contrast, purple and green bacteria use **anoxygenic photosynthesis.** Because they are unable to use water as an electron source, they employ reduced molecules such as hydrogen sulfide, sulfur, hydrogen, and organic matter as their electron source for the generation of NADH and NADPH. Consequently, purple and green bacteria do not produce oxygen but many form sulfur granules. Purple sulfur bacteria accumulate granules within their cells, whereas green sulfur bacteria deposit the sulfur granules outside their cells. The purple nonsulfur bacteria use organic molecules as an electron source. There also are

differences in photosynthetic pigments, the organization of photosynthetic membranes, nutritional requirements, and oxygen relationships.

Normally green and purple bacteria are anaerobic and use H2S and other reduced

electron donors during photosynthesis. Because these bacteria grow best in deeper anaerobic zones of aquatic habitats, they cannot effectively use parts of the visible spectrum normally employed by photosynthetic organisms. There often is a dense surface layer of cyanobacteria and algae in lakes and ponds that absorbs a large amount of blue and red light. The bacteriochlorophyllpigments of purple and green bacteria absorb longer wavelength, far-red light not used by other photosynthesizers. In addition, the bacteriochlorophyll absorption peaks at about 350 to 550 nm enable them to grow at greater depths because shorter wavelength light can penetrate water farther. As a result, when the water is sufficiently clear, a layer of green and purple bacteria develops in the anaerobic, hydrogen sulfide-rich zone.

The second edition of *Bergey's Manual* places photosynthetic bacteria into six major groups. The phylum *Chloroflexi* contains the green nonsulfur bacteria, and the phylum *Chlorobi*, the green sulfur bacteria. The cyanobacteria are placed in their own phylum, *Cyanobacteria*. Purple bacteria are divided between three groups. Purple sulfur bacteria are placed in the γ-proteobacteria, families *Chromatiaceae* and *Ectothiorhodospiraceae*. The purple nonsulfur bacteria are distributed between the α -proteobacteria (five different families) and one family of the β-proteobacteria.

Phylum *Chloroflexi*

The phylum *Chloroflexi* has both photosynthetic and nonphotosynthetic members. *Chloroflexus* is the major representative of the photosynthetic **green nonsulfur bacteria.** It is a filamentous, gliding, thermophilic bacterium that often is isolated from neutral to alkaline hot springs where it grows in the form of orange-reddish mats, usually in association with cyanobacteria. Although it resembles the green bacteria in ultrastructure and photosynthetic pigments, its metabolism is more similar to that of the purple nonsulfur bacteria. *Chloroflexus* can carry out anoxygenic photosynthesis with organic compounds as carbon sources or grow aerobically as a chemoheterotroph. It doesn't appear closely related to any bacterial group based on 16S Rrna studies and is a deep and ancient branch of the bacterial tree.

The nonphotosynthetic, gliding, rod-shaped or filamentous bacterium *Herpetosiphon* also is included in this phylum. *Herpetosiphon* is an aerobic chemoorganotroph with respiratory metabolism and oxygen as the electron acceptor. It can be isolated from freshwater and soil habitats.

Phylum *Chlorobi*

The phylum *Chlorobi* has only one class (*Chlorobia*), order (*Chlorobiales*), and family (*Chlorobiaceae*). The **green sulfur bacteria** are a small group of obligately anaerobic photolithoautotrophs that use hydrogen sulfide, elemental sulfur, and hydrogen

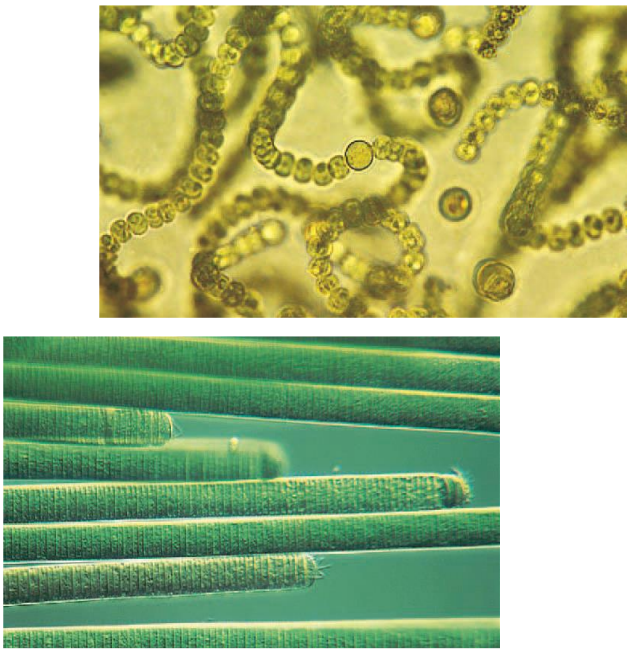
as electron sources. The elemental sulfur produced by sulfide oxidation is deposited outside the cell. Their photosynthetic pigments are located in ellipsoidal vesicles called **chlorosomes** or chlorobium vesicles, which are attached to the plasma membrane but are not continuous with it. The chlorosome membrane is not a normal lipid bilayer or unit membrane. Chlorosomes contain accessory bacteriochlorophyll pigments, but the reaction center bacteriochlorophyll is located in the plasma membrane and must be able to obtain energy from chlorosome pigments.

These bacteria flourish in the anaerobic, sulfide-rich zones of lakes. Although they lack flagella and are nonmotile, some species have gas vesicles to adjust their depth for optimal light and hydrogen sulfide. Those forms without vesicles are found in sulfide-rich muds at the bottom of lakes and ponds. The green sulfur bacteria are very diverse morphologically. They may be rods, cocci, or vibrios; some grow singly, and others form chains and clusters. They are either grass-green or chocolate-brown in color. Representative genera are *Chlorobium, Prosthecochloris,* and *Pelodictyon.*

Phylum *Cyanobacteria*

The **cyanobacteria** are the largest and most diverse group of photosynthetic bacteria. There is little agreement about the number of cyanobacterial species. Older classifications had as many as 2,000 or more species. In one recent system this has been reduced to 62 species and 24 genera. The second edition of *Bergey's Manual of Determinative Bacteriology* describes 56 genera in some detail. The G _C content of the group ranges from 35 to 71%. Although cyanobacteria are true procaryotes, their photosynthetic system closely resembles that of the eucaryotes because they have chlorophyll *a* and photosystem II, and carry out oxygenic photosynthesis. Like the red algae, cyanobacteria use phycobiliproteins as accessory pigments. Photosynthetic pigments and electron transport chain components are located in thylakoid membranes lined with particles called **phycobilisomes**. These contain phycobilin pigments, particularly phycocyanin, and transfer energy to photosystem II. Carbon dioxide is assimilated through the Calvin cycle, and the reserve carbohydrate is glycogen. Sometimes they will store extra nitrogen as polymers of arginine or aspartic acid in cyanophycin granules. Since cyanobacteria lack the enzyme α-ketoglutarate dehydrogenase, they do not have a fully functional citric acid cycle. The pentose phosphate pathway plays a central role in their carbohydrate metabolism. Although many cyanobacteria are obligate photolithoautotrophs, some can grow slowly in the dark as chemoheterotrophs by oxidizing glucose and a few other sugars. Under

anaerobic conditions *Oscillatoria limnetica* oxidizes hydrogen sulfide instead of water and carries out anoxygenic photosynthesis much like the green photosynthetic bacteria. As these examples illustrate, cyanobacteria are capable of considerable metabolic flexibility. Cyanobacteria also vary greatly in shape and appearance (**figure**).



(a) (b)

Figure Oxygenic Photosynthetic Bacteria. Representative cyanobacteria. (**a**) *Nostoc* with heterocysts (_550). (**b**) *Oscillatoria* trichomes seen with Nomarski interference-contrast optics (_250).

A **trichome** is a row of bacterial cells that are in close contact with one another over a large area. In contrast, adjacent cells in a simple chain (such as those commonly found in the genus *Bacillus*) associate by only a small area of contact. Although most appear blue-green because of phycocyanin, a few are red or brown in color because of the red pigment phycoerythrin. Despite this variety, cyanobacteria have typical prokaryotic cell structures and a normal gram-negative type cell wall (**figure**).

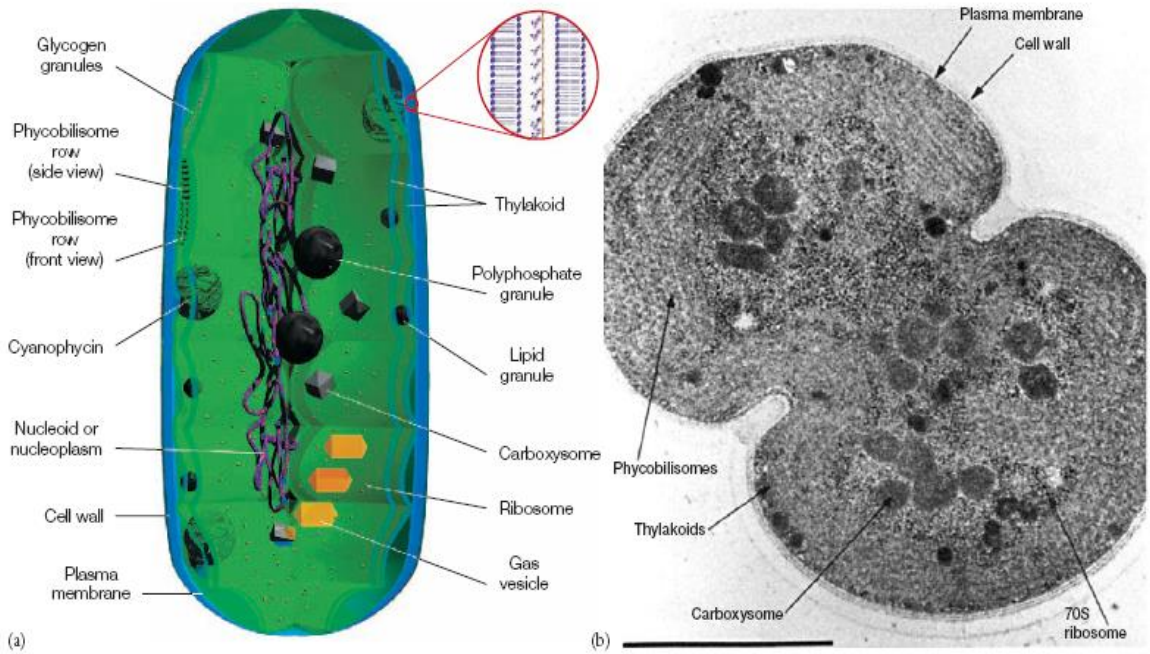


Figure Cyanobacterial Cell Structure. (**a**) Schematic diagram of a vegetative cell.

The insert shows an enlarged view of the envelope with its outer membrane and peptidoglycan. (**b**) Thin section of *Synechocystis* during division. Many structures are visible.

They often use gas vesicles to move vertically in the water, and many filamentous species have gliding motility Although cyanobacteria lack flagella, several strains of the marine genus *Synechococcus* are able to move at rates of up to 25 μm/second by means of an unknown mechanism. Cyanobacteria show great diversity with respect to reproduction and employ a variety of mechanisms: binary fission, budding, fragmentation, and multiple fission. In the last process a cell enlarges and then divides several times to produce many smaller progeny, which are released upon the rupture of the parental cell. Fragmentation of filamentous cyanobacteria can generate

small, motile filaments called **hormogonia.** Some species develop **akinetes,** specialized, dormant, thick-walled resting cells that are resistant to desiccation. Often these germinate to form new filaments. Many filamentous cyanobacteria fix atmospheric nitrogen by means of special cells called **heterocysts**. Around 5 to 10% of the cells can develop into heterocysts when cyanobacteria are deprived of both nitrate and ammonia, their preferred nitrogen sources. When transforming themselves into heterocysts, cyanobacterial cells synthesize a very thick new wall, reorganize their photosynthetic membranes, discard their phycobiliproteins and photosystem II, and synthesize the nitrogen-fixing enzyme nitrogenase. Photosystem I is still functional and produces ATP, but no oxygen arises from noncyclic photophosphorylation because photosystem II is absent. This inability to generate O2 is critical because the nitrogenase is extremely oxygen sensitive. The heterocyst wall slows or prevents O2 diffusion into the cell, and any O2 present is consumed during respiration. The structure and physiology of the heterocyst ensures that it will remain anaerobic; it is dedicated to nitrogen fixation. It obtains nutrients from adjacent vegetative cells and contributes fixed nitrogen in the form of the amino acid glutamine. It should be noted that nitrogen fixation also is carried out by cyanobacteria that lack heterocysts. Some fix nitrogen under dark, anoxic conditions in microbial mats. Planktonic forms such as *Trichodesmium* can fix nitrogen as well.

Prochlorophytes are oxygenic phototrophic procaryotes that have both chlorophyll *a* and *b* but lack phycobilins. Thus although they resemble other cyanobacteria with respect to chlorophyll *a*, they differ in also possessing chlorophyll *b*, the only procaryotes

to do so. Because prochlorophytes lack phycobilin pigments and phycobilisomes, they are grass-green in color. They resemble chloroplasts in their pigments and thylakoid structure, but their 5S and 16S rRNAs place them within the cyanobacteria. The genera are located in subsections I and III. Although the prochlorophytes have been classified separately in the past, the second edition of

Bergey's Manual places them within the cyanobacteria. The three recognized prochlorophyte genera are quite different from one another. *Prochloron* was first discovered as an extracellular symbiont growing either on

the surface or within the cloacal cavity of marine colonial ascidian invertebrates. These bacteria are single-celled, spherical, and from 8 to 30 μm in diameter. Their mol% of G +

C is 31 to 41. *Prochlorothrix* is free living, has cylindrical cells that form filaments, and has been found in Dutch lakes. Its DNA has a higher G + C content (53 mol%). Unlike *Prochloron* it has been cultured in the laboratory. *Prochlorococcus marinus,* less than 1 μm in diameter, has recently been discovered flourishing about 100 meters below the ocean surface. It differs from other prochlorophytes in having divinyl chlorophyll *a* and β-carotene instead of chlorophyll *a* and β-carotene. During the summer, it reaches concentrations of 5 _ 105 cells per milliliter. It is one of the most numerous of the marine

plankton and a significant component of the marine microbial foodweb.

Table Characteristics of the Cyanobacterial Subsections

Subsection	General Shape	Reproduction and Growth	Heterocysts	% G + C	Other Properties	Representative Genera
I	Unicellular rods or cocci; nonfilamentous aggregates	Binary fission, budding	–	31–71	Almost always nonmotile	*Chamaesiphon* *Chroococcus* *Gloeothece* *Gleocapsa* *Prochloron*
II	Unicellular rods or cocci; may be held together in aggregates	Multiple fission to form baeocytes	–	40–46	Only some baeocytes are motile	*Pleurocapsa* *Dermocarpa* *Chroococcidiopsis*
III	Filamentous, unbranched trichome with only vegetative cells	Binary fission in a single plane, fragmentation	–	34–67	Usually motile	*Lyngbya* *Oscillatoria* *Prochlorothrix* *Spirulina* *Pseudanabaena*
IV	Filamentous, unbranched trichome may contain specialized cells	Binary fission in a single plane, fragmentation to form hormogonia	+	38–47	Often motile, may produce akinetes	*Anabaena* *Cylindrospermum* *Aphanizomenon* *Nostoc* *Scytonema* *Calothrix*
V	Filamentous trichomes either with branches or composed of more than one row of cells	Binary fission in more than one plane, hormogonia formed	+	42–44	May produce akinetes, greatest morphological complexity and differentiation in cyanobacteria	*Fischerella* *Stigonema* *Geitleria*

Subsection I contains unicellular rods or cocci that are almost always nonmotile and reproduce by binary fission or budding. Organisms in subsection II are also unicellular, though several individual cells may be held together in an aggregate by an outer wall. Members of this group reproduce by multiple fission to form spherical, very small, reproductive cells, often called **baeocytes,** which escape when the outer wall ruptures. Some baeocytes disperse through gliding motility. The other three subsections contain filamentous cyanobacteria. Usually the trichome is unbranched and often is surrounded by a sheath or slime layer. Cyanobacteria in subsection III form unbranched trichomes composed only of vegetative cells, whereas the other two subsections produce heterocysts in the absence of an adequate nitrogen source (they also may form akinetes). Heterocystous cyanobacteria are subdivided into those that form linear filaments

(subsection IV) and cyanobacteria that divide in a second plane to produce branches or aggregates (subsection V).

Cyanobacteria are very tolerant of environmental extremes and are present in almost all waters and soils. Thermophilic species may grow at temperatures of up to 75°C in neutral to alkaline hot springs. Because these photoautotrophs are so hardy, they are primary colonizers of soils and surfaces that are devoid of plant growth. Some unicellular forms even grow in the fissures of desert rocks. In nutrient-rich warm ponds and lakes, surface cyanobacteria such as *Anacystis* and *Anabaena* can reproduce rapidly to form blooms. The release of large amounts of organic matter upon the death of the bloom microorganisms stimulates the growth of chemoheterotrophic bacteria that subsequently depletes the available oxygen. This kills fish and other organisms.

Some species can produce toxins that kill livestock and other animals that drink the water. Other cyanobacteria, for example, *Oscillatoria*, are so pollution resistant and characteristic of freshwater with high organic matter content that they are used as water pollution indicators. Cyanobacteria are particularly successful in establishing symbiotic relationships with other organisms. They are the photosynthetic partner in most lichen associations. Cyanobacteria are symbionts with protozoa and fungi, and nitrogen-fixing species form associations with a variety of plants (liverworts, mosses, gymnosperms, and angiosperms).

The proteobacteria are the largest and most diverse group of bacteria; currently there are over 380 genera and 1,300 species. Although the 16S rRNA studies show that they are phylogenetically related, proteobacteria vary markedly in many respects. The morphology of these gram-negative bacteria ranges from simple rods and cocci to genera with prosthecae, buds, and even fruiting bodies. Physiologically these bacteria are just as diverse. Photoautotrophs, chemolithotrophs, and chemoheterotrophs are all well represented. There is no obvious overall pattern in metabolism, morphology, or reproductive strategy of the proteobacteria. Comparison of 16S rRNA sequences has revealed what appears to be five subgroups of proteobacteria. The second edition of

Bergey's Manual places all these procaryotes in the phylum *Proteobacteria,* which has five classes: *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria,* and *Epsilonproteobacteria.* Members of the purple photosynthetic bacteria are found among the α-, β-, and γ- proteobacteria. This has led to the proposal that the proteobacteria arose from a photosynthetic ancestor, presumably similar to the purple bacteria. Subsequently photosynthesis would have been lost by various lines, and new metabolic capacities would have been acquired as these bacteria adapted to different ecological niches.

Class *Alphaproteobacteria*

The α **-proteobacteria** include most of the oligotrophic proteobacteria (those capable of growing at low nutrient levels). Some have unusual metabolic modes such as methylotrophy (*Methylobacterium*), chemolithotrophy (*Nitrobacter*), and the ability to fix nitrogen (*Rhizobium*). Members of genera such as *Rickettsia* and *Brucella* are important pathogens; in fact, *Rickettsia* has become an obligately intracellular parasite. Many genera are characterized by distinctive morphology such as prosthecae. The class *Alphaproteobacteria* has six orders and 18 families.

Table Characteristics of Selected α -Proteobacteria

Genus	Dimensions (μm) and Morphology	G + C Content (mol%)	Oxygen Requirement	Other Distinctive Characteristics
Agrobacterium	0.6–1.0 × 1.5–3.0; motile, nonsporing rods with peritrichous flagella	57–63	Aerobic	Chemoorganotroph that can invade plants and cause tumors
Caulobacter	0.4–0.6 × 1–2; rod- or vibrioid-shaped with a flagellum and prostheca and holdfast	62–67	Aerobic	Heterotrophic and oligotrophic; asymmetric cell division
Hyphomicrobium	0.3–1.2 × 1–3; rod-shaped or oval with polar prosthecae	59–65	Aerobic	Reproduces by budding; methylotrophic
Nitrobacter	0.5–0.8 × 1.0–2.0; rod- or pear-shaped, sometimes motile by flagella	60–62	Aerobic	Chemolithotroph, oxidizes nitrite to nitrate
Rhizobium	0.5–0.9 × 1.2–3.0; motile rods with flagella	59–64	Aerobic	Invades leguminous plants to produce nitrogen-fixing root nodules
Rhodospirillum	0.7–1.5 wide; spiral cells with polar flagella	62–64	Anaerobic, microaerobic, aerobic	Photoheterotroph under anaerobic conditions
Rickettsia	0.3–0.5 × 0.8–2.0; short nonmotile rods	29–33	Aerobic	Obligately intracellular parasite

The Purple Nonsulfur Bacteria

All the purple bacteria use anoxygenic photosynthesis, possess bacteriochlorophylls *a* or *b,* and have their photosynthetic apparatus in membrane systems that are continuous with the plasma membrane. Most are motile by polar flagella. With the exception of *Rhodocyclus* (α -proteobacteria), the purple nonsulfur bacteria are located among the α -proteobacteria. The **purple nonsulfur bacteria** are exceptionally flexible in their choice of an energy source. Normally they grow anaerobically as photoorganoheterotrophs; they trap light energy and employ organic molecules as both electron and carbon sources. Although they are called nonsulfur bacteria, some species can oxidize very low, nontoxic levels of sulfide to sulfate, but they do not oxidize elemental sulfur to sulfate. In the absence of light, most purple nonsulfur bacteria can grow aerobically as chemoorganoheterotrophs, but some species

carry out fermentations and grow anaerobically. Oxygen inhibits bacteriochlorophyll and carotenoid synthesis so that cultures growing aerobically in the dark are colorless. Purple nonsulfur bacteria vary considerably in morphology. They may be spirals (*Rhodospirillum*), rods (*Rhodopseudomonas*), half circles or circles (*Rhodocyclus*), or they may even form prosthecae and buds (*Rhodomicrobium*). Because of their metabolism, they are most prevalent in the mud and water of lakes and ponds with abundant organic matter and low sulfide levels. There also are marine species.

Rickettsia* and *Coxiella

In the second edition of *Bergey's Manual,* the genus *Rickettsia* will be located in the order *Rickettsiales* and family *Rickettsiaceae* of the α-proteobacteria, whereas *Coxiella* will be in the order *Legionellales* and family *Coxiellaceae* of the α-proteobacteria. The first edition places these two genera and other bacteria that grow intracellularly in a separate section, and we also will discuss *Rickettsia* and *Coxiella* together because of their similarity in life-style, despite their apparent phylogenetic distance. These bacteria are rod-shaped, coccoid, or pleomorphic with typical gram-negative walls and no flagella. Although their size varies, they tend to be very small. For example, *Rickettsia* is 0.3 to 0.5 μm in diameter and 0.8 to 2.0 μm long; *Coxiella* is 0.2 to 0.4 μm by 0.4 to 1.0 μm. All species are parasitic or mutualistic. The parasitic forms grow in vertebrate erythrocytes, macrophages, and vascular endothelial cells. Often they also live in blood-sucking arthropods such as fleas, ticks, mites, or lice, which serve as vectors or primary hosts. Because these genera contain important human pathogens, their reproduction and metabolism have been intensively studied. Rickettsias enter the host cell by inducing phagocytosis. Members of the genus *Rickettsia* immediately escape the phagosome and reproduce by binary fission in the cytoplasm (**figure**). In contrast, *Coxiella* remains within the phagosome after it has fused with a lysosome and actually reproduces within the phagolysosome. Eventually the host cell

bursts, releasing new organisms. Besides incurring damage from cell lysis, the host is harmed by the toxic effects of rickettsial cell walls (wall toxicity appears related to the mechanism of penetration into host cells). Rickettsia are very different from most other bacteria in physiology and metabolism. They lack the glycolytic pathway and do not use glucose as an energy source, but rather oxidize glutamate and tricarboxylic acid cycle intermediates such as succinate. The rickettsial plasma membrane has carrier-mediated transport systems, and host cell nutrients and coenzymes are absorbed and directly used. For example, rickettsias take up both NAD and uridine diphosphate glucose. Their membrane also has an adenylate exchange carrier that exchanges ADP for external ATP. Thus host ATP may pro vide much of the energy needed for growth. This metabolic dependence explains why many of these organisms must be cultivated in the yolk sacs of chick embryos or in tissue culture cells. Results from genome sequencing show that *R. prowazekii* is similar in many ways to mitochondria. Possibly mitochondria arose from an endosymbiotic association with an ancestor of *Rickettsia.* This order contains many important pathogens. *Rickettsia prowazekii* and *R. typhi* are associated with typhus fever, and *R. rickettsii*, with Rocky Mountain spotted fever. *Coxiella burnetii* causes Q fever in humans. The α-**proteobacteria** overlap the α-proteobacteria metabolically but tend to use substances that diffuse from organic decomposition in the anaerobic zone of habitats. Some of these bacteria use hydrogen, ammonia, methane, volatile fatty acids, and similar substances. As with the α-proteobacteria, there is considerable metabolic diversity; the α-proteobacteria may be chemoheterotrophs, otolithotrophs, methylotrophs, and chemolithotrophs. The subgroup contains two genera with important human pathogens: *Neisseria* and *Bordetella.* The class *Betaproteobacteria* has six orders and 12 families.

Order *Neisseriales*

The second edition places one family, *Neisseriaceae*, within the order and assigns 14 genera to it. The best-known and most intensely studied genus is *Neisseria.* Members of this genus are nonmotile, aerobic, gram-negative cocci that most often occur in pairs with adjacent sides flattened (*figure)*. They may have capsules and fimbriae. The genus is chemoorganotrophic, oxidase positive, and almost always catalase positive. Species are inhabitants of the mucous membranes of mammals, and some are human pathogens. *Neisseria gonorrhoeae* is the causative agent of gonorrhea; *Neisseria meningitidis* is responsible for some cases of bacterial meningitis.

Order *Burkholderiales*

The order contains five families, three of them with well-known genera. The genus *Burkholderia* is placed in the family *Burkholderiaceae.* This genus was established when *Pseudomonas* was divided into at least seven new genera based on rRNA data: *Acidovorax, Aminobacter, Burkholderia, Comamonas, Deleya, Hydrogenophaga*, and *Methylobacterium.* Members of the genus *Burkholderia* are gram-negative, aerobic, nonfermentative, non–spore-forming, mesophilic straight rods. With the exception of one species, all are motile with a single polar flagellum or a tuft of polar flagella.

Table Characteristics of Selected β-Proteobacteria

Genus	Dimensions (μm) and Morphology	G + C Content (mol%)	Oxygen Requirement	Other Distinctive Characteristics
Bordetella	0.2–0.5 × 0.5–2.0; nonmotile coccobacillus	66–70	Aerobic	Requires organic sulfur and nitrogen; mammalian parasite
Burkholderia	0.3–1.0 × 1–5; straight rods with single flagella or a tuft at the pole	59–69.5	Aerobic	Poly-β-hydroxybutyrate as reserve; can be pathogenic
Leptothrix	0.6–1.4 × 1–12; straight rods in chains with sheath, free cells flagellated	69.5–71	Aerobic	Sheaths encrusted with iron and manganese oxides
Neisseria	0.6–1.0; cocci in pairs with flattened adjacent sides	46–54	Aerobic	Inhabitant of mucous membranes of mammals
Nitrosomonas	Size varies with strain; rod-shaped or ellipsoidal cells with intracytoplasmic membranes	45–54	Aerobic	Chemolithotroph that oxidizes ammonia to nitrite
Sphaerotilus	1.2–2.5 × 2–10; single chains of cells with sheaths, may have holdfasts	70	Aerobic	Sheaths not encrusted with iron and manganese oxides
Thiobacillus	0.5 × 1–4; rods, often with polar flagella	52–68	Aerobic	Chemolithotroph, oxidizes reduced sulfur compounds to sulfate

Catalase is produced and they often are oxidase positive. Most species use poly-β-hydroxybutyrate as their carbon reserve. One of the most important species is *B. cepacia*, which will degrade over 100 different organic molecules and is very active in recycling organic materials in nature. This species also is a plant pathogen and causes disease in hospital patients due to contaminated equipment and medications. It is a particular problem for cystic fibrosis patients. The family *Alcaligenaceae* contains the genus *Bordetella*. This genus is composed of gram-negative, aerobic coccobacilli, about 0.2 to 0.5 by 0.5 to 2.0 μm in size. *Bordetella* is a chemoorganotroph with respiratory metabolism that requires organic sulfur and nitrogen (amino acids) for growth. It is a mammalian parasite that multiplies in respiratory epithelial cells. *Bordetella pertussis* is a nonmotile, encapsulated species that causes whooping cough.

The family *Comamonadaceae* contains 15 genera with quite diverse characteristics. Some genera (e.g., *Sphaerotilus* and *Leptothrix*) have a **sheath,** a hollow tubelike structure surrounding a chain of cells. Sheaths often are close fitting, but they are never in intimate contact with the cells they enclose and may contain ferric or manganic oxides. They have at least two functions. Sheaths help bacteria attach to solid surfaces and acquire nutrients from slowly running water as it flows past, even if it is nutrient-poor. Sheaths also protect against predators such as protozoa and *Bdellovibrio*. Two well-studied genera are *Sphaerotilus* and *Leptothrix*. *Sphaerotilus* forms long sheathed chains of rods, 0.7 to 2.4 by 3 to 10 μm, attached to submerged plants, rocks, and other solid objects, often by a holdfast (figure). The sheaths are not usually encrusted by metal oxides. Single swarmer cells with a bundle of subpolar flagella escape the filament and form a new chain after attaching to a solid object at another site. *Sphaerotilus* prefers slowly running freshwater polluted with sewage or industrial waste. It grows so well in activated sewage sludge that it sometimes forms tangled masses of filaments and interferes with the proper settling of sludge. *Leptothrix* characteristically deposits large amounts of iron and manganese oxides in its sheath. This seems to protect it and allow *Leptothrix* to grow in the presence of high concentrations of soluble iron compounds.

Order *Nitrosomonadales*

A number of chemolithotrophs are found in the order *Nitrosomonadales.* Two genera of nitrifying bacteria (*Nitrosomonas* and *Nitrosospira*) are located here in the family *Nitrosomonadaceae* but were discussed earlier along with other genera of nitrifying bacteria. The stalked chemolithotroph *Gallionella* is in this order. The family *Spirillaceae* has one genus, *Spirillum*.

Order *Hydrogenophilales*

This small order contains *Thiobacillus,* one of the best-studied chemolithotrophs and most prominent of the colorless sulfur bacteria. Like the nitrifying bacteria, **colorless sulfur bacteria** are a highly diverse group. Many are unicellular rod-shaped or spiral sulfur-oxidizing bacteria that are nonmotile or motile by flagella (**table**). The second edition disperses these bacteria between

Table Colorless Sulfur-Oxidizing Genera

Genus	Cell Shape	Motility, Flagella	% G + C	Location of Sulfur Deposit[a]	Nutritional Type
Thiobacillus	Rods	+; polar	52–68	Extracellular	Obligate or facultative chemolithotroph
Thiomicrospira	Spirals	+; polar	36–44	Extracellular	Obligate chemolithotroph
Thiobacterium	Rods embedded in gelatinous masses	–	N.A.[b]	Intracellular	Probably chemoorganoheterotroph
Thiospira	Spiral rods, usually with pointed ends	+; polar (single or in tufts)	N.A.	Intracellular	Unknown
Macromonas	Rods, cylindrical or bean shaped	+; polar	67	Intracellular	Probably chemoorganoheterotroph

[a]When hydrogen sulfide is oxidized to elemental sulfur.
[b]N.A., data not available.

two classes; for example, *Thiobacillus* is in the α-proteobacteria, whereas *Thiomicrospira, Thiobacterium, Thiospira, Macromonas, Thiothrix, Beggiatoa,* and others are in the α-proteobacteria. Only some of these bacteria have been isolated and studied in pure culture. Most is known about the genera *Thiobacillus* and *Thiomicrospira.*

Thiobacillus is a gram-negative rod, and *Thiomicrospira* is a long spiral cell (**figure**); both are polarly flagellated. They differ from many of the nitrifying bacteria in that they lack extensive internal membrane systems. The metabolism of *Thiobacillus* has been intensely studied. It grows aerobically by oxidizing a variety of inorganic

sulfur compounds (elemental sulfur, hydrogen sulfide, thiosulfate) to sulfate. ATP is produced with a combination of oxidative phosphorylation and substrate-level phosphorylation by means of adenosine 5′-phosphosulfate. Although *Thiobacillus* normally uses CO2 as its major carbon source, *T. novellus* and a few other strains can grow heterotrophically. Some species are very flexible metabolically. For example, *Thiobacillus ferrooxidans* also uses ferrous iron as an electron donor and produces ferric iron as well as sulfuric acid. *T. denitrificans* even grows anaerobically by reducing nitrate to nitrogen gas. It should be noted that other sulfur-oxidizing bacteria such as *Thiobacterium* and *Macromonas* probably do not derive energy from sulfur oxidation. They may use the process to detoxify metabolically produced hydrogen peroxide. Sulfur-oxidizing bacteria have a wide distribution and great practical importance. *Thiobacillus* grows in soil and aquatic habitats, both freshwater and marine. In marine habitats *Thiomicrospira* is more important than *Thiobacillus.* Because of their great acid tolerance (*T. thiooxidans* grows at pH 0.5 and cannot grow above pH 6), these bacteria prosper in habitats they have acidified by sulfuric acid production, even though most other organisms are dying. The production of large quantities of sulfuric acid and ferric iron by *T. ferrooxidans* corrodes concrete and pipe structures. Thiobacilli often cause extensive acid and metal pollution when they release metals from mine wastes. However, sulfur-oxidizing bacteria also are beneficial. They may increase soil fertility when they release elemental sulfur by oxidizing it to sulfate. Thiobacilli are used in processing low-grade metal ores because of their ability to leach metals from ore.

Class *Gammaproteobacteria*

The γ-**proteobacteria** constitute the largest subgroup of proteobacteria with an extraordinary variety of physiological types. Many important genera are chemoorganotrophic and facultatively anaerobic. Other genera contain aerobic

chemoorganotrophs, photolithotrophs, chemolithotrophs, or methylotrophs. According to

some DNA-rRNA hybridization studies, the α-proteobacteria are composed of several deeply branching groups. One consists of the purple sulfur bacteria; a second includes the intracellular parasites *Legionella* and *Coxiella.* The two largest groups contain a wide variety of nonphotosynthetic genera. Ribosomal RNA superfamily I is represented by the families *Vibrionaceae, Enterobacteriaceae,* and *Pasteurellaceae.* These bacteria use the glycolytic and pentose phosphate pathways to catabolize carbohydrates. Most are facultative anaerobes. Ribosomal RNA superfamily II contains mostly aerobes that often use the Entner-Doudoroff and pentose phosphate pathways to catabolize many different kinds of organic molecules. The genera *Pseudomonas, Azotobacter, Moraxella, Xanthomonas,* and *Acinetobacter* belong to this superfamily. The exceptional diversity of these bacteria is evident from the fact that the second edition of *Bergey's Manual* divides the class *Gammaproteobacteria* into 13 orders, 20 families, and around 160 genera. **Figure** illustrates the phylogenetic relationships between major groups and selected α-proteobacteria, and **table** outlines the general characteristics of some of the bacteria discussed in this section.

The Purple Sulfur Bacteria

The purple photosynthetic bacteria are distributed between three subgroups of the proteobacteria.

Table Characteristics of Selected γ-Proteobacteria

Genus	Dimensions (μm) and Morphology	G + C Content (mol%)	Oxygen Requirement	Other Distinctive Characteristics
Azotobacter	1.5–2.0; ovoid cells, pleomorphic, peritrichous or nonmotile	63.2–67.5	Aerobic	Can form cysts; fix nitrogen nonsymbiotically
Beggiatoa	~1–50 × ~2–10; colorless cells form filaments, either single or in colonies	37–51	Aerobic or microaerophilic	Gliding motility; can form sulfur inclusions with hydrogen sulfide present
Chromatium	1–6 × 1.5–16; rod-shaped or ovoid, straight or slightly curved, polar flagella	48–70	Anaerobic	Photolithoautotroph that can use sulfide; sulfur stored within the cell
Ectothiorhodospira	0.5–1.5 in diameter; vibrioid- or rod-shaped, polar flagella	50.5–69.7	Anaerobic, some aerobic or microaerobic	Internal lamellar stacks of membranes; deposits sulfur granules outside cells
Escherichia	1.1–1.5 × 2–6; straight rods, peritrichous or nonmotile	48–52	Facultatively anaerobic	Mixed acid fermenter; formic acid converted to H_2 and CO_2, lactose fermented, citrate not used
Haemophilus	<1.0 in width; coccobacilli or rods, nonmotile	33–47	Facultative or aerobic	Fermentative; requires growth factors present in blood; parasites on mucous membranes
Leucothrix	Long filaments of short cylindrical cells, usually holdfast is present	46–51	Aerobic	Dispersal by gonidia, filaments don't glide; rosettes formed; heterotrophic
Methylococcus	1.0 in diameter; cocci with capsules, nonmotile	62–63	Aerobic	Can form a cyst; methane, methanol, and formaldehyde are sole carbon and energy sources
Photobacterium	0.8–1.3 × 1.8–2.4; straight, plump rods with polar flagella	40–44	Facultatively anaerobic	Two species can emit blue-green light; Na^+ needed for growth
Pseudomonas	0.5–1.0 × 1.5–5.0; straight or slightly curved rods, polar flagella	58–70	Aerobic	Respiratory metabolism with oxygen as acceptor; some are able to use H_2 or CO as energy source
Vibrio	0.5–0.8 × 1.4–2.6; straight or curved rods with sheathed polar flagella	38–51	Facultatively anaerobic	Fermentative or respiratory metabolism; sodium ions stimulate or are needed for growth; oxidase positive

of the purple nonsulfur bacteria are γ-proteobacteria and were discussed earlier in this chapter. Because the purple sulfur bacteria are γ-proteobacteria, they will be described here. *Bergey's Manual* divides the purple sulfur bacteria into two families: the *Chromatiaceae* and *Ectothiorhodospiraceae.* In the second edition these families are in the order *Chromatiales.* The family *Ectothiorhodospiraceae* contains five genera. *Ectothiorhodospira* has red, spiral-shaped, polarly flagellated cells that deposit sulfur globules externally. Internal photosynthetic membranes are organized as lamellar stacks. The typical purple sulfur bacteria are located in the family *Chromatiaceae*, which is much larger and contains 22 genera. The **purple sulfur bacteria** are strict anaerobes and usually photolithoautotrophs. They oxidize hydrogen sulfide to sulfur and deposit it internally as sulfur granules (usually within invaginated pockets of the plasma membrane); often they eventually oxidize the sulfur to sulfate. Hydrogen also may serve as an electron donor. *Thiospirillum*, *Thiocapsa*, and *Chromatium* are typical purple sulfur bacteria (**figure**). They are found in anaerobic, sulfide-rich zones of lakes.

Large blooms of purple sulfur bacteria occur in bogs and lagoons under the proper conditions (**figure**).

Order *Thiotrichales*

The order *Thiotrichales* contains three families, the largest of which is the family *Thiotrichaceae*. This family has several genera that oxidize sulfur compounds. Morphologically both rods and filamentous forms are present. Two of the best-studied gliding genera in this family are *Beggiatoa* and *Leucothrix* (**figures**). *Beggiatoa* is microaerophilic and grows in sulfide-rich habitats such as sulfur springs, freshwater with decaying plant material, rice paddies, salt marshes, and marine sediments. Its filaments contain short, disklike cells and lack a sheath. *Beggiatoa* is very versatile metabolically. It oxidizes hydrogen sulfide to form large sulfur grains located in pockets formed by invaginations of the plasma membrane. *Beggiatoa* can subsequently oxidize the sulfur to sulfate. The electrons are used by the electron transport chain in energy production. Many strains also can grow heterotrophically with acetate as a carbon source, and some may incorporate CO2 autotrophically. *Leucothrix* (**figure**) is an aerobic chemoorganotroph that forms long filaments or trichomes up to 400 μm long. It is usually marine and is attached to solid substrates by a holdfast. *Leucothrix* has a complex life cycle in which dispersal is by the formation of gonidia. Rosette formation often is seen in culture. *Thiothrix* is a related genus that forms sheathed filaments and releases gonidia from the open end of the sheath (**figure**). In contrast with *Leucothrix*, *Thiothrix* is a chemolithotroph that oxidizes hydrogen sulfide and deposits sulfur granules internally. It also requires an organic compound for growth (i.e., it is a mixotroph). *Thiothrix* grows in sulfide-rich flowing water and activated sludge sewage systems.

Methanogens and Methanogenesis

Methanogenesis is a biological process that produces approximately one billion tons of methane per year. Nature has invented a unique metabolic system during which the step producing the energy necessary for the maintenance of the methanogenic species is associated with the reduction of a methyl group to methane. All methanogens share this biochemical reaction, although the electrons necessary for the reduction may arise, according to the species, from hydrogen, formate, methanol, methylamines or acetate, and in some rare cases, from ethanol or propanol. The phylogenetic diversity of methanogens is evident from their dispersal throughout the Euryarcheota kingdom of the domain Archaea. Most methanogens are capable to reduce a single substrate, CO2, to methane. Only one order of methanogenic Archaea, the Methanosarcinales, has evolved the ability to reduce other compounds to methane. As a result, this highly successful group is found in a number of different environments. For example, Methanosarcina barkeri can produce methane autotrophically by reducing CO2, acetotrophically by cleavage of acetate to methane and CO2, or methylotrophically by the dismutation of methanol, methylated thiols or methyated amines to methane and CO2. To study the biochemistry of methanogens, procedures for mass culture of these organisms that yield kilogram quantities of cells have been developed. Anoxic methods of fractionation of enzymes and coenzymes by FPLC and HPLC procedures as well as the use of anaerobic chambers are routinely used. Reduction of CO2 Most methanogens are able to reduce CO2 to methane via the formyl, methenyl, methylene and methyl stages; the structures and the roles of six new coenzymes have been defined during this process. The reduction of CO2 occurs thanks to a cycle where the C1 group is transferred from the coenzyme of an enzyme to another and is sequentially reduced (Fig).

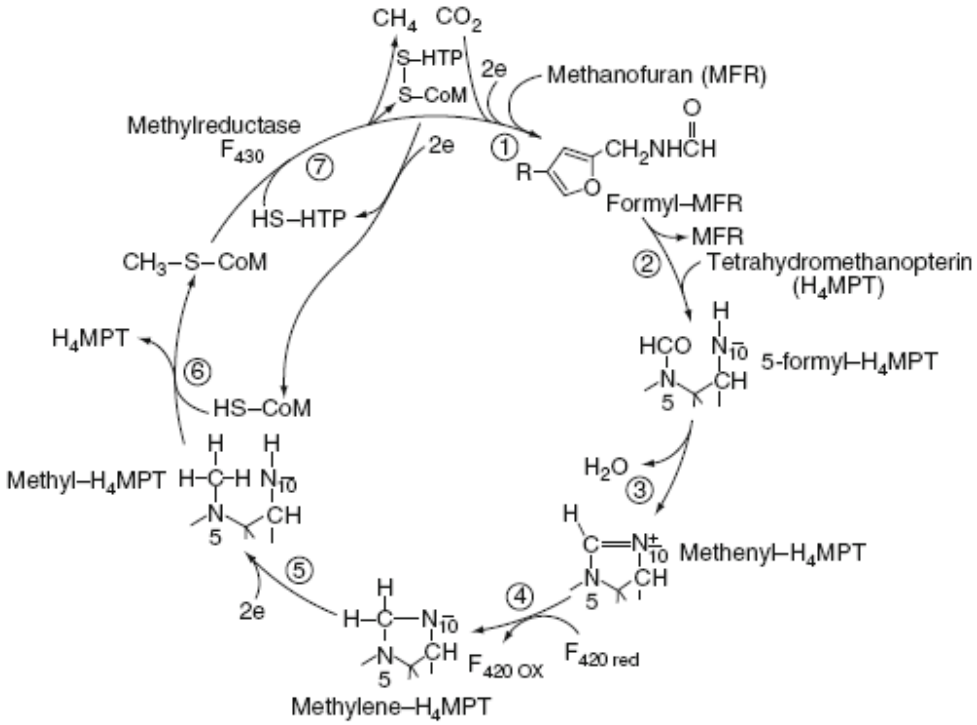


Fig. The methanogenic pathway

Formylmethanofuran is the first stable product of CO2 fixation (Fig., reaction 1). Its formyl group is then transferred to tetrahydromethanopterin, a pterin found so far only among the methanogens. The enzyme catalyzing this reaction is formylmethanofuran: tetrahydromethanopterin formyltransferase (Fig. , reaction 2). The formyl group is then reduced to a methenyl group by 5,10-methenyltetrahydromethanopterin cyclohydrolase (Fig., reaction 3). Reduced deazaflavin (also called coenzyme 420) provides the electrons required to reduce the double bond of the methenyl group, which becomes a methylene. This reaction is catalyzed by methylenetetrahydromethanopterin: coenzyme F420 oxidoreductase (Fig., reaction 4). Most reactions have been documented by homogeneous enzyme preparations. If the methylene group is chemically reduced by borohydride, 5-methyl-tetrahydromethanopterin is obtained, and its methyl group is reduced to methane by cell

extracts. Reaction 7 (Fig., reaction 7) schematizes the methylcoenzyme M methylreductase, the details of which will be found later in this chapter. This last reaction is involved in the activation of the first reaction (synthesis of formyltetrahydrofuran) and thus completes the cycle. Formylmethanofuran Dehydrogenase This enzyme catalyzes the first reaction of the cycle. Methanofuran (for short) is a long linear molecule with a hydrophilic tetracarboxylic part at one of its extremities and a furan ring substituted by a primary amine at the other. In Methanobacterium thermoautotrophicum, methanofuran is 4(N-(4,5,7-tricarboxyheptanoyl-g-glutamyl-g-L-glutamyl)p-(b-aminoethyl) phenoxymethyl(p(aminomethyl) furane. In Methanosarcina barkeri glutamyl units are found instead of the tetracarboxylic structure. The enzyme catalyzes the reversible conversion of CO2 and methanofuran to formylmethanofuran, the formyl group being linked to the primary amine of the furan cycle. CO2 and not bicarbonate is the substrate of the enzyme. Methanobacterium wolfei and M. thermoautotrophicum as well as other methanogens possess two isoenzymes, one containing tungsten (Fwd) and the other molybdenum (Fmd). The primary structures of the four subunits (FwdABCD) of the tungsten-containing enzyme from M. thermoautotrophicum have been determined by cloning and sequencing of the corresponding genes. FwdB contains sequence motifs characteristic of enzymes containing a molybdopter-in-dinucleotide, indicating that this subunit carries the active site. FwdA, FwdC and FwdD show no significant similarity with proteins found in the databases. A Northern blot analysis demonstrates that the four fwd genes form a transcriptional unit with three genes fwdE, fwdF and fwdG. Two proteins have been inferred from fwdE and fwdG, respectively, of 17.8 and 8.6 kDa. Each contains [4Fe-4S] binding motifs fwdF encodes a 38.6 kDa protein containing eight such motifs, suggesting that the gene product is a polyferredoxin. The seven fwd genes have been expressed in E. coli, yielding proteins of the expected size. The fwd operon is located in a region of the genome coding for molybdenum enzymes and for proteins involved in the synthesis of molybdopterins. The group of «molybdate genes»

comprises three open phases (fmdECB). The fmdB gene codes for the subunit binding molybdopterin guanine dinucleotide and carries the active site. It is thus functionally equivalent to FwdB. The 50 terminal part of fmdC codes for a protein similar to FwdC, the C-terminal part of which is similar to FwdD. FmdC is thus functionally equivalent to FwdCþ FwdD. The fmd operon does not contain a fmdA gene coding for a FmdA subunit. Since the FmdA protein has the same apparent molecular weight and the same N-terminal sequence as FwdA, one supposes that FmdA et FwdA are encoded by fwdA belonging to the fwd operon. Actually, it has been found that M. thermoautotrophicum cells grown in the presence of tungstate or molybdate transcribe the fwdHFGDACB operon whereas the fmd ECB gene cluster is transcribed only by cells grown in the presence of molybdate. The molybdenum enzyme from M. barkeri contains about 30 molecules of nonheme iron and 30 molecules of labile sulfur. The FmdB subunit, which carries the molybdenum active site binds one [4Fe-4S] cluster fmdF codes for a protein with four tandemly repeated motifs; this protein is predicted to be a polyferredoxin containing up to 32 iron atoms in eight [4Fe-4S] clusters. The fact that FmdF forms a stable complex with the other subunits of formylmethanofuran dehydrogenase is in favor of a function of polyferredoxin during catalysis. A malE-fmdF fusion has been constructed and expressed in E. coli and has led to the production of the polyferredoxin apoprotein in preparative amounts. mylmethanofuran: Tetrahydromethanopterin Formyltransferase Formylmethanofuran: tetrahydromethanopterin formyltransferase (FTR) from M. thermoautotrophicum has been cloned and its sequence determined. The active enzyme has been synthesized under the control of the lac promoter. The ftr gene codes for an acidic protein (MW ¼ 31,401) the sequence of which presents no homology with the sequences present in the databases, including those having pterin substrates. FTR transfers the formyl group of formylmethanofuran to tetrahydromethanopterin of known structure, which is the central transporter in methanogenesis to which the C1 structure will remain successively linked as the formyl, methenyl, methylene and methyl derivatives. Methenyltetrahydromethanopterin

Cyclohydrolase This enzyme has been obtained in the homogeneous state from M. thermoautotrophicum. Its subunit has a molecular weight of 41 kDa. Since the native protein has been found by exclusion chromatography to have a molecular weight of 82 kDa, it is thought to be a homodimer. The correspondiing mch gene has been cloned, sequenced and overexpressed in E. coli: the deduced sequence of the subunit indicates a molecular weight of 37 kDa. Mch has been crystallized and its structure determined at a resolution of 2 A°. It shows a new type of a/b-fold with two domains separated by a pocket formed by a long conserved sequence. One phosphate ion is in the pocket whereas another one is adjacent to the pocket. The latter is displaced by the phosphate residue of the substrate, formyl-H(4)MPT according to a hypothetical model. 5, 10-Methylenetetrahydromethanopterin Dehydrogenase Coenzyme F420 is the phosphodiester N-(N-lactyl–L-glutamyl)L-) glutamic acid of 7,8-didemethyl-8-hydroxy-5-deazariboflavin -50 phosphate. F420 is the first described deazaflavin. In most methanogens, two hydrogenase systems can catalyze the reduction of Coenzyme 420 by H2.

1. The first type of hydrogenases catalyzes the reversible conversion of N5, N10- methylene-H4MPT to N5, N10-methenyl-H4MPT. These hydrogenases do not contain nickel, nor iron or iron-sulfur-clusters and are considered to be metalfree. They catalyze the reaction via a special reaction mechanism.

2. The second type of hydrogenases catalyzes the splitting of H2 and reduces a deazaflavine, the cofactor F420. Therefore these enzymes are called F420-reducing hydrogenases. The cofactor F420 is an important electron carrier in methanogens, but it is not unique to Archaea. The F420-reducing hydrogenases also react with artificial dyes such as benzylviologen. Typically these hydrogenases are composed of three subunits. The large subunit (or subunit A) contains the primary reaction centre. The thiol-groups of four cysteines are ligands of the bimetallic active site. Two of these cysteines are conserved in the aminoterminal part, the other two in the carboxy-terminal

part of the subunit. The conserved motif -Asp-Pro-Cys-X-X-Cys-X-X-His- is found in the carboxyterminal region of the large subunit. The molecular hydrogen is split and the electrons are transferred to the third subunit (subunit B) via [FeS]-clusters, which are localized in the small subunit (or subunit G). This subunit probably contains one flavin and transfers the electrons to the cofactor F420. In most methanogens, two hydrogenase systems can catalyze the reduction of Coenzyme 420 by H2: (1) a nickel Fe–S flavoprotein composed of three different subunits and (2) the 5N, 10N-methylenetetrahydromethanopterin dehydrogenase, composed of a H2-forming methylenetetrahydromethanopterin dehydrogenase and a F420-dependent methylenetetrahydromethanopterin dehydrogenase, both metalfree and without an apparent prosthetic factor. In nickel-limited chemostat cultures of M. thermoautotrophicum, the specific activity of the first system is negligible whereas that of H2-forming methylenetetrahydromethanopterin dehydrogenase is six times higher, and that of F420-dependent methylenetetrahydromethanopterin dehydrogenase is four times higher than in cells where nickel is not limiting. These experiments suggest that, when nickel is limiting, the reduction of F420 by H2 is catalyzed by the second system. The methylenetetrahydromethanopterin dehydrogenase from M. thermoautotrophicum has been purified to homogeneity. It is a homohexamer (MW of the subunit: 36 kDa). Its coding gene mer codes for an acid polypeptide of 321 residues. The enzyme catalyzes the reversible oxidation of 5,10-methylene-5,6,7,8-tetrahydromethanopterin to the methenyl derivative. There is a strict requirement for the electron carrier F420 as co-substrate. 5, 10-Methylenetetrahydromethanopterin F420 Oxidoreductase 5,10-methylenetetrahydromethanopterin oxidoreductase from has been purified to homogeneity. It catalyzes the reduction of the 5,10-methylene derivative to 5-methyltetrahydromethanopterin (Fig., reaction 5). Here again, the electron carrier F420 is a specific co-substrate. The reaction is reversible; however, the reduction of the methylene derivative is thermodynamically favored. The enzyme is a monomer (MW ¼ 35,000). It contains neither flavin nor Fe–S clusters. The Methylreductase: Methyl

Coenzyme M Reductase The coupling of CH3-S-CoM methylreductase with the reduction of CO2 to CH4 has been discovered by R.P. Gunsalus in 1980; in the presence of CH3-S-CoM, the rate of reduction of CO2 to CH4 is increased 30-fold and the amount of methane produced 11-fold. The effect can be reinitiated by further additions of CH3-S-CoM. If 14CO2 is used, the synthesis of 14C formyl-methanofuran can be detected only if CH3-S-CoMis added. The transient intermediate has been identified: it is the mixed disulfide CoM-SS- HTP (Fig., reactions 6 and 7) which is the product of the methylreductase reaction. In the absence of CH3-S-CoM, the mixed disulfide CoM-S-S-HTP stimulates the reduction of CO2 42-fold, activates the biosynthesis of formylmethanofurane biosynthesis and is reduced to HS-CoM and HS-HTP by cell extracts. CoenzymeM(HS-CoM) is mercaptoethane sulfonic acid, its methylated form (CH3-S-CoM) being the substrate of the methylcoenzyme M methylreductase system (Fig., reaction 7). This system is a complex of several protein fractions and cofactors. It has been first resolved in three fractions called A, B and C, according to their elution order during an anion exchange chromatography. A and C are protein fractions whereas B is 7-mercaptoheptanoylthreonine phosphate (HS-HTP), a small dialysable molecule which the organic acid chemists have synthesized. Fraction A consists of three proteins A1, A2 and A3 and FAD. Furthermore, ATP is required for the enzyme activity, whereas coenzyme F420 and vitamin B12 stimulate, but are not absolute requirements. The methylcoenzyme M reductase system is extremely sensitive to oxygen. The methylation of coenzyme M is catalyzed by a methyltransferase composed of several subunits, one of which is MtsA (the methylcobalamin:coenzyme M methyltransferase proper) another (MtsB) being homologous to a class of corrinoid proteins involved in methanogenesis. Component C of M. thermoautotrophicum, the methylreductase proper, is stable in the presence of oxygen. Its molecular weight is of about 300,000 and consists of three different subunits of respectively 68,000, 45,000, and 38,500 kDa, corresponding to an a2b2g stoichiometry. The genes of the three subunits of Methanococcus voltae, Methanococcus vannielii, and Methanosarcina

barkeri have been cloned and sequenced. They are part of an operon which contains in addition two open reading frames, Orf1 and

Orf2 which codes for two polypeptides (16 and 21 kDa). Component C amounts to 10% of the cell proteins and is highly conserved in all methanogens. It presents a characteristic yellow color with a peak at 420 nm and a shoulder at 440 nm. The isolated chromophore, factor F430, is a tetrahydrocorphin, a highly reduced porphinoid, which chelates a nickel atom and which is the first biological Ni-tetrapyrrole structure to have been isolated. The displacement from 421 to 430 nm is representative of the interactions between the co-factor and component C (reconstitution studies show that a molecule of F430 is bound to each of the two large subunits of Component C). Extended x-ray absorption fine structure spectroscopy (EXAFS) and Raman resonance spectroscopy reveal structure differences between two free forms of F430. The first is a hexacoordinated structure with long Ni–N bonds (2.1 A °) and a plane cycle. The nickel atom of the second form is tetracoordinated with shorter Ni–N bonds (1.9 A °) which suggests a bent form of the corphine nucleus. The structure of bound F430 differs from the two free forms: the nickel atom is hexacoordinated and under the form of “EPR silent” Ni(II) The reduction of free F430 is difficult and occurs under non physiological conditions. In addition to the two molecules of F430, component C binds also two molecules of coenzyme M which are incorporated in the CH3-S-CoM form only when the methylreductase functions. Finally, component C binds also two molecules of HS-HTP. As noted above, the reduction of CH3-S-CoM requires in addition to component C other protein fractions (A1, A2 and A3). A2 is a colorless monomer (MW ¼ 59,000) with no known enzymatic activity. Component A1 is a complex fraction containing several unidentified proteins A1 has often been considered as the hydrogenase of the methylreductase system because it co-migrates on a phenyl-Sepharose column with the hydrogenase which specifically reduces F420. However, all the efforts to replace H2 by reduced F420 have met no success. Component A3 is extremely oxygen-sensitive. Methylcoenzyme M methylreductase requires the presence of 7-

mercaptoheptanoylthreonine phosphate. The latter is part of a cytoplasmic factor. A hydrolytic fragment of this cytoplasmic factor has been characterized as uridine 50-(O-2-

acetamido-2-deoxy-b-mannopyranuronosic acid substituted by 2-acetamido-2-deoxy-a-glucopyranosyl diphosphate, by mass spectrometry and by high resolution NMR. It is thought that this UDP-disaccharide serves as an anchor for 7-mercaptoheptanoyl threonine phosphate at the active site of the methylreductase complex. Two essential observations have been made:

(1) HS-HTP is the electron donor for the reduction of CH3-S-CoM. (2) The SH-HTP/HS-CoM heterodisulfide has been identified as the other product of the reduction of CH3-S-CoM to methane.

$$CH_3 - S - CoM + HS\text{-}HTP \rightarrow CH_4 + CoM\text{-}S\text{-}S\text{-}HTP$$

Simplification of the Methylreductase System Homogeneous preparations of component C from M. thermoautotrophicum catalyze the reduction of CH3-S-CoM by dithiothreitol. This rection requires catalytic amounts of HS-HTP and is stimulated by vitamin B12. No other protein component nor ATP are required. The EPR spectrum of these preparations have a peak corresponding to the Ni atom of F430 in the Ni(I) or Ni(III) state. The hyperfine study of the signal shows an interaction of the four nitrogen atoms of the tetrahydrocorphine ring. Furthermore, a correlation has been established between the intensity of the EPR signal and the specific activity of the component C preparations. The component C yielding the EPR signal is the active form Ca of the enzyme and contains Ni (I) in its prosthetic group, the inactive component C being EPR silent. Ca alone can demethylate CH3-S-CoM in the presence of HS-HTP, which demonstrates unequivocally that it is the methylreductase per second. A model of the methylreductase has been proposed, in which a role for each of the enzymes and the

coenzymes of the system is given. Reaction 1 describes the conversion of Ci into Ca by reduction of Ni (II) to Ni (I), the components A2, A3 and ATP being involved and the necessary electrons being provided by H2 or by titanium (III) citrate. ATP is supposedly required to induce a conformational change of Ci necessary for the reduction of Ni (II). Reaction II is the methylreductase proper, catalyzed by Ca. During reaction III, the heterodisulfide CoM-S-S-HTP is reduced, either chemically into HS-CoM et HS-HTP by titanium (III) citrate or dithiothreitol in presence of B12, or enzymatically by a reductase linked to a hydrogenase. The heptanoyl chain of HS-HTP is very specific. The hexanoyl and octanoyl analogues are not substrates, but strong inhibitors of the methylreductase. Structure of the Methylreductase The methylreductase contains eight different subunits which have all been cloned and sequenced. Sodium dodecylsulfate electrophoresis of the purified complex has shown the presence of eight polypeptides of respective molecular weights of 34 (MtrH)), 28 (MtrE), 24 (MtrC), 23 (MtrA), 21 (MtrD), 13 (MtrG), 12.5 (MtrB) and 12 kDa (MtrF). Cloning and sequencing of the corresponding genes has revealed the presence of eight mtr genes organized in a 4.9-kbp segment in the order mtrED-CBAFGH. A Northern blot analysis reveals a transcript of 5 kbp Since mtrE and mtrH DNA probes hybridize with the transcript, the eight genes form an operon. This composition is to be compared with the tridimensional structure of the methylreductase which, as noted above, is a 300 kDa protein hexameric protein. It has been crystallized in the inactive EPR silent state. Two molecules of the coenzyme F430 Niporphinoid are sandwiched between subunits a, a0, and g on one hand and a0, a, and g0 on the other, forming two identical active sites. The substrate methylcoenzyme M can reach each site by a narrow channel which closes after the second substrate HS-HTP (coenzyme B) is fixed. Thanks to another structure crystallized with the heterodisulfide, a reaction mechanism is proposed which involves a free radical intermediate and an organonickel

compound. The crystal structure of methyl-coenzyme M reductase from M. thermoautotrophicum has revealed the presence of five amino acids post translationally

modified in the subunit, near the active site. Four of them are C- N- et S-methylations, among which 2-methylglutamine and 5-methylarginine had never been encountered before. These modifications have been confirmed by mass spectroometry of chymotryptic peptides. When methyl-coenzyme M reductase was purified from cells grown in presence of L-[methyl-3D] methionine, it was shown that the methyl group of the modified amino acids originates from the methyl group of methionine Biosynthesis of Some Cofactors Involved in Methanogenesis Methanogenesis, the anaerobic production of methane from CO2 or simple carbon compounds, requires specific organic coenzymes. Methanofuran The biosynthesis of the 2-(aminomethyl)-4-(hydroxymethyl)furan (F1) and of the 1,3,4,6-hexane tricarboxylic(TCA) subunits of methanofuran has been studied using gas chromatography and mass spectrometry to follow stable isotope incorporation into these subunits. It was rconcluded that F1 is generated from the condensation of dihydroxyacetone phosphate with pyruvate. The resulting dihydroxy- substituted tetrahydrofuran after elimination of 2 mol of water would produce the phosphate ester of 2-carboxy-4-(hydroxymethyl)furan. Reduction of the carboxylic acid to an aldehyde and subsequent transamination would produce the phosphate ester of F1.

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POSSIBLE QUESTION

UNIT

2 marks

1. What is methanogenesis?
2. Write an account on sexual sporulation in Fungi
3. Define Biofilms? Write their significance.
4. Write a note on lipid catabolism
5. Outline the green sulphur bacteria.
6. Write about the Photosynthetic pigments
7. What do you know about fatty acid and their role in metabolism of bacteria?
8. Elaborate on bacterial photosynthetic pigments
9. Describe photophosphorylation.
10. Write the steps involved in the methanogenesis

8 Marks

1. Compare and contrast aerobic and anaerobic respiration
2. Derive pyruvic acid from glucose by suitablemetabolic pathway.
3. Describe in detail about photophosphorylation.
4. Describe spore formation in *Streptomycetes*
5. Describe the arrangement of cell wall in *Staphylococcus*
6. Detail account on Bioluminescence importance and applications
7. Detail account on Quorum sensing – mechanism, importance and applications.
8. Different between oxygenic and anoxygenic bacteria
9. Differentiate oxygenic and anoxygenic photosynthesis
10. Elaborate on bacterial photosynthetic pigments and its role in energy generation

Unit V Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
_____ are present in sulfur-rich hot springs	*Sulfolobus*	*Sulfobacillus*	*Sulfococcus*	*Sulfothrix*	*Sulfolobus*
Absorption peak of phycoerythrin found in red algae is	590-676 nm	690-767 nm	490-576 nm	586-976 nm	490-576 nm
Anaerobic treatment of wastewater containing high sulphate levels causes production of	H_2O	H_2S	HCL	H_2SO_4	H_2S
Anoxygenic photoautotrophic bacteria is	Green bacteria	Purple sulphur bacteria	Sulfolobus	Cyanobacteria	Purple sulphur bacteria
Anoxygenic photosynthesis is seen in	Red algae	Brown algae	Fungi	Blue-green algae	Blue-green algae
Bacteria that utilize malate as electron donor is	Photolithotrophic	Photo heterotrophic	Photo autotrophic	Photoorganotrophic	Photoorganotrophic
Bacteriochlorophyll absorbs light having a maximum wavelength of	660 to 715 nm	725 to 800 nm	800 to 925 nm	above 925 nm	800 to 925 nm
Bacteriochlorophyll are located in	Chlorosomes	Mesosomes	Metasomes	Ribosomes	Chlorosomes
Best described pathway involve the use of _____ as terminal electron acceptor	Carbon dioxide	Oxygen	Hydrogen	Nitrogen	Carbon dioxide
Bioluminescent bacteria found predominantly in symbiosis with various marine animals	*Vibrio parahaemolyticus*	*Vibrio vulnificus*	*Vibrio fischeri*	*Vibrio harveyi*	*Vibrio fischeri*
Calvin cycle is found to take place in _____ of plants, algae and cyanobacteria	Mitochondria	Chloroplast	Protoplast	Cytoplasm	Chloroplast
Coenzymes M and F420 are found in	Ethanotrophs	Methanotrophs	Methanogenic bacteria	Ethanogenic bacteria	Methanogenic bacteria

Common class of signaling molecule seen in Gram negative bacteria for quorum sensing	N-Acyl Homoserine Lactones	Acetyl Homoserine Lactones	N-Acyl Homoserine Lactose	O-Acyl Homoserine Lactones	N-Acyl Homoserine Lactones
Conversion process of inorganic carbon (carbon dioxide) to organic compounds by living organisms	Carbon metabolism	Carbon degradation	Carbon fixation	Carbon reduction	Carbon fixation
Cyanobacteria contains a special water soluble pigment called	Phycobiliproteins	G-coupled proteins	Lipoproteins	Pseudoproteins	Phycobiliproteins
Cyclic oxidative phosphorylation is known as	Photosystem I	Photosystem II	Photosystem III	Photosystem IV	Photosystem I
Different types of Bacteriochlorophyll are	c to h, 6 types	a to g, 7 types	b to f, 5 types	d to g, 4 types	a to g, 7 types
Electrons expelled from photosystem I are accepted by	Ferrous	Ferrodoxin	Ferrous dioxide	Ferritin	Ferrodoxin
Enzyme that catalyze the oxidation of luciferin	Lucidase	Luciferinase	Lucigenase	Luciferase	Luciferase
Essential metal that is found in active site of methyl–coenzyme M reductase, an enzyme common to all methanogenic pathways	Nickel	Lead	Aluminium	Iron	Nickel
Example for anoxygenic photoautotrophic bacteria is _____.	Cyanobacteria	Green bacteria	Purple sulphur bacteria	None	Purple sulphur bacteria
Example for purple non sulfur bacteria belongs to	*Chlorobium*	*Pseudomonas*	*Chromatium*	*Rhodospirillum*	*Rhodospirillum*
Example for purple sulfur bacteria	*Rhodospirillum*	*Chromatium*	*Chlorobium*	*Zymomonas*	*Chromatium*
Fungi with bioluminescent property	*Panellus stipticus*	*Dictostellum antennum*	*Farmosum rubrum*	*Lanthanum acrimonum*	*Panellus stipticus*

Gene specific for sulfur oxidation	Thiosulfate oxygenase	ThioSulfate hydrogenase	ThioSulfora se	ThioSulfanase	Thiosulfate oxygenase
Global warming potential of methane is ____ times greater than carbon dioxide	5	10	15	25	25
Green sulfur bacteria belongs to the family	*Chromatium*	*Rhodospirillu m*	*Chlorobium*	*Nitrosomonas*	*Chlorobium*
Green sulfur bacteria exist in _____ rich zone lakes	Sulfur	Iron	Copper	Nickel	Iron
H_2S is produced by	Non-sulfur bacteria	Sulfate degrading	Sulfate reducing bacteria	Sulfate oxidizing bacteria	Sulfate reducing bacteria
H_2S is utilized by	Green sulfur bacteria	Purple sulfur bacteria	Green sulfur bacteria	Green non sulfur bacteria	Purple sulfur bacteria
How many protons are picked during the passage of electrons through the carriers of photosystem I.	8	12	16	4	4
In *Roridomyces roridus* luminescence occurs only in	Mycelium	Spores	Conidiopho res	Vesicles	Spores
It is thought that the chloroplasts in plants and algae evolved from				Cyanobacteria	Cyanobacteria
Levels of oxidation of sulfate depends on	Sulfate concentration	Nitrogen concentration	Hydrogen concentratio n	Oxygen concentration	Oxygen concentration
Main phycobilin of Cyanobacteria is	Phycoerythrins	Phycocyanins	Allocyanins	Chromatins	Phycocyanins
Methanogenesis is	Oxidation of methane	Reduction of methane	Production of methane	Assimilation of methane	Production of methane

Methanogenesis is an	Aerobic process	Anaerobic process	Microaerobic process	Facultative anaerobic process	Anaerobic process
Methanogens are also	Thermophiles	Halophiles	Alkaliphiles	All of the above	All of the above
Methanogens are also found in	Surface layer of soil	Water bodies	Atmosphere of higher altitudes	Rumen of animals	Rumen of animals
Methanogens belong to a group of	Archaea	Actinomycetes	Higher fungi	Pathogenic bacteria	Archaea
Methyltransferases are involved in the metabolism of methylamines by	*Methanococcus*	*Methanosarcina*	*Methanolobus*	*Methanobacter*	*Methanosarcina*
Microbes that use of CO2 as their main source of carbon	Heteotroph	Aututrophs	Chemotroph	Lithotroph	Aututrophs
Most abundant protein on earth.	RuBisCO	Bacteriochlorophyll	Carboxylase	Oxygenase	RuBisCO
Organic compound is utilized by	Green non sulfur bacteria	Purple sulfur bacteria	Green sulfur bacteria	Photosynthetic bacteria	Green sulfur bacteria
Organism that synthesis chlorophyll b in addition to chlorophyll a.	Actinomycetes	*Penicillium*	*Pseudomonas*	Cyanobacteria	Cyanobacteria
Organisms that grow by fixing carbon are called	Nutrotrophs	Carbotrophs	Heterotrophs	Autotrophs	Autotrophs
Other than CO_2, _____ also acts as a terminal electron acceptor in methanogenesis	Hydrochloric acid acid	Acetic acid	Nitric acid	Oxalic acid	Acetic acid
Oxidation of ethanol was strictly _____ dependent	H_2	O_2	N_2	Co_2	Co_2
Photosynthetic bacteria that have the	Green sulfur	Purple bacteria	Cyanobacter	Halobacteria	Green sulfur

most different types of chlorophyll is	bacteria		ia		bacteria
Photosystem I is otherwise known as _____.	Z pathway	Non-cyclic oxidative phosphorylation	Cyclic oxidative phosphorylation	Photosystem I & II	Cyclic oxidative phosphorylation
Pigment used in photosystem I is	P_{700}	P_{500}	P_{400}	P_{600}	P_{700}
Process of conversion of light energy from the sun to chemical energy with in ATP is known as _____.	Photophosphorylation	Substrate level phosphorylation	Oxidative phosphorylation	Chemiosmosis	Photophosphorylation
Proton motive force is needed to drive formation of	GTP	UTP	ATP	NADPH	ATP
Rapid interconversion of carbon dioxide and water to bicarbonate and protons or vice versa is catalyzed by	Carbonic reductase	Carbonic hydrogenase	Carbonic anhydrase	Carbonic hydrolase	Carbonic anhydrase
Reaction center bacteria chlorophyll absorb maximally at _____.	810nm	800nm	840nm	820nm	840nm
Regulation of gene expression in response to fluctuations in cell-population density	Metabolic repression	Phosphorescence	Cell adaptation	Quorum sensing	Quorum sensing
Sulfate oxidizing bacteria are capable of oxidizing	Sulfate to sulfide	Sulfide to sulphuric acid	Elemental sulfur to Sulfide	Sulfide to elemental sulfur	Sulfide to elemental sulfur
Terminal electron acceptor in methanogenesis is	Oxygen	Nitrogen concentration	Water	Carbon	Carbon
Vesicles produced by green photoautotrophic bacteria is	Cetntromere	Mesosome	Chlorosome	Magnetosome	Chlorosome
Which uses sulfate as terminal	*Desulfovibrio*	*Methano*	*Nitrobacillu*	*Hydrogenomo*	*Desulfovibrio*

electron acceptor		*bacterium*	*s*	*nas*	