T-15

PMB 271 Cintroduction to pharmaceutical MCB

EMMY

GENERAL STRUCTURE OF BACTERIAL CELL/BACTERIAL SPORE, ITS STRUCTURE AND RESISTANCE TO INACTIVATING AGENTS

What is bacteriology?

Bacteriology is a branch of microbiology that is concerned with the study of bacteria (as well as archaea) and related aspects. It's a field in which bacteriologist study and learn more about the various characteristics (structure, genetics, biochemistry and ecology etc) of bacteria as well as the mechanism through which they cause diseases in humans and animals.

Bacteria belong to kingdom called prokaryote (monera) which means cells or organisms that lacks a nucleus and other membrane- enclosed organelles and usually have its DNA in a single circular molecule. Based on their molecular studies, the member prokaryote (monera) were grouped into two domains – Archaea and Bacteria. These were classified based on the differences in the sequences of nucleotides in the cell's ribosomal RNA (r RNA).

Domain Archaea (Archaebacteria: meaning primitive bacteria) which includes methanogens – bacteria that produce methane gas; salt-loving bacteria (extreme halophiles); thermoacidophiles (acid- loving bacteria, which grow at high temperatures). The cell walls of archaea contain no peptidoglycan; they live in extreme environments and include- methanogens; extreme halophiles, hyperthermophiles.

Domain Bacteria (eubacteria)/ true bacteria: They are prokaryotic cells that are common in human daily life, encounter many more times than the archaebacteria. Eubacteria can be found almost everywhere and kill thousands upon thousands of people each year, but also serve as antibiotics producers and food digesters in our stomachs. Their cell walls contain peptidoglycan and they are sensitive to antibacterial agents but are resistant to most antibiotics that affect Eukaryotes. Bacteria / eubacteria include – (1) Mycoplasmas (2) Cyanobacteria (3) Gram positive bacteria (4) Gram negative bacteria. Cyanobacteria are photosynthetic bacteria that can produced oxygen during photosynthesis.

Bacteria are small and simple in structure when compared with eukaryotes, yet they often have characteristics shapes and sizes.

Two key features of prokaryotic cells are their shape (cell morphology) and size. Based on size, bacterial cells are usually measured in microns.

Primitive

Basic Shapes of bacteria:

Prokaryotes typically have shapes and are extremely small cells. The cell shapes and Gram staining are useful in bacterial identification. Bacterial cells exist in different shapes. The term morphology means cell shape and this is useful in identification of a bacterial cell. Under the light microscope, bacteria may have one of the following morphological forms:

(1) Cocci (spherical in shape): are roughly spherical cells. They can exist as individual cells, but also are associated in characteristic arrangements that are frequently useful in bacterial identification. It can appear in pair, in row, clusters.

(a) In pair (diplococci) – arise when cocci divide and remain together to form pairs e.g. *Neisseria gonorrhaeae*

(b) Long chain of cocci or in row of cocci – result when cells adhere after repeated division in one plane; this pattern is seen in the genera streptococcus, Enterococcus and Lactococcus.

(c) Cluster of four (tetrads) – which divide in the planes to form square groups of four cells called (tetrads). E.g. Micrococcus.

(d) Irregular cluster (grape - like) e.g. staphylococcus which divides in random planes to generate irregular grape - like clumps. In the genus, sarcina, cocci divide in three planes producing cubical packets of eight cells.

(2) Bacillary: rod (cylinder) shaped cells, approximately 0.4 to 1.5 µm wide and 1.5 to 8 µm long, occurring singly or in chains. Many rods do occur singly; they may remain together after division to form pairs or chains e.g. *Bacillus megaterium* is found in long chain). Some of the rod are curved to form commas (curved rods) example *vibrio cholerae.*

(3)Coccobacillary: These appear to be as much coccal-shaped as rod-shaped. These are usually short, stubby rods (between 0.5 and 1µm in length with a diameter only slightly less than the length) with rounded corners and hence, an overall elliptical shape.

Bacteria can assume a great variety of shapes, although they often are simple sphere/rods. Actinomycetes characteristically form long multinucleate filaments or hyphae that may branch to produce a network called a *mycelium.*

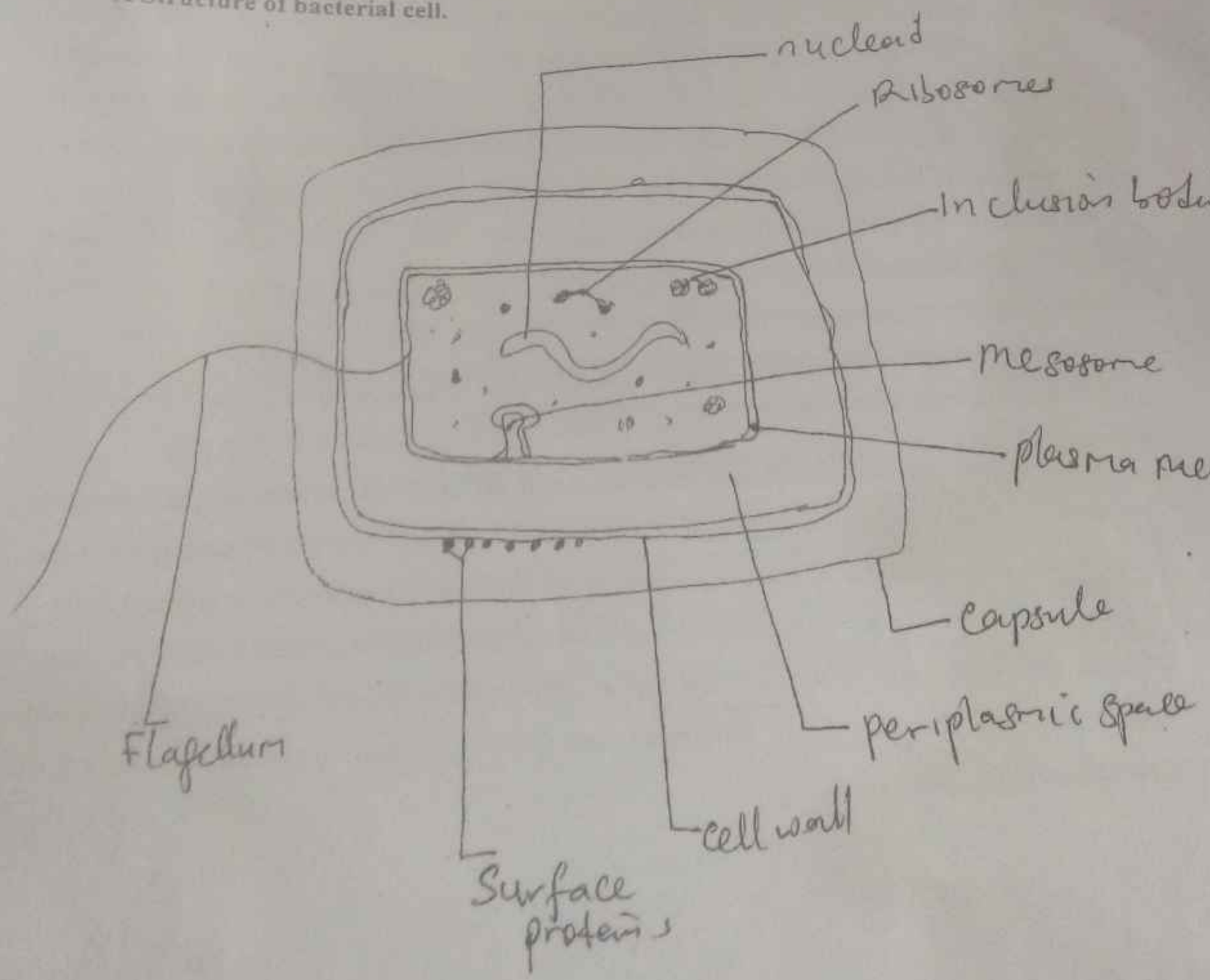
(4)Many bacteria are shaped like long rods twisted into **spirals or helices; they are called spirilla** if rigid and spirochetes when flexible. Finally, some bacteria are variable in shape and lack a single, characteristics form. These are pleomorphic

Size: Bacteria vary in size as much as in shape. The smallest are about 0.3 μm in diameter.

Prokaryotic cell organization/General Structure of bacterial cell:

Prokaryotic 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 prokaryotic 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.

A Structure of bacterial cell.



Functions of prokaryotic 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 and other substance

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.

(1) **Bacterial cell wall:** The cell wall is one of the most important parts of a prokaryotic cell, that give them shape and rigidity and protect them from osmotic lysis. 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. The cell wall of bacteria is made up of **peptidoglycan or murein or mucopeptide layer**. The peptidoglycan layer is the structure that group bacteria into gram- positive and Gram – negative. The gram positive cell wall consists of a single 20 to 80 nm thick homogeneous peptidoglycan layer lying outside the plasma membrane while 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**. All the structure outside the plasma membrane is the envelope e.g. capsule.

A space is seen between the plasma membrane and the outer membranes of gram negative bacteria is called **periplasmic space**. The substance that occupies the periplasmic space is the **periplasm**. Size estimates of the periplasmic space in gram – negative bacteria range from 1 nm to as great as 71 nm. The periplasmic space of gram negative bacteria contains many proteins that participate in nutrient acquisition, for example, hydrolytic enzymes attacking nucleic acid and phosphorylated molecules, and binding proteins involved in transport of materials into the cell.

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. The archaeobacteria differ from other prokaryotes in many respects. Although they may be either gram positive or gram negative, their cell wall are distinctive in structure and chemical composition. The walls lack peptidoglycan and are composed of proteins, glycoproteins, or polysaccharides.

Peptidoglycan Structure

Peptidoglycan or murein is an enormous polymer composed of many identical subunits which include: (1) the backbone which is composed of alternating– N – acetyl glucosamine and N – acetylmuramic acid residues. (2) A set of identical tetrapeptide side chain or a peptide chain of four alternating D - and L –amino acids which 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. Long chains of peptidoglycan are biosynthesized adjacent to one another to form a sheet surrounding the cell. The glycosidic bonds connecting the sugars in the glycan strands are covalent bonds, but these provide rigidity to the structure in only one direction. In gram – negative bacteria, peptidoglycan cross-linkage occurs by peptide bond formation from the amino group of DAP meso – diaminopimelic acid of one glycan chain to the carboxyl group of the terminal D – alanine on the adjacent glycan chain.

In gram – positive bacteria, cross – linkage occurs by way of a peptide inter bridge, the kinds and the numbers of amino acids in the inter bridge varying form organism to organism. For example, in the gram-positive *Staphylococcus aureus*, the interbridge peptide is composed of five glycine residues, a common interbridge amino acid.

Peptidoglycan can be destroyed by certain agents. One such agent is the enzyme lysozyme, a protein that cleaves the β-1,4-glycosidic bonds between N-acetylglucosamine and N-acetylmuramic acid in peptidoglycan, thereby weakening the wall; water can then enter the cell and cause lysis. Lysozyme is found in animal secretions including tears, saliva, and other body fluids, and functions as a major line of defense against bacterial infection.

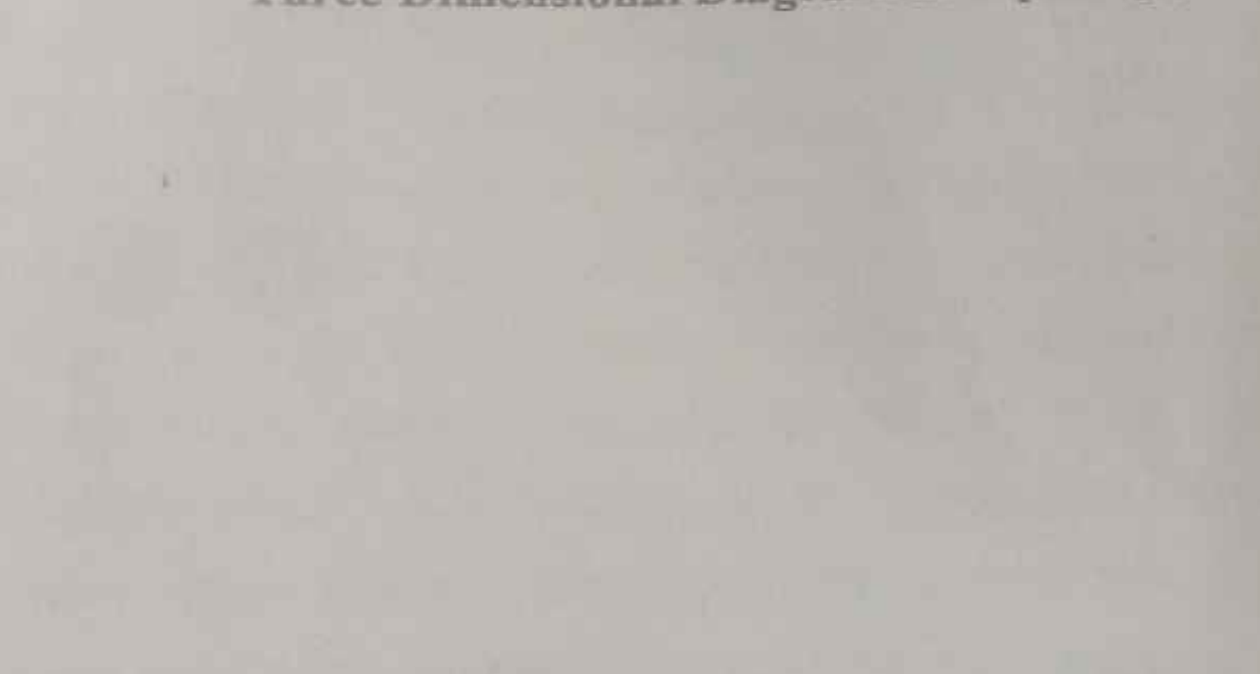
Peptidoglycan is present only in species of Bacteria—the sugar N-acetylmuramic acid and the amino acid analog DAP have never been found in the cell walls of Archaebacteria or Eukaryotic organisms. Another exception is mycoplasmas.

(3) A set of identical peptide cross bridges: This is called pentaglycine peptide chain. Chains of linked peptidoglycan subunits are joined by cross-links 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, inter-connected network. These sacs have been isolated from gram-positive bacteria and are strong enough to retain their shape and integrity. Whether gram positive or negative, the back bone is the same but the tetrapeptide side chain and the crossbridge will vary from species to species.

The tetrapeptide side chain: the tetrapeptide side chain of all the species however, have certain important feature in common. At position 1, L – alanine attached to N – acetylmuramic acid. The position three ie the L – Lyine is the most variable position. Most of the Gram negative bacteria will carry diaminopimelic acid (DAP) at position three. But the Gram positive bacteria usually carry either L-Lysine, DAP or any other L- amino acid at position three. The tetrapeptide side chain include: ⓐ L-Alanine ⓑ D-glutamic acid ⓒ L-Lysine or diaminopimelic acid (DAP) ⓓ D-alanine.

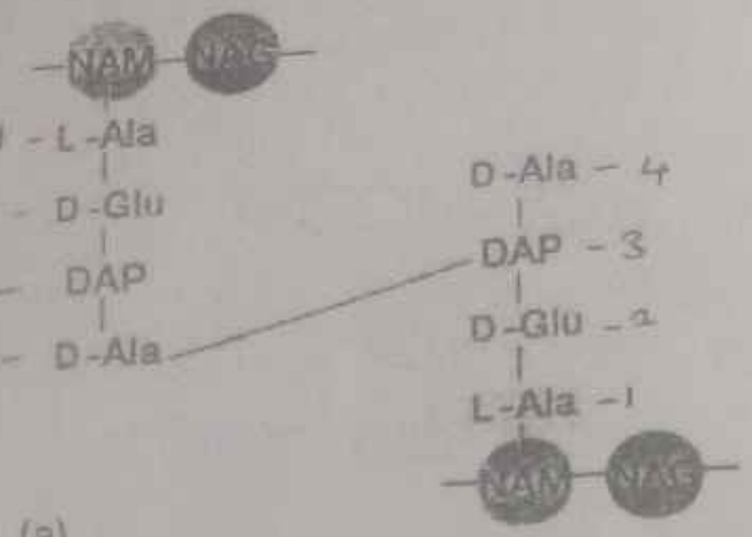
Crossbridge: They also vary in composition from species to species. Among the Gram positive, they are usually found as connective sheets cross- linked in 3 – dimensions, but in case of Gram negative bacteria they will form only 2 – dimensional layers.

The backbone of Gram positive and Gram negative bacteria are the same.

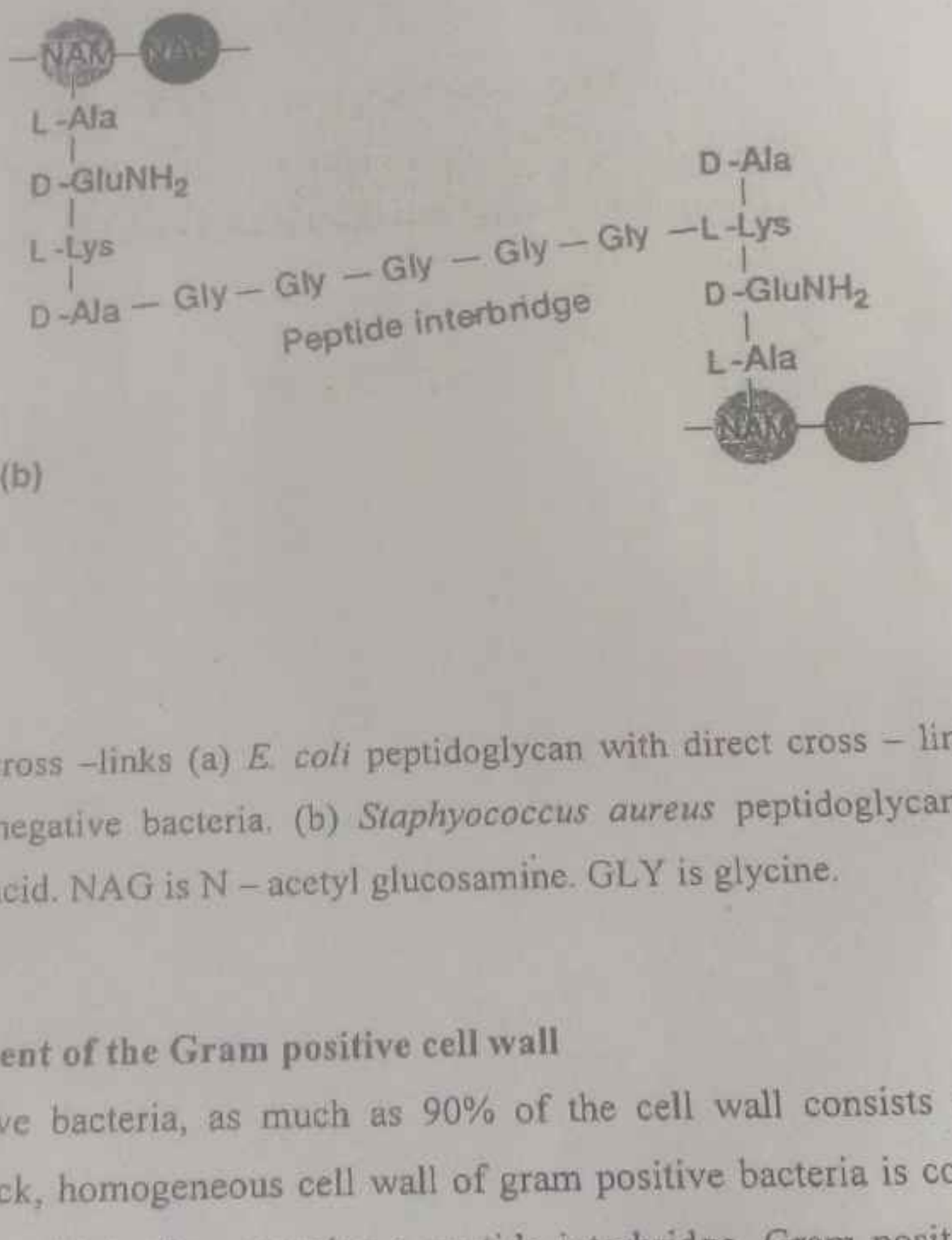


Three-Dimensional Diagram of Peptidoglycan Layer

Peptidoglycan Cross-links in gram negative (a) and gram positive (b) cell walls



(a)



(b)

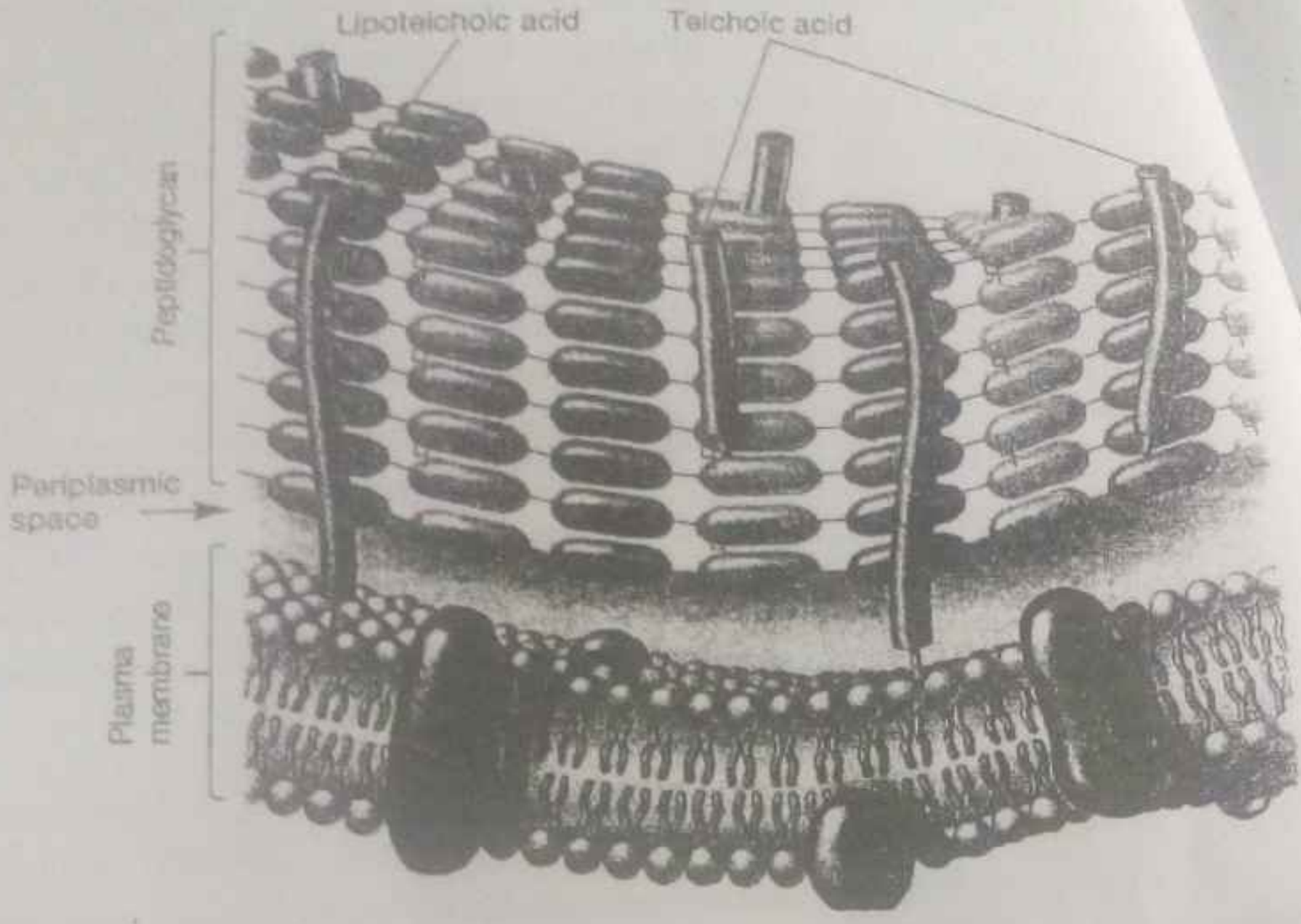
Peptidoglycan cross –links (a) *E. coli* peptidoglycan with direct cross – linking, typical of many gram – negative bacteria. (b) *Staphyococcus aureus* peptidoglycan. NAM is N – acetylmuramic acid. NAG is N – acetyl glucosamine. GLY is glycine.

Special component of the Gram positive cell wall

In Gram- positive bacteria, as much as 90% of the cell wall consists of peptidoglycan. Normally the thick, homogeneous cell wall of gram positive bacteria is composed primarily of peptidoglycan, which often contains a peptide interbridge. Gram positive cell walls also contain large amount of teichoic acids, polymers of glycerol or ribitol joined by phosphate groups. This teichoic acid is not present in gram negative bacteria. Certain teichoic acids are

covalently bound to membrane lipids; thus they have been called Lipoteichoic acids. The teichoic acid forms 50 % of dry weight of the cell wall and 10 % of the total weight of the dry cell. It is a water soluble polymers. They are usually linked on the muramic acid of the mucopeptide chain. Teichoic acids appear to extend to the surface of the peptidoglycan. Because they are negatively charged, they help give the gram-positive cell wall its negative charge. The functions of teichoic acids are still unclear, but they may be important in maintaining the structure of the wall. The periplasmic space of gram-positive bacteria lies between the plasma membrane and the cell wall, and is smaller than that of gram-negative bacteria. The periplasm has relatively few proteins; this is probably because the peptidoglycan sac is porous and any proteins secreted by the cell usually pass through it. Enzymes secreted by gram-positive bacteria are called **exoenzymes.** They often serve to degrade polymeric nutrients that would otherwise be too large for transport across the plasma membrane. Those proteins that remain in the periplasmic space are usually attached to the plasma membrane.

Staphylococci and most other gram-positive bacteria have a layer of proteins on the surface of the peptidoglycan. These proteins are involved in interactions of the cell with its environment. Some are noncovalently attached by binding to the peptidoglycan, teichoic acids, or other receptors. For example, the S-layer proteins bind noncovalently to polymers scattered throughout the cell wall. Enzymes involved in peptidoglycan synthesis and turnover also seem to interact noncovalently with the cell wall. Other substances found in Gram positive bacteria are (i) C polysaccharide (ii) M – protein. This polysaccharide includes sugar like mannose, ribose, galactose, rhamnose as well as some acid sugars like glucoranic acid. Other surface proteins are covalently attached to the peptidoglycan. Many covalently attached proteins, such as the M protein of pathogenic streptococci, have roles in virulence, such as aiding in adhesion to host tissues and interfering with host defenses. In staphylococci, these surface proteins are covalently joined to the pentaglycine interbridge of the peptidoglycan. An enzyme called sortase catalyzes the attachment of these surface proteins to the peptidoglycan. Sortases are attached to the plasma membrane of the cell.



Gram- Positive Cell Wall

Lysozyme and Protoplast: Peptidoglycan can be destroyed by certain agents, such as enzyme Lysozyme, a protein that breaks the β 1, 4- glycosidic bonds between N – acetylglucosamine and N – acetylmuramic acid in peptidoglycans, there by weakening the wall. Water then enters the cell and the cell swells and eventually bursts (cell lysis). Lysozyme is found in animal secretions including tears, saliva, and other body fluids and functions as a major line of defense against bacterial infection. If a solute that does not penetrate the cell, such as sucrose, is added to a cell suspension containing lysozyme, the solute concentration outside the cell balances the concentration inside (these conditions are called isotonic). Under isotonic conditions, if lysozyme is used to digest peptidoglycan, water does not enter the cell and lysis does not occur. Instead, a protoplast (a bacterium that has lost its cell wall) is formed. If such sucrose – stabilized protoplasts are placed in water, they immediately lyse. A protoplasts are cells that are free of residual cell wall material, whereas spheroplasts contain pieces of wall material attached to the otherwise membrane – enclosed structure.

Special component of the Gram negative cell walls

Gram negative cell walls are much more complex than gram positive walls. The thin peptidoglycan layer next to the plasma membrane may constitute not more than 5 to 10% of the wall weight. The cell wall of the Gram negative bacteria contains three polymers outside the peptidoglycan layer. These polymers are **lipoprotein, outer membrane and Lipopolysaccharide**. The outer membrane lies outside the thin peptidoglycan layer. The most abundant membrane protein is the lipoprotein or murein lipoprotein, a small lipoprotein conveniently joined in 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. The outer membrane and plasma membrane appear to be in direct contact at many locations in the gram – negative wall.

The outer membrane consists of lipopolysaccharide (LPS). These large, complex molecules contain both lipid and carbohydrate, and consist of three parts: (1) Lipid A (2) the core polysaccharide (3) the 0 specific side chain

The LPS 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.

0 specific side chain or antigen – is a short polysaccharide chain extending outward from the core. It has several peculiar sugar and varies in composition between bacterial strains. Although 0 side chains are readily recognized by host antibodies, gram negative bacteria may thwart host defences by rapidly changing the nature of their 0 side chains to avoid detection.

Important of LPS – (1) it protect the cell wall from direct attack (i.e. help it to avoid host defences). The lipopolysaccharide has the following functions:

(a) it contributes to the negative charge on the bacterial surface because of its negatively charged sugars and phosphates of the core polysaccharide. The core region of the LPS is strongly negatively charged and functions as a selective permeability barrier for negatively charged antibiotics resulting in decreased susceptibility.

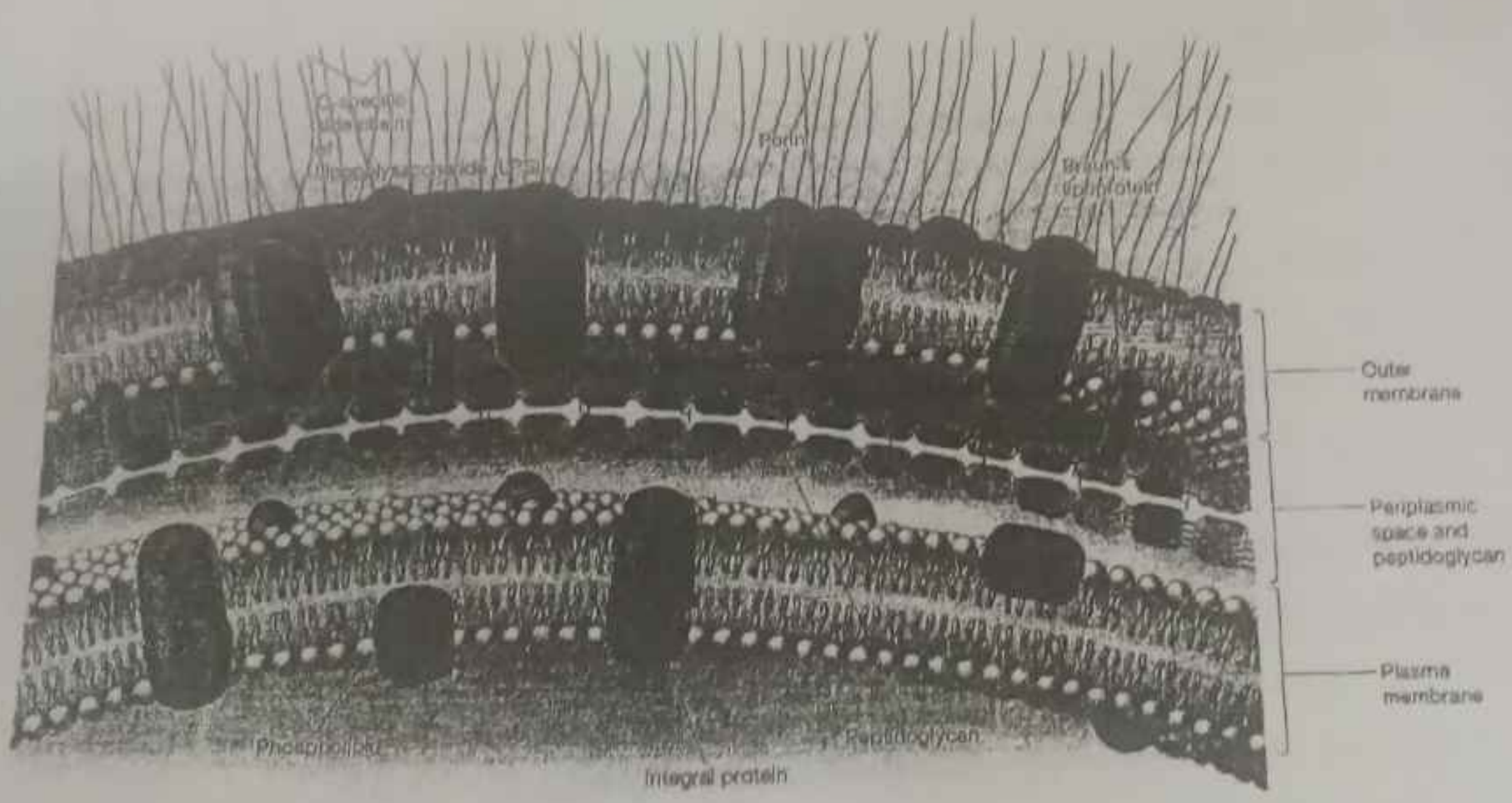
(b) It helps stabilize outer membrane structure because lipid A is a major constituent of the exterior leaflet of the outer membrane,

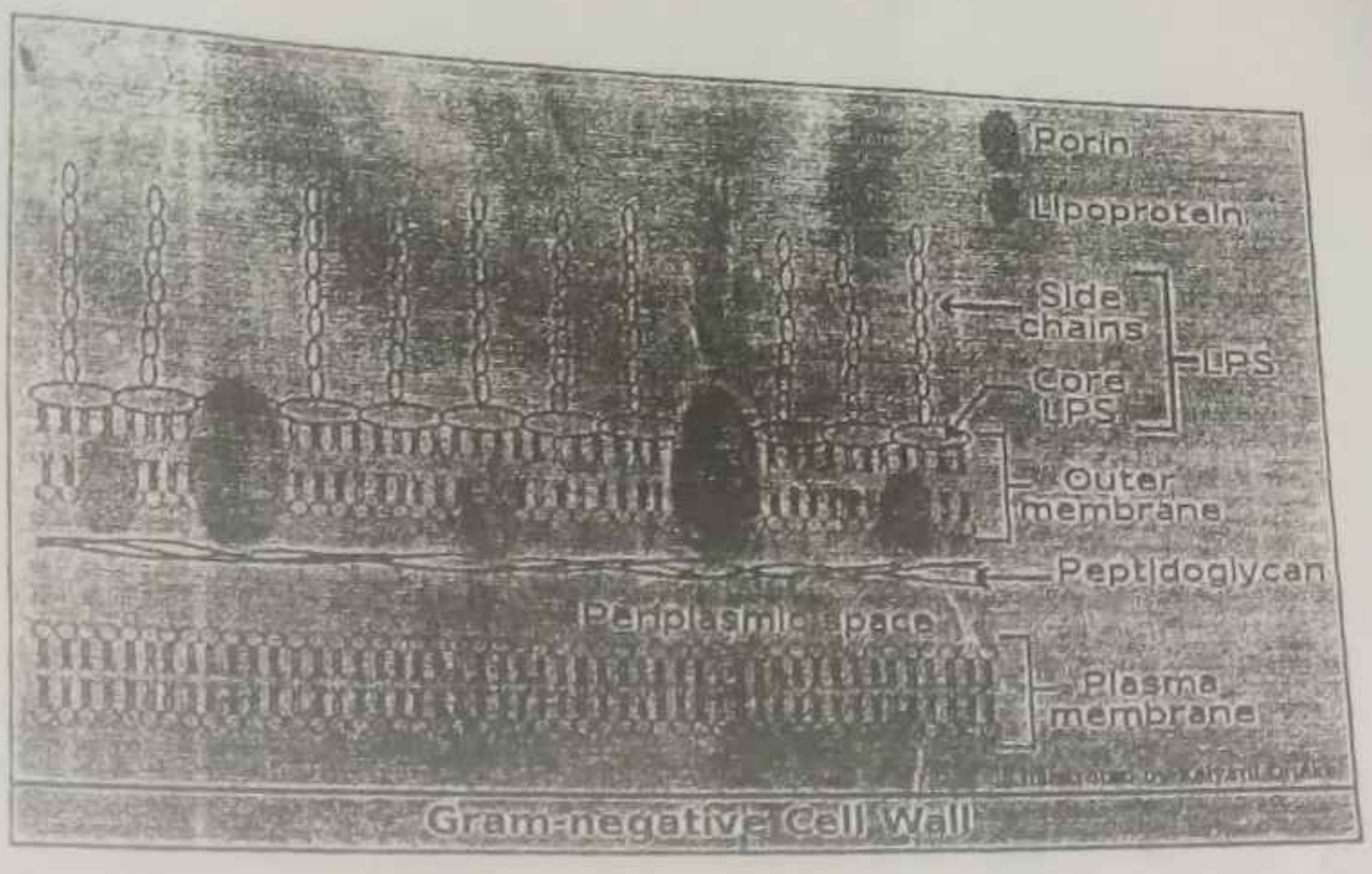
(c) A major function of LPS is that it helps create a permeability barrier, and

(d) LPS also plays a role in protecting pathogenic gram-negative bacteria from host defenses.

Lipid A is a major constituent of the outer membrane, and the LPS helps stabilize membrane structure. 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 infection.

Most important function of outer membrane is to serve as a protective barrier. It prevents/slows the entry of bile salts, antibiotics and other toxic substances that might kill or injure the bacterium. The outer membrane is more permeable than the plasma membrane and permits the passage of small molecule like glucose and other monosaccharaides. This is due to the presence of special porin proteins. Three porin molecules cluster together and span the outer member to form a narrow channel through which molecules smaller than about 600 to 700 daltons can pass. Larger molecules such as vitamin B_{10} must be transported across the outer membrane by specific carriers. The outer membrane also prevents the loss of constituents like periplasmic enzymes.





Mechanism of Gram staining

The difference between gram – positive and gram – negative bacteria is due to the physical nature of their cell walls. The peptidoglycan itself is not stained; instead it seems to act as a permeability barrier preventing loss crystal violet. Gram – positive bacteria have very thick cells walls consisting of several layers of peptidoglycan, these become dehydrated by the alcohol, causing the pores in the walls to close and preventing the insoluble crystal violet – iodine complex from escaping. 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 decolourized with ethanol, the alcohol is thought to shrink the pores of the thick peptidoglycan. Thus the dye – iodine complex is retained during the short decolourization 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 gram – negative wall to increase its porosity further. For these reasons, alcohol more readily removes the purple crystal violet – iodine complex from gram negative bacteria.

The cell wall and osmotic protection: The cell wall usually is required to protect bacteria against destruction by osmotic pressure. Solute are much more concentrated in bacterial cytoplasm than in most microbial habitats, which are hypotonic. During osmosis, water

moves across selectively permeable membranes such as the plasma membrane from dilute solutions (higher water concentration) to more concentrated solutions (lower water concentration). Thus water normally enters bacterial cells and the osmotic pressure may reach 20 atmospheres. The plasma membrane cannot withstand such pressures and the cell will swell and be physically disrupted and destroyed, a process called Lysis, without the wall that resists cell swelling and protects it. Solutes are more concentrated in hypertonic habitats than in the cell. Thus, water flows outward, and the cytoplasm shrivels up and pulls away from the cell wall. This phenomenon is known as plasmolysis and is useful in food preservation because many microorganisms cannot grow in dried foods and jellies as they cannot avoid plasmolysis.

Comparative Activities of Gram-negative and Gram-positive Bacteria

The various glaring comparative activities of both Gram-negative and Gram-positive bacteria are enumerated below:

(1) It has been duly demonstrated that the outer membrane of Gram-negative bacteria prominently behaves as a solid barrier to the smooth passage of certain critical substances, for instance: antibiotics, bile salts (Alkali salts of bile *viz.*, sodium glycocholate, and sodium taurocholate), and dyes into the cell. Hence, the Gram-negative organisms are comparatively much less sensitive to these substances than the Gram-positive ones.

(2) Adequate treatment of Gram-negative bacteria with an appropriate chelating agent, such as: Ethylene diaminetetra acetic acid (EDTA), that eventually affords the release of a substantial amount of lipopolysaccharides renders ultimately the cells more sensitive to the drugs and chemical entities. Thus, the presence of lipopolysaccharide on the surface of the cell also helps the bacteria to become resistant to the phagocytes (A cell (*e.g.*, leukocyte or macrophage) having the ability to ingest and destroy particulate substances *viz.*,bacteria, protozoa, cells and cell debris, colloids, and dust particles) of the host.

(3) The resistance acquired in (2) above is almost lost only if the host enables to synthesize the antibodies that are particularly directed against the O-side chain. There exists a vast diversification in the specific structure of the O-side chain; and, therefore, gives rise to the somatic antigenic specificity very much within the natural bacterial populations. Evidently, the ensuing antigenic diversity exhibits a distinct selective advantage specifically for a pathogenic bacterial species, because the animal host is not in a position

to possess higher antibody levels strategically directed against a relatively large number of varieties of O-side chains.

Bacteria that have no Cell wall

Protoplasts, Spheroplasts, L-forms and the **Mycoplasmas** are wholly or partially devoid of a cell wall. Such cells can be created experimentally by removing the cell wall by enzymatic digestion using lysozyme. Lysozyme is an enzyme found in human nasal secretions, tears, and in the white of chicken eggs that attacks bacterial cell walls by specifically breaking the β-1-4 glycosidic bond between N-acetyl muramic acid and N-acetyl glucosamine in the peptidoglycan. Once the peptidoglycan is hydrolysed, the cell loses its rigid shape and becomes sensitive to osmotic pressure.

1. A **protoplast** is a bacterium without a cell wall. The cell wall is lost due to the action of lysozyme enzymes. Because a protoplast is without a cell wall, it is usually lysed. A protoplast is metabolically active but unable to reproduce. Protoplast is mostly occurred in Gram-positive bacteria
2. **Spheroplast** is a bacterium with a damaged cell wall. The damage is caused by the action of a toxic chemical or an antibiotic such as penicillin. Spheroplasts show a variety of forms. They are able to change back to their normal form when the toxic agent is removed, for example when grown on a culture medium.
3. **L-forms** are mutant bacteria without cell walls. They are produced when the surrounding becomes unfavourable. They are able to reproduce and can be grown on special culture media with a high osmotic pressure. L-forms arise from both Gram-positive and Gram-negative bacteria. Some have partial cell wall whereas others are completely cell wall deficient.
4. **Mycoplasmas** - L-forms often have a morphological resemblance to **Mycoplasmas**, but the two groups of bacteria are the major groups of bacteria that have never produced cell wall. Instead of having a rigid cell structure the **Mycoplasms** assume many shapes, varying from small cocci to extended tubules or filaments. In the past, they were thought to be viruses because their small size and plasticity enabled them to pass through filters that retained other bacteria.

(2) Bacterial Cell membrane/ plasma membrane: Membranes contain both proteins and lipids, although the exact proportions of protein and lipid vary widely. Most membrane associated lipids are structurally asymmetric with polar and non-polar ends and are called amphipathic. The polar end 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.

Cells membranes are very thin structures, about 5 to 10 nm thick and can only be seen with the electron microscope. 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 protein are loosely connected to the membrane and can be easily removed. They are soluble in aqueous solution 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 solution when freed of lipids. Integral protein, like membrane lipids, are amphipathic; their hydrophobic regions are buried in the lipid while the hydrophilic portions project from the membrane surface. Integral proteins can diffuse laterally around the surface to new locations, but do not flip – flop or rotate through the lipid layer.

Functions of Plasma Membranes

(1) The plasma membrane retains the cytoplasm, particularly in cells without cell walls, and separates it from the surroundings.

(2) 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.

(3) 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.

(4) It is the location of a variety of crucial metabolic processes: respiration, photosynthesis, and the synthesis of lipids and cell wall constituents.

(5) The membrane contains special receptor molecules that help bacteria detect and respond to chemicals in their surroundings. *the plasma membrane is essential to the survival of microorganisms.

(3) **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) the plasma membrane and everything within is called the protoplast; thus the cytoplasmic matrix is a major part of the protoplast. These include:

a) Inclusion bodies – which include granules of organic or inorganic material. Some inclusion bodies are not bounded by a membrane and lie free in the cytoplasm. For e.g. polyphosphate granules, cyanophycin granules and some glycogen granules. Other inclusion bodies such as gas vacuoles, carboxysomes sulphur granules are membrane – enclosed. Some are protein in nature, whereas others contain Lipid A.

Ribosomes: The cytoplasmic matrix often is packed with ribosomes; they also are loosely attached to the plasma membrane. Ribosomes are made up of protein and ribonucleic acid (RNA). They are the site of protein synthesis. The prokaryotic ribosomes are smaller than eukaryotic ribosomes. They commonly are called 70 S ribosomes, have dimensions of about 14 to 15 nm, a molecular weight of approximately 2.7 million, and are constructed of a 50 S and a 30 S subunit. The S in 70 S and similar values stand for Svedberg unit. This is unit of the sedimentation coefficient, a measure of the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged; the greater it's Svedberg value or sedimentation coefficient. The sedimentation coefficient is a function of a particle's molecular weight, volume and shape. The weight of a 70 S ribosome equals the sum of the 50 S and 30 S subunit weights even though the sum of 50 and 30 is 80, not 70.

Nucleoid: The prokaryotic chromosome, almost always a single circle of double – stranded deoxyribonucleic acid (DNA), is located in an irregularly shaped region called the nucleoid. The nucleoid appearance varies with the method of fixation and staining, fibres often are seen in electron micrographs and are probably DNA. 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. Chemical analysis reveals that the nucleoids are composed of about 60% DNA, some RNA and a small amount of protein.

Many bacteria possess plasmids in addition to their chromosome. These are circular, double-stranded DNA molecules that can exist and replicate independently of the chromosome or may be integrated with it; in either case they are inherited or passed on to the progeny. 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.

Components External to the cell wall:

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

1 Capsules and Slime layers

Many bacteria manufacture and export high molecular weight polymers that adhere to the exterior of the cell wall to form a capsule or slime layer. In general, a bacterial capsule has a uniform thickness and can be thicker than the cell itself, whereas material adhering to the cell wall in a diffused arrangement is called a slime layer. Capsule usually refers to the layer both intimately and tightly attached to the cell wall; whereas, the slime coating (layer) is contrarily the loose structure which often gets diffused right into the corresponding available growth medium. Slime layer is easily removed or washed off whereas capsule is not. Capsules may be polysaccharide (as in *Diplococcus pneumoniae* and *Leuconostoc dextranicum*), polypeptide (*Bacillus anthracis*) or more complex consisting of combinations of polysaccharide/polypeptide (*B. megaterium*), polysaccharide/protein/phospholipid (*Shigella dysenteriae*).

Capsule formation is a genetic property of the species but depends also on the environment, e.g. the presence of saccharides, $C0_2$ or serum, and on the age of the culture. Lactic acid bacteria produce dextran only when excess sucrose is available. Capsular structure is highly specific and is used diagnostically. While slime layers help bacteria to colonize surfaces, capsules contribute to the virulence (invasiveness) of pathogenic bacteria since the encapsulated cells are protected from phagocytosis by white blood cells. Many bacteria that contribute to dental caries produce slime that helps them adhere to the tooth surface. Cells

whose capsules formation is due to mutation (genetic change), usually appear "smooth" or "mucoid" while the non-encapsulated cells appear as "rough" colonies.

Functions of Capsules: In reality capsules may serve *five* cardinal functions exclusively depending on their respective bacterial species as described under:

(*a*) They may afford adequate protection against temporary drying by strategically bound to water molecules.

(*b*) They may cause absolute blockade of attachment to bacteriophages.

(*c*) They may be antiphagocytic in nature.

(*d*) They may invariably promote attachment of bacteria to surfaces, such as: *Streptococcus mutans* — a bacterium that is directly linked to causing dental caries, by means of its 'ability to adhere intimately onto the smooth surfaces of teeth on account of its specific secretion of a water-insoluble capsular glucan.

(*e*) In the event when the capsules are essentially made up of compounds bearing an 'electrical charge', for instance: a combination of sugar-uronic acids, they may duly help in the promotion of the stability of bacterial suspension by preventing the cells from aggregating and settling out by virtue of the fact that such cells having identical charged surfaces would have a tendency to repel one another predominantly.

Examples of bacteria that have capsules are *streptococcus pneumonia*, *Klesbsiella pneumonia*. glycocalyx – also aids bacterial attachments to surfaces of solid objects in aquatic environments or to tissue surface in plant and animal host.

2 Fimbriae and Pili

Many prokaryotes have short, fine, hair-like appendages that are thinner than flagella. They are usually called **fimbriae** (singular- fimbria). Although many people use the terms fimbriae and pili interchangeably, they are distinct from each other. A cell may be covered with up to 1,000 fimbriae but they are only visible in an electron microscope due to their small size. Fimbriae are about 3 – 10 nm in diameter. At least some types of fimbriae attach bacteria to solid surfaces such as rocks in streams and host tissues. Therefore, they play a role in the adherence of symbiotic bacteria to host cells. The virulence of certain pathogenic bacteria depends on the production not only of toxins but also of "**colonization antigens**" which are now recognized to be fimbriae that provide the cells with adherent properties. Fimbriae do play a major role in causing and spreading human infection to an appreciable extent by permitting the pathogenic bacteria to get strategically attached to various epithelial cells lining the genital, urinary, intestinal, or respiratory tracts specifically. It is worthwhile to

mention here that this particular attachment exclusively checks and prevents the bacteria from being washed away critically by the incessant flow of either mucous or body fluids thereby allowing the infection to be established rather firmly.

Fimbriae are responsible for more than attachment. **Type IV fimbriae** are present at one or both poles of bacteria cells. They can aid in attachment to objects and also required for the twitching motility that occur in some bacteria such as *P. aeruginosa, Neisseria gonorrhoeae* and in some strains of *E. coli*. There is evidence that the fimbriae actually retract to move these bacteria.

Pili on the other hand are short hair-like structures that project out from the bacterial cell wall, especially Gram-negative rods of enteric origin. They differ from fimbriae in the following ways. Pili often are larger than fimbriae (around 9 – 10 nm in diameter). Pili are genetically determined by conjugative plasmids and are required for conjugation. Like flagella, they are composed of protein sub-unit; they are however shorter and finer than flagella.

Pili that are involved in the transfer of genetic information between cells and function as receptors during some viral infections are called sex pili. Sex pili occur sparsely on 'male' (F^+) cells only and enable them to attach to 'female' (F^-) cells. The pilus (singular form of pili) then contracts to form a cytoplasmic bridge through which genetic material is transferred from the male donor (F^+) to the female recipient (F^-). This mating process is known as conjugation. It is important medically because it is possible for the genetic materials which determine antibiotic resistance to be passed from one bacterium to another. Sex pili also possess receptors to which viruses (bacteriophages) can become attached. Such bacteriophages can also transfer genetic characteristic from one bacterial strain to another by transduction.

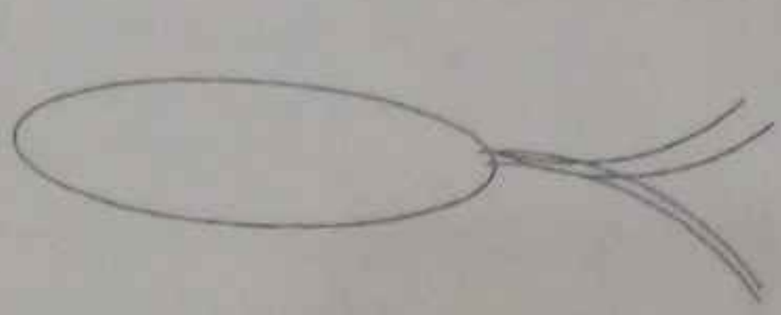
3 ***Flagella*** – Flagella are threadlike locomotors appendages extending outward from the plasma membrane and cell wall. They are slender, rigid structure, about 20 nm across and up to 15 or 20 um long. Flagella are so thin they cannot be observed directly with a bright-field microscope, but must be stained with special techniques designed to increase their thickness. Flagella help the bacterial in movement.

Bacterial species often differ distinctively in their patterns of flagella distribution. Three types of arrangements of flagella are known namely: (i) monotrichous bacteria (trichous

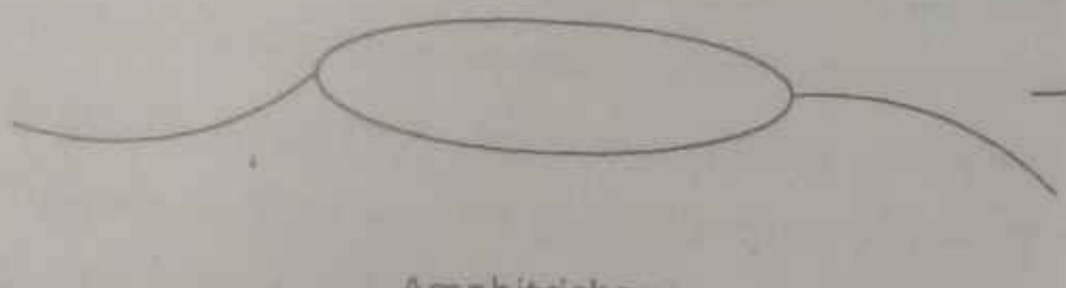
means hair) have one flagellum; if it is located at the end, it is said to be a **polar flagellum.** Amphitrichous bacteria (amphi means on both sides) have a single flagellum at each pole. In contrast, lophotrichous bacteria (lopho means tuft) have a cluster of flagella at one or both ends. Flagella are spread fairly evenly over the whole surfaces of **peritrichous** (peri means around) bacteria. Flagellation patterns are very useful in identifying bacteria.

Diagrams of different types of flagella arrangement

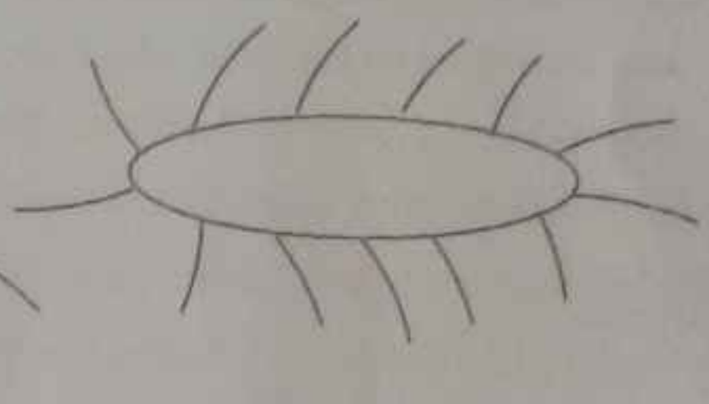
Monotrichous



Lophotrichous



Amphitrichous



Peritrichous

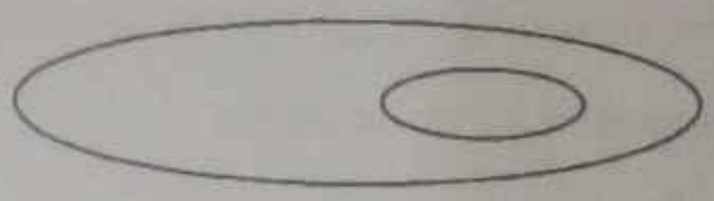
Ultrastructure of flagella

Transmission electron microscope studies have shown that the bacterial flagellum is composed of three parts:

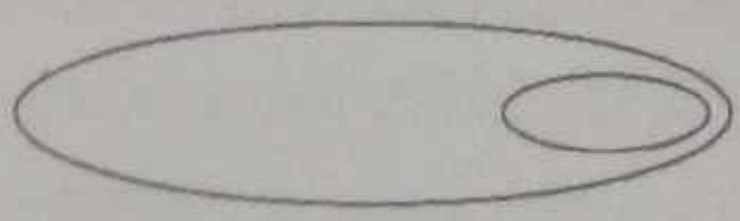
1) The longest and most obvious portion is the **filament** which extends from the cell.
2) A basal body – is embedded in the cell.
3) A short, curved segment, **the hook**, links the filament to its basal body and acts as a flexible coupling. The filament is a hollow, rigid cylinder constructed of a single protein called **flagellin,** which ranges in molecular weight from 30,000 to 60,000.

4 Bacterial endospore: A number of gram positive bacteria can form a special resistant, dormant structure called an **endospore**. Microorganisms sense and adapt to changes in their environment. When favoured nutrients are exhausted, some bacteria may become motile to seek out nutrients, or they may produce enzymes to exploit alternative resources. One example of an extreme survival strategy employed by certain low G+C Gram-positive bacteria is the formation of endospores. This complex developmental process is often initiated in response to nutrient deprivation. It allows the bacterium to produce a dormant and highly resistant cell to preserve the cell's genetic material in times of extreme stress. Endospores are produced by certain bacteria from the Firmicute phylum, namely members of the genera *Bacillus*, *Clostridium* and *Sporosarcina* (cocci). They characteristically form endospores (because the spores are formed within vegetative cells) under certain environmental conditions. One cell generally produces one spore, which may be located centrally, terminally or sub terminally according to the species. Generally, spores are formed when the conditions are unfavourable for growth; when conditions again become favourable for growth, the spore germinates to produce one vegetative cell again. Unlike in fungi where spore formation is a means of reproduction; they are resting cells highly resistant to desiccation, heat, ultraviolet radiation, gamma radiation, enzymatic destruction and chemical disinfectants. Endospores usually survive boiling for one hour or more. Bacterial spores are of paramount importance to the microbiologist because of their ability to withstand high temperatures and chemical treatments which makes them very difficult to control. Thus, it is the presence of spores which determines the treatment required to sterilize materials. The structures of spores and their modes of occurrence and germination may be diagnostic of the species. e.g. *B. polymyxa* and *Cl. perfringens* form central spores, *B. subtilis* and *B. anthracis* form sub terminal spores while *Cl. tetani* and *B. circulans* form terminal spores.

Central endospore



Sub terminal endospore



Terminal endospore

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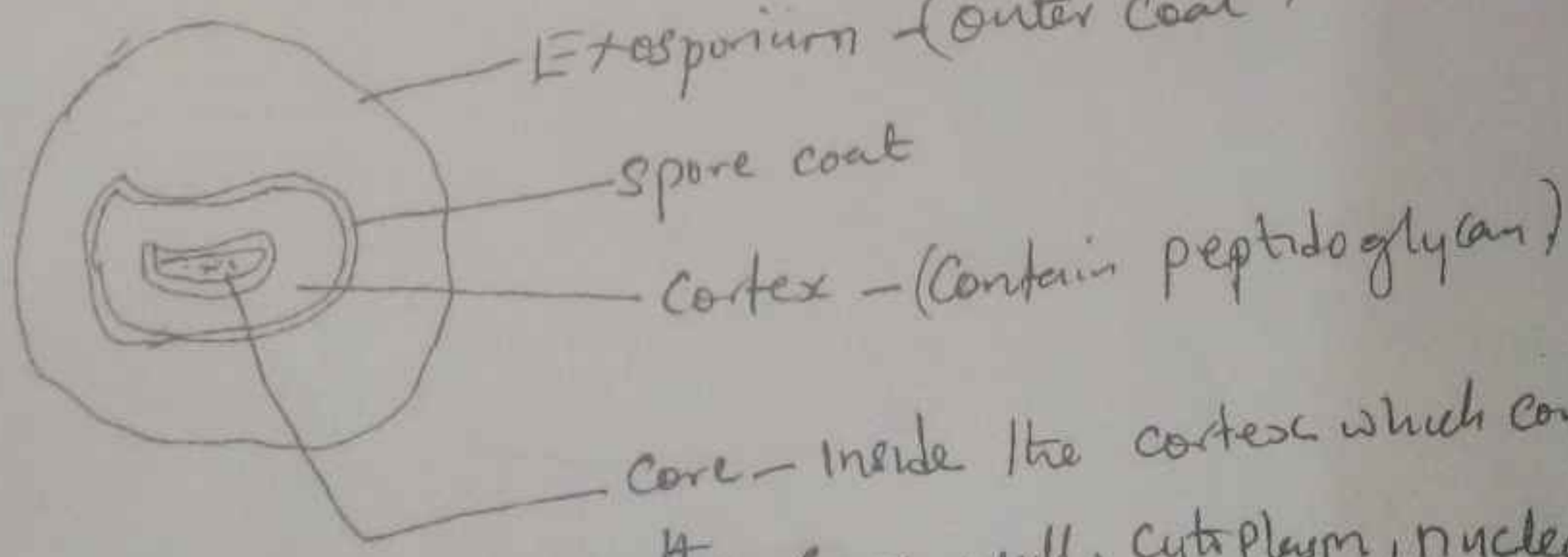
Endospores develop within vegetative bacterial cells of several genera: Bacillus and clostridium. Some bacteria that have spores are found on the soil eg *Clostridium tetani*, which causes tetanus, *Clostridium botulism* that cases food poison. These structures are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, chemical disinfectants and desiccation. Some endospores have remained viable for over 500 years. Endospore forming bacteria are dangerous pathogens, and are of great practical importance in food, industrial and medical microbiology. Endospores often survive boiling for an hour or more; therefore autoclaves must be used to sterilize many materials.

Endospores can be examined with both light and electron microscopes. Because spores are impermeable to most stains, they often are seen as colourless areas in bacteria treated with simple stains.

Spore position in the mother cell or sporangium frequently differs among species, making it of considerable value in identification.

Endospore structure:

Endospores stand out under the light microscope as strongly refractile structures. Endospores are impermeable to most dyes, so occasionally they are seen as unstained regions within cells that have been stained with basic dyes such as methylene blue. To stain endospores, special stains and procedures must be used. In the classical endospore-staining protocol, malachite green is used as a stain and is infused into the spore with steam. The structure of the endospores are seen with the electron microscope differs distinctly from that of the vegetative cell. The endospore is structurally more complex in that it has many layers that are absent from the vegetative cell. The outermost layer is the **exosporium**. Within this are the **spore coats**, composed of layers of spore-specific proteins. Below the spore-coat is the **cortex**, which consists of loosely cross-linked peptidoglycan, and inside the cortex is the **core**, which contains the core wall; cytoplasmic membrane, cytoplasm, nucleoid, ribosomes and other cellular essentials. Thus, the endospore differs structurally from the vegetative cell primarily in the kinds of structures found outside the core wall. The endospores contain one substance called dipicolinic acid which is absent in the vegetative cells. This substance has been found in the endospores of all endospore- forming bacteria and is located in the core. Endospores are also enriched in calcium (Ca^{2+}), most of which is complexed with dipicolinic acid. The calcium – dipicolinic acid complex of the core represents about 10 % of the dry weight of the endospore. The complex functions to reduce water availability within the endospore intercalates in DNA, and in so doing stabilizes DNA to heat denaturation. Dipicolinic acid is a spore – specific chemical that appears to help in the ability for endospores to maintain dormancy.



Structure of Endospore