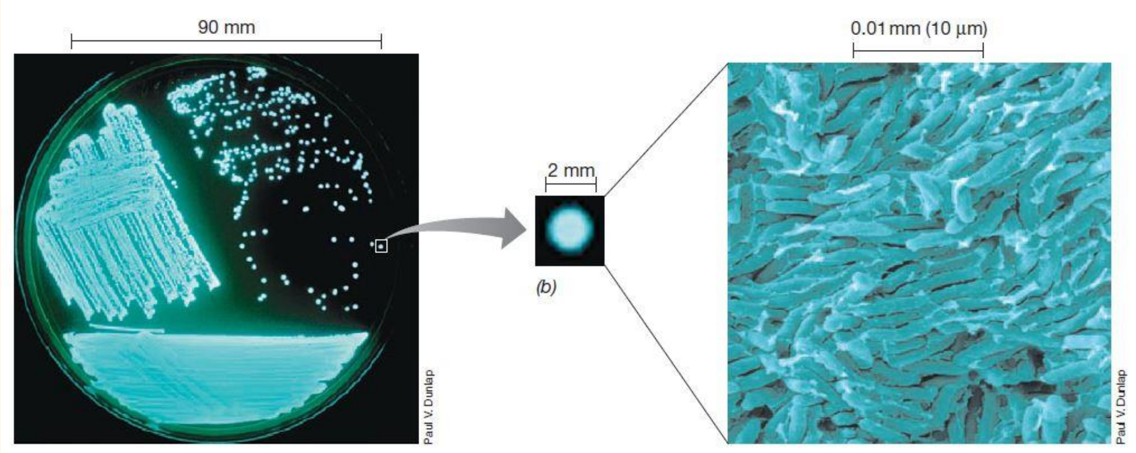
M.Sc. 4th Semester Study Material (I)

Subject: HUMAN PHYSIOLOGY

Topic : Microbial Biochemistry	
Paper	**PHY 403 (Special Paper)**
Unit	**37 (Module II)**
Name of Guest Lecturer	**Suparna Majumder**

A. MEMBRANE CHEMISTRY OF GRAM POSITIVE AND NEGATIVE BACTERIA:

Microorganisms are single-celled microscopic organisms that are essential for the well-being and functioning of other life forms and the planet.



Microbial cells. *(a)* Bioluminescent (light-emitting) colonies of the bacterium *Photobacterium* grown in laboratory culture on a Petri plate. *(b)* A single colony can contain more than 10 million (10^7) individual cells. *(c)* Scanning electron micrograph of cells of *Photobacterium.*

The **cytoplasmic membrane** surrounds the cytoplasm and separates it from the environment. If the cytoplasmic membrane is compromised, the integrity of the cell is destroyed, the cytoplasm leaks into the environment, and the cell dies. The cytoplasmic membrane is structurally weak and confers little protection from osmotic lysis, but it is an ideal structure for its major function on the cell: selective permeability.

►**Composition of Membranes:** The general structure of the cytoplasmic membrane is a phospholipid bilayer. Phospholipids are composed of both hydrophobic (fatty acid) and hydrophilic (glycerol–phosphate) components. As phospholipids aggregate in an aqueous solution, they naturally form bilayers. In a phospholipid membrane, the fatty acids point inward toward each other to form a hydrophobic environment, and the hydrophilic portions remain exposed to the external environment or the cytoplasm. Common fatty acids in the cytoplasmic membrane include those with 14 to 20 carbon atoms. The cytoplasmic membrane is only 8−10 nanometers wide but is still visible in the transmission electron microscope, where it appears as two dark lines separated by a light line. This *unit membrane*, as it is called (because each phospholipid leaf forms half of the "unit"), consists of a phospholipid bilayer with proteins embedded in it. Although in a diagram the cytoplasmic membrane appears rigid, it is actually somewhat fluid, having a consistency approximating that of a low-viscosity oil. Thus, some freedom of movement exists for proteins embedded in the membrane. The cytoplasmic membranes of some *Bacteria* are strengthened by sterol-like molecules called *hopanoids*. Sterols are rigid and planar molecules that function to strengthen the membranes of eukaryotic cells, and hopanoids serve a similar function in *Bacteria*.

●**Membrane Proteins:** The protein content of the cytoplasmic membrane is quite high, and membrane proteins typically have hydrophobic surfaces in regions that span the membrane and hydrophilic surfaces in regions that contact the environment and the cytoplasm. The *outer* surface of the cytoplasmic membrane faces the environment and in gram-negative bacteria interacts with a variety of proteins that bind substrates or process larger molecules for transport into the cell. The *inner* surface of the cytoplasmic membrane touches the cytoplasm and interacts with proteins and other molecules in this milieu. Many membrane proteins are firmly embedded in the membrane and are called *integral* membrane proteins. Other proteins have one portion anchored in the membrane and extra membrane regions that point into or out of the cell. Still other proteins, called *peripheral* membrane proteins, are not membrane embedded but nevertheless remain associated with membrane surfaces. Some of these peripheral membrane proteins are lipoproteins, molecules that contain a lipid tail that anchors the protein into the membrane. Peripheral membrane proteins typically interact with integral membrane proteins in important cellular processes such as energy metabolism and transport. Membrane proteins that need to interact with each other in some process are typically grouped together into clusters to allow them to remain adjacent to one another in the semifluid environment of the membrane.

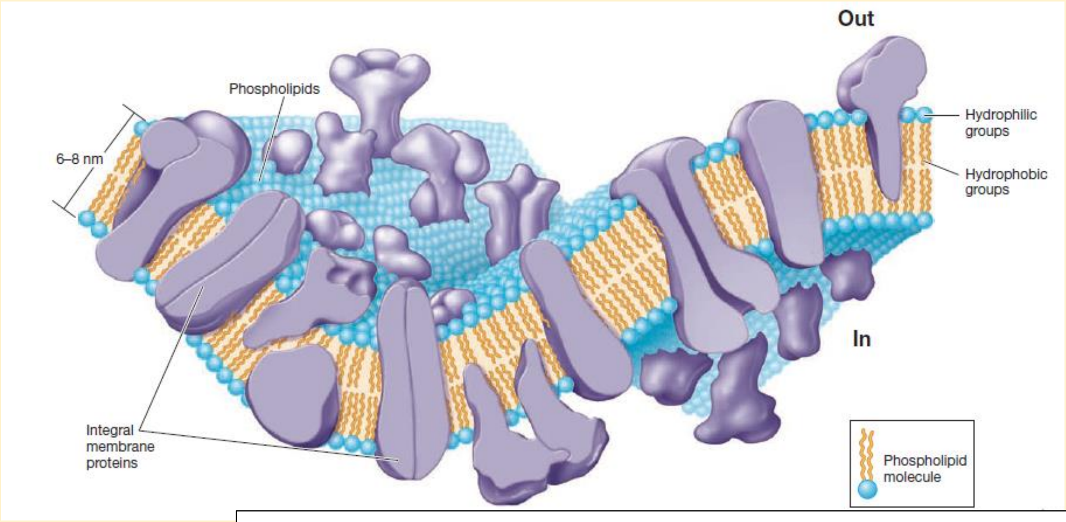
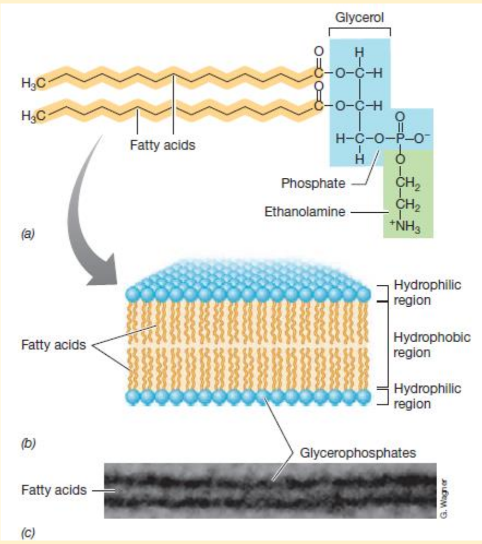


Fig: Cell membrane structure of bacteria



Phospholipid bilayer membrane.
(a) Structure of the phospholipid phosphatidylethanolamine.
(b) General architecture of a bilayer membrane; the blue spheres depict glycerol with phosphate and/or other hydrophilic groups.
(c) Transmission electron micrograph of a membrane. The light inner area is the hydrophobic region of the model membrane shown in part b.

CELL WALL: Prokaryotic cells almost always are bounded by a fairly rigid and chemically complex structure present between the cell membrane and capsule/slime layer called the cell wall. Peptidoglycan is the main component of the cell wall and is responsible for the shape and strength of the cell. It is a disaccharide and contains two sugar derivatives—*N*-acetylglucosamine and *N*-acetylmuramic acid—joined together by short peptide chains. *N*-acetylmuramic acid carries a tetrapeptide side chain consisting of D- and L-amino acids (D-glutamic acid and L-alanine) with mesodiaminopimelic acid (Gram-negative bacteria) or L-lysine (Gram-positive bacteria). Tetrapeptide side chains are interconnected by pentaglycine bridges. Most Gram-negative cell walls lack interpeptide bridge. Cell wall provides shape to the cell and protects bacteria from changes inosmotic pressure, which within the bacteria cell measures 5–20 atmospheres. Bacterial cells can be classified into Gram-positive or Gram-negative based on the structural differences between Gram-positive and Gram-negative cell walls. The cell walls of the Gram-positive bacteria have simpler chemical structures compared to Gram-negative bacteria.

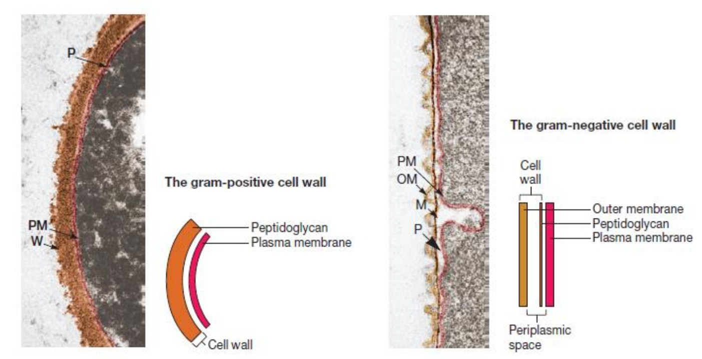


Fig: Cell wall of Gram +ve and Gram –ve bacteria

●**Gram-positive cell wall:** The Gram-positive cell wall is thick (15–80 nm) and more homogenous than that of the thin (2 nm) Gram-negative cell wall. The Gram-positive cell wall contains large amount of peptidoglycan present in several layers that constitutes about 40–80% of dry weight of the cell wall. The Gram-positive cell wall consists primarily of teichoic and teichuronic acids. These two components account for up to 50% of the dry weight of the wall and 10% of the dry weight of the total cell.

1. **Teichoic acids:** Teichoic acids are polymers of polyribitol phosphate or polyglycerol phosphate containing ribitol and glycerol. These polymers may have sugar or amino acid substitutes, either as side chain or within the chain of polymer. Teichoic acids are of two types—wall teichoic acid (WTA) and lipoteichoic acids (LTA). They are connected to the peptidoglycan by a covalent bond with the six hydroxyl of *N*-acetylmuramic acid in the WTA and to plasma membrane lipids in LTA.

Teichoic acids have many functions:

■They constitute major surface antigens of those Gram positive species that possess them. In *Streptococcus pneumoniae*, the teichoic acids bear the antigenic determinants called *Forssman antigen*. In *Streptococcus pyogenes*, LTA is associated with the M protein that protrudes from the cell membrane through the peptidoglycan layer. The long M protein molecules together with the LTA form microfibrils that facilitate the attachment of *S. pyogenes* to animal cells;

■They are also used as antigen for serological classification of bacteria;

■They serve as substrates for many autolytic enzymes;

■They bind magnesium ion and may play a role in supply of this ion to the cell;

■They play a role in normal functioning of the cell wall and provide an external permeability barrier to Gram-positive bacteria; and

■ Membrane teichoic acid serves to anchor the underlying cell membrane.

2. Teichuronic acid: Teichuronic acid consists of repeat units of sugar acids (such as *N* acetylmannuronic or D-glucuronic acid). They are synthesized in place of teichoic acids when phosphate supply to the cell is limited. Gram-positive cell wall also contains neutral sugars (such as mannose, arabinose, rhamnose, and glucosamine) and acidic sugars (such as glucuronic acid and mannuronic acid), which occur as subunits of polysaccharides in the cell wall.

●Gram-negative cell wall

The Gram-negative cell wall is much more complex than the Gram-positive cell wall. Peptidoglycan content in the Gram-negative cell wall is significantly less than the Grampositive cell wall. Only 1–2 layers of peptidoglycan (2–8 nm) are present just outside the cell membrane. The Gram-negative cell wall outside the peptidoglycan layer contains three main components—(*a*) lipoprotein layer, (*b*) outer membrane, and (*c*) lipopolysaccharides.

Lipoprotein layer: The lipoprotein layer is mainly composed of Braun's lipoprotein. Braun's lipoprotein is a small lipoprotein that is covalently joined to the underlying peptidoglycan and embedded in the outer membrane by its hydrophobic end. The lipoprotein stabilizes the outer membrane of the Gram negative cell wall.

Outer membrane: The outer membrane is a bilayered structure; its inner part resembles in composition with that of the cell membrane, while its outer part contains a distinctive component called lipopolysaccharide. The outer membrane and plasma membrane appear to be in direct contact at many sites in the Gram-negative wall. The outer membrane has a variety of proteins as follows:

(a) ***Porins*****:** The outer membrane has special channels consisting of protein molecules called porins. These porins have many functions: ■They permit the passive diffusion of low-molecular weight hydrophilic compounds, such as sugars, amino acids, and certain ions;

■They exclude hydrophobic molecules; and

■They serve to protect the cell.

(b) ***Outer membrane proteins (OMPs)*****:** These include the following:

■ Omp C, D, F, and PhoE & Lam B are the four major proteins of the outer membrane that are responsible for most of the transmembrane diffusion of maltose and maltodextrins.

■ Tsx, the receptor for T6 bacteriophage, is responsible for the transmembrane diffusion of nucleosides and some amino acids.

■ Omp A protein anchors the outer membrane to the peptidoglycan layer. It is also the sex pilus receptor in F-mediated bacterial conjugation.

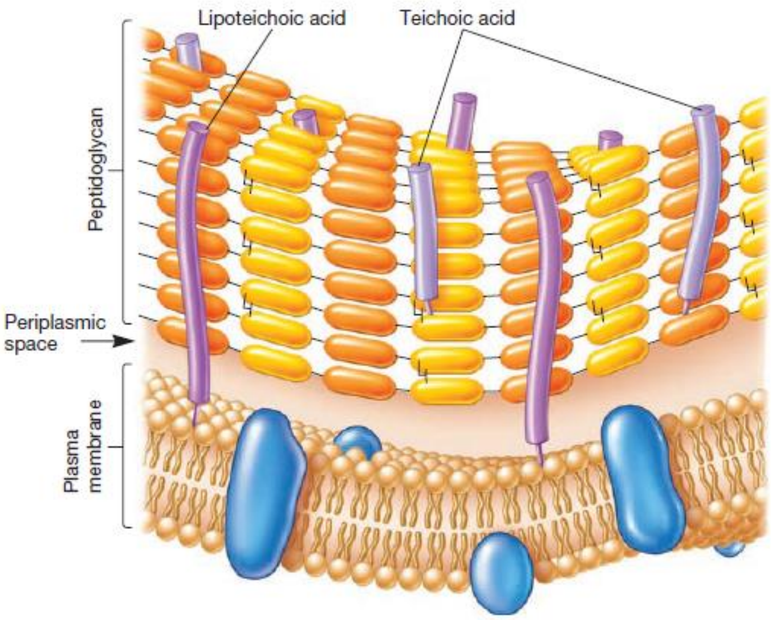
The outer membrane also contains proteins that are involved in the transport of specific molecules, such as vitamin B^{12} and iron-siderophore complexes; it also contains a limited number of minor proteins, such as enzymes, phospholipases, and proteases.

Lipopolysaccharides: Lipopolysaccharides (LPS) are complex molecules present in the outer membrane of the Gram-negative bacteria. Structurally, the LPS consist of three main components— lipid A, the core oligosaccharide, and the O polysaccharide or O-antigen.

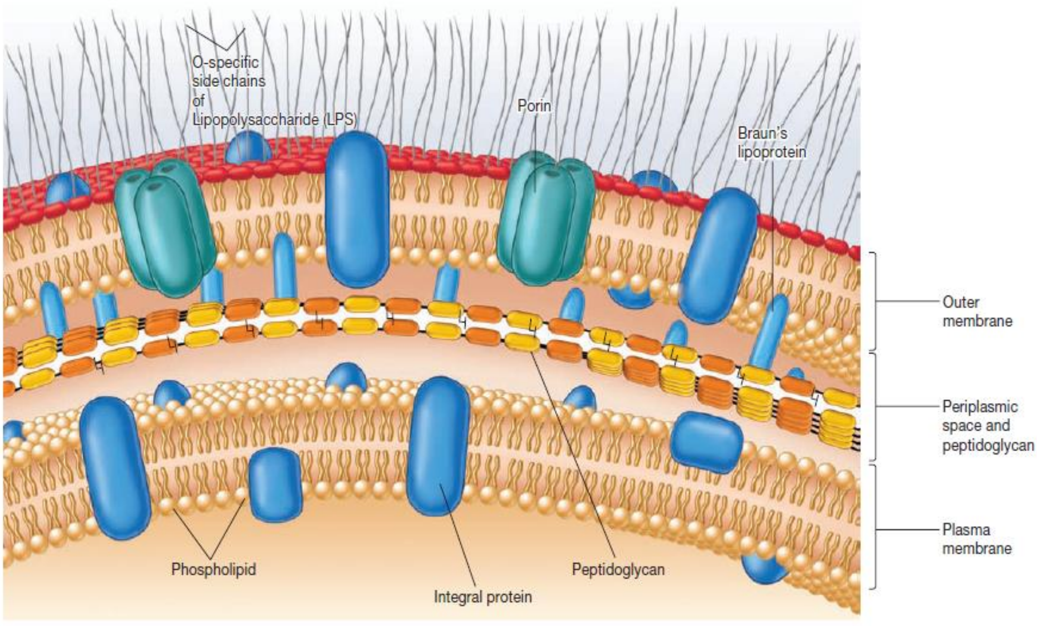
■ ***Lipid A*****:** This consists of phosphorylated glucosamine disaccharide units, to which a number of long-chain fatty acids are attached. This also consists of hydroxymyristic acid, a unique fatty acid, which is associated with endotoxic activity of the LPS. There is a little variation in the structure of the lipid A among different species of the Gram-negative bacteria. However, it remains the same within the bacteria of the same species.

■ ***Core oligosaccharide*****:** The core oligosaccharide includes two characteristic sugars—ketodeoxyoctanoic acid (KDO) and a heptose—both joined together by lipid A. This is genus specific and similar in all Gram-negative bacteria. Lipooligosaccharides (LOS) are smaller glycolipids. They have relatively short, multiantennary (i.e., branched) glycans pre sent in bacteria (e.g., *Neisseria meningitidis*, *N. gonorrhoeae*, *Haemophilus influenzae*, and *Haemophilus ducreyi*) that colonize mucosal surfaces. They exhibit extensive antigenic and structural diversity even within a single strain. LOS is an important virulence factor. Epitopes on LOS have a terminal *N*-acetyllactosamine (Gal (β) l-4-GlcNAc) residue, which is immunochemically similar to the precursor of the human erythrocyte i antigen. Sialylation of the *N*-acetyllactosamine residue *in vivo* provides the organism with the environmental advantages of molecular mimicry of a host antigen and the biologic masking thought to be provided by sialic acids.

■ ***O polysaccharide or O-antigen*****:** It is the portion extending outwards from the core. It has several peculiar sugars and varies in composition between bacterial strains, conferring species-specifi c antigen specificity. It is exposed to host–immune system. Gram-negative bacteria may thwart host defenses by rapidly changing the nature of their O side chains to avoid detection.



The Gram-Positive Envelope.



The Gram-Negative Envelope.

►POSSIBLE QUESTIONS:

1. Give a brief description of the ultrastucture of bacterial membrane.
2. Differentiate between Gram +ve and Gram –ve cell wall properties.
3. What do you mean by OMPs?
4. Why techoic acid is recognized as an important component of cell wall of Gram +ve bacteria?

►HOME ASSIGNMENT:

Prepare the questions and write about role of peptidoglycan in bacterial cell wall which you have to submit after opening the college.