Cell- The Unit of Life Part 1

Tools and Technique.

The preparation of the cells or cells parts for their study requires very specialized methods. The search continues for new instruments. So as to provide better understanding of cell structure upto molecular level, the following tools and techniques helped the biologists to know more about the cells.

(1) **Microscopy :** (*Gk. Micros* = small ; *Skopein* = to see) It is practice of using microscopes for the study of finer details of small objects including cells and tissues. Microscope are instruments consisting of lenses (made of glass / Lithium fluoride / electromagnetic lens) which magnify and resolve small objects not visible to unaided eye for the study of their details.

(i) **Magnification :** Is the power of enlargement, which is the ratio of

$$\text{Magnification} = \frac{\text{Size of the image with the instrument}}{\text{Size of the image with unaided eye}}$$

Magnification of a microscope is roughly equal to the multiple of magnifying power of objective lens and ocular lens (eye piece) *e.g.*, if the magnification power of an ocular lens is $10\,X$ and of the objective is $40\,X$, then the total magnifying power of a microscope is $10 \times 40 = 400\,X$ (the magnification power of a microscope is represented by the symbol 'X').

(ii) **Resolving power :** It is the ability of a system to distinguish two close objects as two distinct objects. Its values is calculated by *Abbe* equation –

$$L_m = \frac{0.61\lambda}{NA}$$

Here, λ – is wavelength of used light, NA – Numerical Aperture, $(NA = n\sin\theta)$

Numerical aperture is multiple of refractive index of medium (n) and $\sin\theta$, which is sine of angle substended by optical axis and outer ray covered by objective. The value for best objective $\sin 70° = 0.94$.

The resolving power of a microscope depends on the kind of illumination used. It is equal to one half of the wavelength of the illuminating light. The wavelength of visible light is 3,900 Å to 7,800 Å. Taking the average as 5,850 Å, the resolving power of the light microscope is about 3,000 Å or $0.3\,\mu m$. Therefore, we can not see objects smaller than $0.3\,\mu m$ even with the light microscope. Many cell organelles are smaller than $0.3\,\mu m$. These were unknown till electron microscope was invented.

The resolving power of human eye is $100\mu m$ or microns (0.1 *mm*). This means that two points less than $100\,\mu m$ apart appear as one point to our eyes.

Father of microscopy is *Leeuwenhoek*. He built first 270 *X* magnification microscope in 1672.

(2) **Types of microscopes**

(i) **Simple microscope :** It is also known as magnifying glass and consists of a convergent lens. The image formed by it is virtual, enlarged and on same side of lens.

Robert Hooke designed a primitive microscope and discovered cells with it. It was the first tool ever used to observe biological objects. Its magnification power was 14 – 42 times only, so it is considered as simple microscope.

(ii) **Compound microscope or Light microscope :** The first compound microscope was assembled by *Zacharias Janseen* and *J. Janseen*, the Dutch spectacles makes in 1590. The first compound microscope was prepared by *Kepler* and *Galileo* in 1611. However, it was not used for laboratory study. It is simplest, widely used microscope having three lens *i.e.* condensor, which collects the light rays and precisely focuses them on the objects; objective lens, which magnifies the image by three objective lenses, *i.e.* low power, high power and oil immersion lenses.

In a compound microscope an object can be magnified upto 1000 times and the magnification is independent of intensity of light, size of microscope and numerical aperture. The light microscope is also called *bright field* microscope because it forms the image when light is transmitted through the object. The specimen formed is darker than the surrounding bright field. The light microscope can be used to examine the live, unstained materials as well as the preserved and stained specimens.

(iii) **Fluorescent microscope :** It was developed by *Coons* (1995). It is observed that when ultraviolet light is irradiated on certain chemical substances, they absorb it and emit visible light. These chemical substances are called fluoro-chromes. The fluorescent substances *e.g.*, quinine sulphate, rhodamine and auramine are used to stain the cellular objects and these objects are easily visible as fluorescent areas when illuminated with ultraviolet light. Property of emission of long wave radiation soon after getting excited by shorter wave called, fluorescence.

(iv) **Polarizing microscope :** It was invented by *Tolbart*. In this microscope the plane polarised light is used as a source of illumination. Unlike the ordinary light, plane polarised light vibrates only in one direction and the cellular objects are easily visible as they appear bright against the dark ground. Polarizing microscope is helpful in studying the spindle fibres in the cells.

(v) **Ultraviolet microscope :** It was invented by *Caspersson*. In this microscope the source of illumination is ultraviolet radiations having shorter wavelengths (1500 Å – 3500 Å) as compared to ordinary visible light. In this microscope, the lenses are made of fluoride, lithium fluoride or quartz instead of glass.

Ultraviolet microscope is helpful in quantitative determination of all those cell components which absorb ultraviolet rays, *e.g.*, those places where high concentration of nucleic acids is found, appear as darker regions than the remaining cell components.

(vi) **Phase contrast microscope**

(a) Discovered by *Dutch man Fredericke Zernicke* (1935).

(b) Source of illumination is visible light.

(c) It is used to study living cells and tissues without staining and effect of chemical and physical agents on the living cells.

(d) The optical system of the phase contrast microscopy converts these phase variations into visible variations in light intensity or contrast.

(e) It also used to study spindle formation, pinocytosis, karyokinesis, cytokinesis etc.

(f) It is draw back is low magnification power so subcellular organelles smaller than $0.2\,\mu$, like ribosomes, lysosomes, ER, cannot be visualised.

(vii) **Interference microscope (Morten et.al.)**

(a) It's principle is similar to that of the phase contrast microscope and gives / studies quantitative data.

(b) Nomarski interference contrast microscope is useful to study mitosis /cell components in living state.

(c) It gives better image of living structures. It also used to measure thickness of the cell and determination of several light absorbing chemicals like nucleic acid, proteins, lipids etc.

(d) It helps to measure dry weight and water contents of the specimen.

(viii) **Dark field microscope**

(a) *Zsigmondy* (1905) invented this microscope.

(b) It is based on the fact that light is scattered at boundaries between regions having different refractive index.

(c) The object smaller than those seen with ordinary light microscope can be detected but can not be resolved.

(d) It makes use of visible light.

(ix) **Electron microscope :** This was developed by *M. Knoll* and *E. Ruska* (1931) in Germany. It is a large sized instrument which has an internal vacuum, high voltage (50,000 – 1,00,000 volts), a cooling system, a fast beam of electrons (0.54 Å wavelength), a cathod filaments of tungsten and electromagnetic lens (which having a coil of wire enclosed in soft iron casing) for focusing.

Thus an electron microscope essentially comprises an electron gun and electron lenses. The electron gun is the source of electrons consisting of a heated tungsten filament. It is preferred because it can be heated upto 3000°C. The electron beam can be reflected by magnetic field. Therefore, a very powerful magnetic coil acts as lens. The focal length of the electromagnetic lenses change with the wavelength of illumination. Since the wavelength is controlled by the voltage, it should be controlled and made constant. Three types of magnetic lenses are used namely projector, objective and condenser. The magnetic field produced is concentrated by soft iron casing. When the filament is heated to incandescence, it emits electron. The electrons then move to positively charged anode. The entire microscope column operates under conditions of high vacuum. It is due to this fact that we can not observe living objects through an electron microscope (EM). For viewing objects under EM, ultrathin sections are prepared through an ultramicrotone.

Electron microscope can magnify the objects upto 2,00,000 times (now possible upto 2,50,000 – 4,00,000) and direct study of objects is possible on this microscope. The resolving power of electron microscope is 10 Å which is 100 times more than the light microscope. The images obtained in electron microscope have usually black gray and white shades. Computer is used to enhance contrast and develop colour. The most recent technique for examining objects through electron microscope is freeze fracture. The material is frozen quickly in liquid nitrogen (–196°C). This material during microtomy tends to along lines of weakness.

Electron microscope are of two types: –

(a) **Transmission electron microscope (TEM) :** It was the first microscope developed by *Ruska* (1932). It produces two dimensional images. Study of living cells can not be done through this microscope because of high voltage, which is required to operate it, kills the living materials.

Magnification of TEM is 1–3 *lakh* and resolving power is 2–10Å. Because of them transmission electron microscope has helped in the discovery of a number of small cell organelles *e.g.*, ER, ribosomes, centrioles, microtubules etc. Details structure of larger cell organelles could also be known only with the help of TEM. *e.g.*, chloroplast (thylakoids), mitochondria (elementary particles, DNA, ribosomes) etc. Study of virus, mycoplasma and other small entities could also be made possible with the advent of electron microscope.

(b) **Scanning electron microscope (SEM) :** This microscope was invented by *Knoll* (1935). It is used to see the surface view of structures by forming an image with the secondary electrons reflected by those structures and it gives three dimensional image. The specimen to be studied is first super cooled (in liquid propane at –180°*C*) and dehydrated in alcohol (at –70°*C*). It is then coated with gold, platinum or some other metal for creating a reflecting surface for electrons. Magnification of SEM varies from 15 – 2,00,000. Resolution power is 5 – 20 *nm*.

Difference between compound and electron microscope

Compound microscope	Electron microscope
Source of illumination is visible part of electromagnetic radiation.	Source of radiation is a beam of electrons.
It does not require vacuum.	The apparatus is enclosed in vacuum chamber.
Glass lenses are used.	Not used.
It does not use magnets.	It uses high power magnets.
High voltage electricity is not required.	High voltage used to generate beam of electrons.
The specimen used may be 6 *mm* or more in thickness.	The specimen must be ultra thin, about 100 Å in thickness.
Contrast is achieved by dyes like heamatoxylin, fast green, saffranin, etc.	Contrast is achieved by using heavy metals likes lead acetate and phosphotungstate.
The human eye can directly observe the image formed by the instrument.	Can not be seen directly by eye, instead, a fluorescent screen or a photographic plate is used.
Radiation risk is absent.	Radiation risk is always there.
The specimen or object is kept in liquid medium or a resin.	The specimen must be completely dried.

(x) **Advanced high power microscope**

(a) **Scanning probe microscope :** The microscope is capable of resolving the outer texture of the material to the minutest detail since it has the potential to image even a single atom. Magnification is upto 100 million.

(b) **Scanning tunnelling microscope :** It has a tiny tungsten probe for moving over the surface of specimen. The microscope is used to detect defect in electrical conductors and computer chips.

(c) **Atomic force microscope :** It has an extremely fine diamond probe for moving over the surface of biochemicals. Oscillations produced in the probe are changed into images by a computer. The microscope is useful in viewing detailed structure of biological molecules, *e.g.*, DNA, proteins, etc.

(3) **Units of measurement used in microscopy**

1 micron (μ) = 10^{-6} or one millionth

1 micrometer (μm) = $10^{-6}\,m,\ 10^{-4}\,cm, 10^{-3}\,mm = 1000\,nm$

1 Nanometer (nm) = $10^{-9}\,m,\ 10^{-7}\,cm, 10^{-6}\,mm, 10^{-3}\,\mu m = 10\,\text{Å}$

1 Angstrom (Å) = $10^{-10}\,m,\ 10^{-8}\,cm,\ 10^{-7}\,mm,\ 10^{-4}\,\mu m,\ 10^{-1}\,nm, 0.1\,nm$

1 Picometer (pm) = $10^{-12}\,m, 10^{-3}\,nm$

1 Fempometer (fm) = $10^{-15}\,m, 10^{-6}\,nm$

(4) **Cytochemistry :** A number of dyes or stains are known to colour specific parts. Certain dyes can be used even in case of living materials. They are called vital stains, *e.g.,* neutral red, methylene blue. Fuelgen or Schiff's reaction was developed by *Fuelgen* and *Rossenbeck* (1924). Identification and localization of chemical compounds of a cell is studies in cytochemistry. It is based on four main analytical techniques.

(i) Separation of cell fractionation by conventional bio-chemical techniques.

(ii) Isolation of minute amount of tissue and even single cells by micro and ultramicromethod.

(iii) Direct detection of cell components in the cell by chemical staining.

(iv) Use of measurement of physical parameters.

Cytochemical stains : Some of the important stains are as follows.

Stain	Used for staining	Final colour
Acetocarmine	Chromosomes	Pink
Acid fuchsine	Cortex, cellular walls, mitochondria	Magenta
Aniline blue	Fungal hyphae	Blue
Basic fuchsine	Nucleus	Magenta red
Crystal violet	Bacteria	Violet
Chloro zinc iodine test	Cellulose	
Eosin	Cytoplasm	Pink
Feulgen's stain	DNA	Purple or Red
Geimsa orcein	Nucleus	
Haematoxyline	Nuclei, cell wall and cellulose	Violet
Iodine solution	Starch	Blue
Janus green	Fungi and mitochondria	Green
Methylene blue	Yeast and Glogi complex	Blue
Ninhydrin	Protein and amino acid	
Nephthal yellow	Protein	
Phloroglucinol *+HCl*	Lignin	Red
Pyronin *Y*	RNA	
Ruthenium red	Pectin	Red
Saffranin	Nuclei, lignified tissue	Red
Schiff's reagent	DNA	
Sudan- III or IV	Suberin, cutin, oil	Red
Sudan black	Fatty substance	Black
Toludine blue	RNA	Blue
Cotton blue	Fulngi	Violet

(5) **Cell fractionation :** In isotonic medium cells components are separated, it is two step process.

(i) **Homogenisation :** Cell products are separated in isotonic medium (0.25 *M* sucrose solution) either with the help of homogeniser of ultrasonic vibrations kept at 0 – 4°C. A homogenised cell is called homogenate.

(ii) **Differential centrifugation :** Homogenisation product is rotated (centrifuged) at different speeds. The sediment or pellete of each speed is collected. *e.g.,* nuclei at $1000 \times g$ (g= force of gravity) for 10 minutes, chloroplast and mitochondria at $10{,}000 \times g$ for 15 minutes. The particle settle according to their sedimentation ratios. Sedimentation coefficient is expressed in svedberg unit '*S*' related with molecular weight of the particles. For mitochondria it is the best technique.

The various cell organelles and macromolecules sediment in the following order.

Nucleus → Chloroplast → Mitochondria → Ribosome → DNA → mRNA → tRNA

(6) **Chromatography :** Discovered by *Michael Tswett* (1906). This technique is used to separate the molecules of different substances present together. Mixture of molecules is run over an adsorption medium. Chromatography may be following types.

(i) **Adsorption or Column chromatography :** The stationary phase consist of a column of charcoal, silica, alumina, calcium carbonate or magnesium oxide. The solution is made to percolate through this column when different chemicals get adsorb at various levels. The technique is useful for separation of tissue lipids.

(ii) **Thin layer chromatography :** The stationary phase consists of a thin plate of cellulose powder or alumina. As a few drops of mixture are poured over it, the different chemicals spread to different distances. The method is useful in separation of amino acids, nucleotides and other low molecular weight products.

(iii) **Paper chromatography :** A paste of mixture is applied near one end of a chromatographic paper (or Whatman 1). The lower end below the paste is dipped in a solvent. As the solvent rises in chromatographic paper, the different chemicals of the mixture spread to different distances. The paper can be rotated to obtain two dimensional chromatogram.

(iv) **Ion exchange chromatography :** Beads of cellulose and other materials having negative and positive charges are placed in a column. The mixture (mobile phase) is poured over the column. As the mixture passes through the column, its constituents separate according to their charges. The technique is used in purification of insulin, plasma fractionation and separation of proteins.

(v) **Gel fractionation chromatography (Molecular sieve chromatography) :** Dextran gel sephadex is available with various pore size. A mixture is poured over a column of sephadex. The various chemicals pass through the pores and come out of the column with heavier larger molecules do so first followed by progressively smaller sized molecules provided the pores are larger than the size of largest molecules. The technique is used in determining the molecular weight of proteins by calibrating the column with proteins of different molecular weights.

(vi) **Affinity chromatography :** Satationary phase consists of column of ligands (molecules that bind to other specific molecules at particular sites). Mixture is allowed to pass through the column. Chemical linkages are established between ligands and their specific chemicals. Others pass out of the column. The technique is used in separation of enzymes, immunoglobulins, *m*RNA, etc.

(7) **Electrophoresis :** It is an another technique of separation. In which patricles of different charges and sizes are separated under the influence of electric field. *e.g.,* nucleic acids, proteins, amino acid, nucleotides can be separated by this method. The technique was discovered by Russian phyicist *Alexender Reuss* in 1807**.**

A base material is used for the passage of molecules of the mobile phase. In PAGE (ploy acrylamide gel electrophoresis) the base material is polyacrylamide or polymer of acrylamide and methylene bisacrylamide. In agarose gel electrophoresis, the base material is agarose. The base material dip in solution having cathode at one end anode at the other end. As the electric current is switched on, the chemicals of the mixture separate and pass to different distances. The gel functions as a sieve. In two dimensional electrophoresis, molecules are separated in two directions. In immunoelectrophoresis antibodies coupled with radioisotopes, specific enzymes or fluorescent dyes

are used in detection of particular proteins. The technique is highly sensitive. It can separate molecules in picogram and nanogram quantities and distinguish proteins which differ from each other in only one amino acid.

(8) **Autoradiography**

(i) It is a technique of studying the route of chemicals in chemical reactions taking place inside the cell and organisms with the help of radioactive isotope.

(ii) Commonly used radio isotopes are ^{14}C, ^{3}H, ^{32}P.

(iii) In this technique the radioisotopes are incorporated into the precursor molecule. Then the labelled precursor molecules introduced into the cells and their path is followed with the help of their radiation.

(iv) Radioactive precursors emit radiations and their position in the cell is located by bringing the cell in contact with a photographic plate or film.

(v) ^{32}P is used for the study of nucleic acids.

(vi) Melvin Calvin detected the intermediate involved in Calvin cycle of photosynthesis by the use of ^{14}C.

(9) **X-ray crystallography :** It was developed by the *Bragg* (1913). The wavelength of *X*–rays depends upon the distance between atoms. Therefore, they can be used as a tool for determining the arrangement of atoms in various biological molecules. When the *X*–rays pass through a molecule, they are scattered by the atoms. The diffraction pattern of the *X*–rays is photographed. The nature of diffraction is related to the orientation of the atoms in the molecule. By using this technique *Wilkins et al.,* 1953 found out details of the DNA molecule for which he was also awarded Nobel Prize along with *Watson* and *Crick* in 1962. *Kendrew*, 1957 by using the same technique studied the molecules of myoglobin.

(10) **Pulse-labelling technique :** Some of the biological molecules undergo changes after their synthesis. We can cite here the case of RNA. The transcription of *hn*RNA from DNA ultimately leads to the formation of *m*-RNA. These changes can be studied through pulse-labelling technique. Here, first the radioactive precursors are introduced in the system for a short time. These precursors are then chased away by non-radioactive precursors by any process of maturation.

(11) **Intracellular electrodes :** The concentration of various ions in different parts is now studies by using a glass microelectrode. It has silver wire dipped in *KCl* solution. This technique is used for studying the movement of ions through ions channels. The ions channels are intrinsic membrane proteins. For studying this passive transport of ions through ion channels *Neher and Sakman* developed a Patch clamp technique for which they are awarded Nobel prize in 1991.

Important Tips

- *The term microscope was coined by Faber 1625.*
- *R.B. Tolles made oil immersion lens in 1830.*
- *The structure of membrane is observed by freeze fracturing an freeze etching technique.*
- *Electron microscope is used to study ultrastructure of organelles.*
- *For microscopic examination of sections are cut with the help of a machine called Microtome or ultramicrotome. It was first developed by W.His.*
- *X-ray microscope was developed by Kirkpatrick.*

- *Three dimensional image are obtained with the help of scanning electron microscope and X-ray microscope. Where as all other microscopes give two dimensional image.*
- *A staining technique, which is used for distinguishing cell structure known as metallic impregnation technique. e.g., golgibody by osmium chloride and silver salts, flagella by silver salts.*
- *Tracer isotopes / radioactive isotopes : Which functions like normal elements but emit radiations. They can, therefore, be located by Geiger muller counter or scintillalion counter and autoradiography, e.g.,* ^{3}H, ^{14}C, ^{32}P, ^{35}S, *etc.*

Cell as a unit of life.

(1) **Cytology :** (G.k. *kyios* = cell ; *logas* = study) is the branch of biology. Which comprises the study of cell structure and function. "*Cell is the structure and functional unit of all living beings*".

All living organisms are composed of repeated structural units called cells. Each cell is independent in performing all necessary processes of life and is the least complex unit of matter which can be called living. *Robert Hooke* (1665) discovered hollow cavities (empty boxes) like compartments in a very thin slice of cork (cell wall) under his microscope. He wrote a book "*Micrographia*" and coined the term cellula, which was later changed into cell. *Grew and Malpighi* also observed small structures in slice of plants and animals. *Leeuwenhoek* was the first to see *free cells*. He observed bacteria, protozoa, RBCs, sperms, etc. under his microscope.

(i) **Cell theory :** H.J. Dutrochet (1924) a French worker gave the idea of cell theory.

The actual credit for cell theory goes to two German scientists, a Botanist *M.J. Schleiden* (1838) and a *Zoologist T. Schwann*(1839).They gave the concept "all living organisms are composed of cell". *Schleiden* and *Schwann* both supported the theory of "spontaneous generation". They also mentioned that "the new cell arises from nucleus by budding". Main postulates of cell theory are :

(a) Living beings are made of cells. They may be unicellular, colonial or multicellular.

(b) Cell is a mass of protoplasm having nucleus.

(c) Cells are similar in structure and metabolisms.

(d) The functions of an organism are due to activities and interactions of cells.

(ii) **Exceptions to the cell theory :** Viruses, viroids and prions are an exception to the cell theory as they are obligate parasites (sub–cellular in nature). *Paramecium, Rhizopus, Vaucheria* are some examples, which may or may not be exceptions to the cell theory.

(iii) **Modification of cell theory :** Modification of cell theory was done by *Rudolf Virchow* (1885). He proposed the "law of cell lineage" which states that cell originates from pre-existing cells. *i.e. (omnis cellula-e-cellula)*. It is also called "cell principle" or "cell doctrine". It states : –

(a) Life exists only in cells.

(b) Membrane bound cell organelles of the protoplasm do not survive alone or outside the protoplasm.

(c) Cells never arise *de novo*. The new cells are like the parent cell in all respect.

(d) All cells have similar fundamental structure and metabolic reactions.

(e) Cells display homeostasis and remain alive.

(f) Functions of an organism as a whole are the sums of the activities and interactions of its constituent cell units. An organism can not show functions which is absent in its cells.

(g) Genetic information is stored in DNA and expressed within the cells.

(h) DNA controls structure and working of a cell.

(iv) **The cell as a self contained unit :** Autonomy of a cell is believed due to presence of DNA and its expressibility, otherwise, cell components have different shape and function. It has two positions.

(a) **Autonomy in unicellular organisms :** Unicellular organisms lead to a totally independent life due to different shape, size and role of different organelles shows division of labour. All these display homeostasis. Unicellular organisms are more active due to large surface volume ratio.

(b) **Autonomy in multicellular organisms :** In multicellular organisms life activities are displayed by each of the cells independently. Multicellular organisms have one thing advantage over unicellular organisms is division of labour.

(v) **Cellular totipotency :** Totipotency was suggested by *Haberlandt* (1902)**.** When cells have tendency or ability to divide and redivide the condition of the cell is called totipotent and this phenomenon is called *totipotency*.

(vi) **Steward's experiment :** *Steward et.al.* showed the phenomenon of cellular totipotency in carrot culture. Small fragments (phloem) of mature carrot roots were placed in liquid medium in special containers and growth factors like "coconut milk" was added. The culture developed into clumps or embryoids. When these were shifted to semisolid media, full plants were formed. The plants flowered normally and even bore the seeds.

(vii) **Surface volume ratio :** *Metabolically active cells are small*, as small cells have higher nucleocytoplasmic ratio for better control and higher surface volume ratio for quicker exchange of materials between the cell and its outside environment. Larger cells have lower surface volume ratio as well as lower nucleocytoplasmic ratio. Surface volume ratio decreases by one half if cell size doubles.

Differences between plant cell and animal cell

Plant cell	Animal cell
Cell wall present.	Cell wall absent.
Nucleus usually lies near periphery due to vacuole.	Nucleus present near the centre.
Centrosome is usually absent from higher plant cells, except lower motile cells.	Usually centrosome is present that helps in formation of spindle fibres.
Plastids are present, except fungi.	Plastids are absent.
Mitochondria is generally spherical or oval in shape.	Generally tubular in shape.
Single large central vacuole is present.	Many vacuoles occurs, which are smaller in size.
Number of mitochondria from 200 – 2000.	Number of mitochondria is approximately 1600 – 16000 in liver cells.
Cytoplasm during cell division usually divides by cell plate method.	Cytoplasm divides by furrowing or cleavage method.
Plant cells are capable of forming all the amino acids coenzymes and vitamins.	Animal cells cannot form all the amino acids, coenzymes and vitamins.
There is no contractile vacuole.	Contractile vacuole may occur to pump excess water.
Sodium chloride is toxic to plant cells.	Tissue fluid containing sodium chloride bathes the animal cells.
Plant cells are generally well over 100 μm long.	Generally much smaller than 100 μm.
Spindle formed during cell division is anastral.	Spindle formed during cell division are amphiastral.

Lysosomes present in less number.	Lysosomes present in more number.
Chromosomes are larger in size.	Chromosomes are smaller in size.

Important Tips

- *Jan swammerdam : First to see red blood cells of frog.*
- *Marcello Malpighi : Observed small utricles in slice of plant and animal tissue.*
- *N. Grew : Initiated cell concept*
- *Lamarck : All living beings are formed of cells.*
- *Corti : First to point out living substance filled inside the cell. It was called "Sarcode" by Dujardin.*
- *In vivo (in life) study : Study of cells in their natural environment within the intact organism.*
- *In vitro (cultural condition) study : Study of isolated life system in laboratory and cultural condition .*
- *Max Shultze proposed protoplasm theory.*
- *Sachs proposed organismic theory.*
- *Crystallo : colloidal theory (Fischer), substances dispersed and dissolved in water forming both true solution as well as colloidal solution.*
- *Energy transducers : Photosynthetic cells are called energy transducers because they convert radiant energy to chemical energy and store it as food energy.*
- *Intrinsic information is primary while hormonal information is extrinsic and secondary information.*
- *Largest organelles is nucleus. Largest cytoplasmic organelle is mitochondria in animal cells and chloroplast in plant cell.*
- *Smallest component is microfilament but smallest organelle is ribosome.*
- *Viruses do not have cellular structure.*
- *Monerians and protistians are not divisible into cells they are rather acellular.*
- *Certain organisms are multinucleated eg., Rhizopus, Vaucheria, etc.*
- *Fibre of ramie, Boehameria nivea longest plant cell (55 cm in size).*
- *The shrunken state of RBC caused by exosmosis is called crenation.*
- *In human beings cell of kidney are smallest and of nerve fibre largest.*
- *Pyrenoid is a proteinaceous body around which starch is stored in green algae.*
- *The smallest cell considered so far is of PPLO (Pleuropneumonia like organisms) or Mycoplasma gallisepticum i.e. 0.1 μ.*
- *The largest cell is an egg of ostrich.*
- *Acetabularia a unicellular green alga is about 10 cm in length.*
- *In the alga caulerpa (Siphonales) the length of cell may be up to one metre.*
- *The bacteriophages or viruses are still smaller in size (but cannot be considered as cells because of sub – cellular nature).*

Structure of the cell .

(1) Introduction

(i) Study of cell is called cytology.

(ii) Study of metabolic aspects of cell component is called cell biology.

(iii) *Leeuwenhoek* : First to see free cells called them "wild animalcules" and published a book "The secret of nature".

(iv) *Robert Hooke* is known as father of cytology.

(v) *C.P. Swanson* is known as father of modern cytology/ cell doctrine.

(vi) *A.K. Sharma* is known as father of cytology in India.

(vii) Dougherty classified cells based on plan as prokaryotic and eukaryotic.

(2) **Mesokaryon :** *Dodge* gave the term 'Mesokaryon' for dinoflagellates. These are intermediate type of cell organisation in dinophyceae of algae. In mesokaryotic there is present a true or eukaryotic nucleus with definite nuclear membrane and chromosomes. Chromosomes are not well organised and basic proteins or histones are absent. Nuclear membrane is persistent during cell division. Chromosomes are permanently attached to nuclear membrane. They show dinomitosis *e.g.*- Dinophysis Heterocapsa, Dinothrix etc.

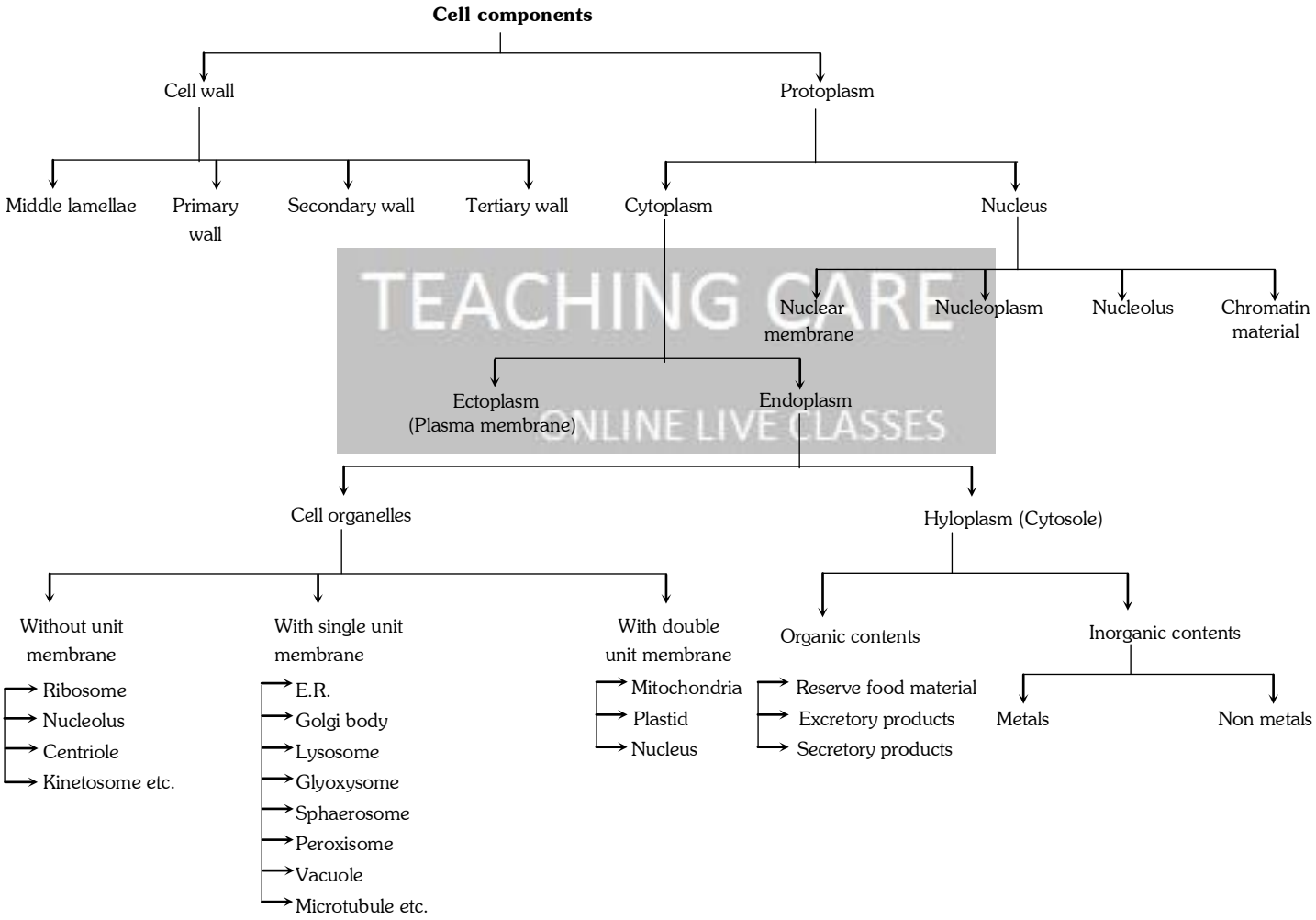
(3) **Types of cell :** Chatton gave the term prokaryote and eukaryote. Depending upon the nature of nucleus cells are classified. A primitive ill defined or incipient nucleus is present in prokaryotes, where as in eukaryotes. Well organised nucleus is present.

Differences between Prokaryotic and Eukaryotic cell

Prokaryotic cell	Eukaryotic cell
It is a single membrane system.	It is a double membrane system.
Cell wall surrounds the plasma membrane.	Cell wall surrounds the plasma membrane in some protists, most fungi and all plant cell. Animal cell lack it.
Cell wall composed of peptidoglycans. Strengthening material is mureir.	It is composed of polysaccharide. Strengthening material is chitin in fungi & cellulose in others plants.
Cell membrane bears *respiratory enzymes*.	It lacks respiratory enzymes.
Cytoplasm lacks cell organelles *e.g.*, Mitochondria, ER, Golgi body etc.	Cytoplasm contains various cell organelles.
Ribosomes are 70 S type.	Ribosomes are 80 S type.
There are no streaming movements of cytoplasm.	Cytoplasm show streaming movements.
Endocytosis and exocytosis do not occur.	Endocytosis and exocytosis occur in animal cells.
Mitotic spindle is not formed in cell division.	Mitotic spindle is formed in cell division.
The mRNA does not need processing.	The mRNA needs processing.
Nuclear material is not enclosed by nuclear envelope and lies directly in cytoplasm. It is called nucleoid.	It is enveloped by nuclear envelope. Nucleus is distinct from cytoplasm.
DNA is circular and not associated with histone proteins.	Nuclear DNA is linear and associated with histone proteins extranuclear DNA is circular and protein free.
Replication of DNA occurs continuously through out cell cycle.	Replication of DNA occurs during *S*- Phase of cell cycle only.
These have small size (0.5 to 10 μm) and have much less DNA.	These are relatively large (10 – 15 μm) and have much more DNA.

Sexual reproduction absent but parasexuality present.	Sexual reproduction is present.
Plasmids and pili occur in many prokaryotes Example – *E. coli*	There are no plasmids and pili in eukaryotic cells Example – *Spirogyra, Chlorella*
Cell division mostly amitotic.	Cell division is typically mitotic.
Plasma invaginates and from finger like process. Mesosome which take part in respiration	Absent

(4) Cell compartmentation map



Cell wall.

(1) **Discovery :** It was first discovered by *Robert Hooke* in 1665.

Cell wall is the outer most, rigid, protective, non living and supportive layer found in all the plant cells, bacteria, cyanobacteria and some protists. It is not found in animal cells.

(2) **Chemical composition :** Mainly cell wall consists of two parts, matrix and cellulosic fibres (microfibriles). Matrix consists of hemicellulose, pectin, glycoproteins, lipids and water. A cellulose molecule is long unbranched

chain of glucose molecules. There are about 6,000 glucose units in each chain. In most of the plants cell wall is made up of cellulose $(C_6H_{10}O_5)_n$, a polymer made-up of unbranched chain of glucose molecule linked by $\beta,1-4$ glycosidic bond. About 100 molecules of cellulose form a micelle, about 20 micelle form a microfibril and approx 200 microfibril form a fibril. The cell wall of bacteria and the inner layer of blue green algae is made-up of mucopeptide and not of cellulose. The mucopeptide is a polymer of two amino sugars namely N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) held alternately in β –1,4- linkage. In higher fungi, the cell wall is made up of chitin, polymer of glucosamine.

Pectin is a mixture of polymerised and methylated galacturans, galacturonic acid and neutral sugars. Hemicellulose is a mixture of polymerised xylans, mannans, glucomannans, galactans, xyloglucans and arabinogalactans. Glycoproteins are known to influence metabolic activities of the wall. A glycoprotein called extensin or expansin takes part in loosening and expansion of cell was through incorporation of cellulose molecules to cellulose microfibrils.

Plant cell wall may have lignin for strength (*e.g.*, woody tissue), silica for stiffness and protection (*e.g.*, epidermal cells of grasses, *Equisetum*), cutin for preventing loss of water (*e.g.,* epidermal cells), wax as component of cuticle and surface bloom as water repellent (floating leaves) and checking transpiration, suberin for impermeability (*e.g.,* cork cells, endodermal cells), etc.

(3) **Structure :** Cell wall consists of middle lamella, primary wall, secondary wall, tertiary wall.

(i) **Middle lamella :** Middle lamella is the outermost region which functions as a cementing layer between two cells. It is absent on the outer free surface. It ruptures to create intercellular spaces. Middle lamella is formed of calcium and magnecium pectate. Fruit softening is due to gelatinisation of pectic compounds of middle lamella. Pectin is used as commercial jellying agent. Which is present outside the primary wall.

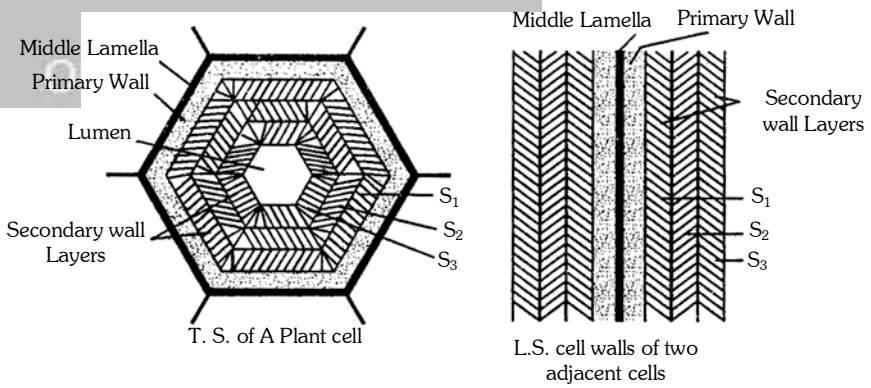


Fig : Layers of cell wall in T.S. and L.S. of a cell

(ii) **Primary wall :** A young plant cell forms a single layer of wall material. This layer is known as the primary cell wall. The primary wall is thin, elastic and capable of expansion in a growing cell. It grows by intussusception. Meristematic and parenchymatous cells have primary cell wall only. The cells of leaves and fruits too have only primary wall.

(iii) **Secondary wall :** In mature cell, more layers of wall material are added internal to the primary wall. These are called the secondary cell wall. Growth by addition of new wall material on the primary wall is called accretion. The secondary wall is thick and rigid. It usually consists of three layers, which are often named S_1, S_2 and S_3. It is found in collenchyma and sclerenchyma cells, xylem vesseles.

(iv) **Tertiary wall :** Sometimes tertiary wall is laid down on secondary wall, *e.g.,* tracheids of gymnosperms. It is composed of cellulose and xylan, another ploysaccharides.

(4) **Origin :** A cell wall is organised at telophase stage of cell division. The plane and place of cell wall is determined by the microtubules. Fragments of ER and vesicles of golgi body alligned at the equator, called as *phragmoplast, later which forms the cell plate*. The synthesis of cellulose takes place by the help of enzyme *cellulose synthase* present in the plasma membrane.

The cell plate forms the cell wall. A cell posses three phases of growth namely cell formation, cell elongation and cell maturation. The formation of new cells occurs by mitotic activity. The cell elongation is initiated by an increase in cell turgor. It is brought about by special proteins called *expansion*. They are of two types α – expansion and β – expansion. As a result, lacunae or gaps appear in between the cellulose micelle. There are two possibilities for the deposition of new wall material.

(i) **By intussuception :** As the cell wall stretches in one or more directions, new cell wall material secreted by protoplasm gets embedded within the original wall.

(ii) **By apposition :** In this method new cell wall material secreted by protoplasm is deposited by definite thin plates one after the other.

Differences between primary and secondary cell wall

Primary cell wall	Secondary cell wall
Primary wall is laid inner to middle lamella	Secondary wall is laid inner to primary wall.
It is formed in a growing cell.	It is formed when the cells have stopped growing.
It is capable of extension.	Extensibility is absent except in collenchyma cells.
It is single layered.	It is three or more layered.
Cellulose content is comparatively low (5 – 20%).	Cellulose content is comparatively high (20 – 90%).
Cellulose microfibrils are shorter, wavy and loosely arranged.	They are longer, closely arranged straight and parallel.
Protein content up to 5%.	Protein content up to 1%.
Hemicellulose content is high up to 50%.	It is 25% of the total.
Lipid content up to 5 – 10%.	Lipid is absent.
Primary wall is comparatively thin 1 – 5 μm.	It is comparatively thick 5 – 10 μm

(5) **Thickenings of cell wall :** In many secondary walls specially those of xylem the cell wall becomes hard and thick due to the deposition of lignin. With the increasing amount of lignin, deposition protoplasm is lost. First the lignin is deposited in middle lamella and primary wall and later on in secondary wall. Like cellulose lignin is permeable to water and substances dissolved in it. Lignin is deposited at specific places of the cell walls due to which xylem tracheids and trachea take up following forms:

(i) **Annular thickenings :** Deposition of lignin takes place in the form of rings on the inner surface of protoxylem

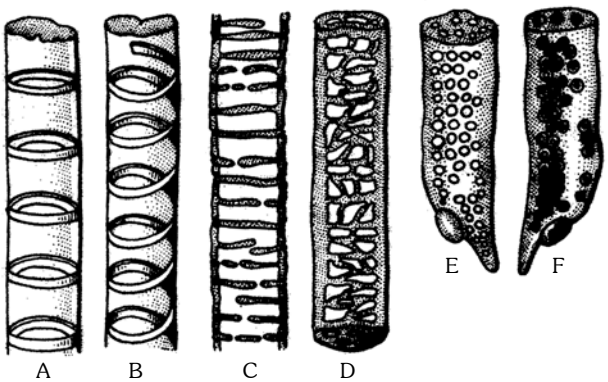


Fig : Different types of secondary wall thickenings – (a) annular (b) spiral (c) scalariform (d) reticulate (e) pitted-simple pits (f) pitted-bordered pit

cells. These rings are placed one above the other leaving some space in between each other.

(ii) **Spiral thickenings :** In these thickenings deposition of lignin takes place in the form of complete spiral bands and are formed in tracheids and trachea of protoxylem.

(iii) **Scalariform (Ladder like) thickenings :** In these thickenings lignin is deposited in the form of transverse rods of the ladder. The unthickened areas between the successive thickenings appear as elongated transverse pits. This type of thickening is common in protoxylem.

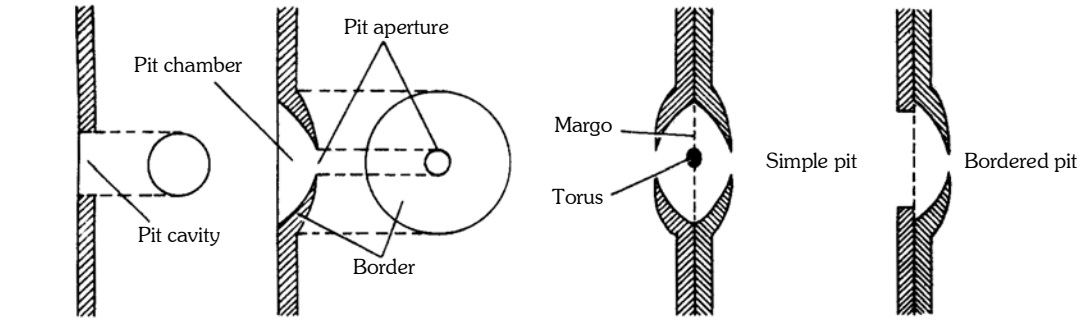
(iv) **Reticulate (Net like) thickenings :** The lignin is deposited in the form of a net or reticulum. The unthickened areas are irregular in shape. These are found in metaxylem.

(v) **Pitted thickenings :** These are found in metaxylem. In such thickening the whole inner wall is more or less uniformly thickened leaving here and there some unthickened areas called pits.

(6) **Pits :** Secondary walls may have irregular thickenings at some places and these places are called pits. Pits are of two types :–

(i) **Simple pit :** In which pit chamber is uniform in diameter.

(ii) **Bordered pit :** In which pit chamber is flask shaped in tracheids of gymnosperm and vessels of angiosperms.



A. Simple pit **B. Bordered pit** **C. Bordered pit pair** **D. Half bordered pit**

(7) **Plasmodesmata :** *Tangle* (1879) first of all discovered them and were studied elaborately by *Strasburger* (1901). A number of plasmodesmata or cytoplasmic strands are present in pit through which the cytoplasm of one cell is in contact with another. Endoplasmic reticulum plays a role in origin of plasmodesmata.

(8) **Intercellular spaces :** In mature cells certain spaces or cavities are produced which are of 3 types.

(i) **Schizogenous cavities** : In mature cells, the cell walls separate from each other and form a cavity. *e.g.*, resin canals in Pinus.

(ii) **Lysogenous cavities** : It is formed by the break down of cell walls *e.g.*, *Citrus* oil cavities.

(iii) **Schizo-lysogenous cavities :** Both the above processes are involved in this cavity formtion *e.g.*, protoxylem of maize.

(9) **Function of cell wall :** The cell wall serves many functions –

(i) It maintain shape of the cells.

(ii) It protect the cells from mechanical injury.

(iii) It wards off the attacks of pathogens (viruses, bacteria, fungi, protozoans).

(iv) It provides mechanical support against gravity. It is due to the rigid cell walls that the aerial parts of the plants are able to keep erect and expose their leaves to sunlight.

(v) The cell wall prevents undue expansion of the cell when water enters by osmosis to compensate for the lack of contractile vacuole. This prevents bursting of cells.

(vi) It allows the materials to pass in and out of the cell.

(vii) Though permeable, the cell wall plays some regulatory role on the passage of materials into and out of the cell.

(viii) Many enzymic activities associated with metabolism are known to occur in the cell wall.

(ix) Cutin and suberin deposits check loss of water form the cell surface by evaporation.

(x) The cell wall helps in the maintenance of balance of intracellular osmotic pressure with that of its surroundings.

(xi) Pores in the cell walls permit plasmodesmata to link up all the protoplasts into a system called symplast (symplasm).

(xii) The walls of xylem vessels, tracheids and sieve tubes allow movement of materials.

(xiii) The wall in some cases has a role in defence and offence by means of spines.

(xiv) Growth of the cell wall enables the cells to enlarge in size.

(xv) Cell wall and intercellular spaces constitute a nonliving component of plant body known as apoplasm.

Important Tips

- *Peptidoglycane = murein = mucopeptide is the only cell wall material of prokaryotes. It's sugar portion consists of NAG and NAM.*
- *In fungi cell wall is made up of chitin (polymer of N- acetyl glucosamine). In bacteria it is composed of protein lipid polysaccharide having N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM).*
- ***Cell wall proteins –***

  HRGP –*Hydroxy proline rich glycoprotein → Phloem and cambium.*

  PRP– *Proline rich protein → Xylem, fibres, cortex.*

  GRP– *Glycine rich protein → Xylem.*

Plasma membrane.

(1) **Definition :** Every living cell is externally covered by a thin transparent electron microscopic, elastic regenerative and *selective permeable membrane* called plasma membrane. It is quasi fluid in nature. According to *Singer* and *Nicolson* it is "protein iceberg in a sea of lipid". A cell wall lies external to plasmalemma in plant cells, many monerans, some protists and fungal cells. Membranes also occur inside the cells. They are collectively called

biomembranes. The term cell membrane was given by *C. Nageli* and *C. Cramer* (1855) for outer membrane covering of the portoplast. It was replaced by the term plasmalemma or plasma membrane by *Plowe* (1931).

(2) **Chemical composition :** Proteins lipoprotein (Lipid +Protein) are the major component forming 60% of the plasma membrane. *Proteins provide* mechanical strength and responsible for transportation of different substances. Proteins also act as enzyme. *Lipids account may* 28%-79% depending upon the type of cell and organism involved (in humans, myelin 79%). Because of the presence of lipids, membranes are always continuous, unbroken structures and are deformable and their over all shape can change. The lipids of plasma membrane are of three types namely *phospholipids*, *glycolipids* and *sterols*. A glycolipid may be cerebroside or ganglioside. The sterol found in the membrane may be cholesterol (Animals), phytosterol (Plants) or ergosterol (Microorganisms). A lipid molecule is distinguishable into a head of glycerol and two tails of fatty acids.

Carbohydrates form 2%–10%. Oligosaccharides are the main carbohydrates present in plasma membrane. The carbohydrates of plasma membrane are covalently linked to both lipid and protein components. The common sugars found in the plasma membrane are *D* – glucose, *D* – mannose, *D* – glactose, *N* – acetyl glucosamine, *N* – acetyl galoactosamine (Both are amino sugars) and sialic acid. Generally the terminal sugar of oligosaccharides is sialic acids (Also known as *N* – acetylneuraminic acid NANA) which gives them a negative charge.

(3) **Ultra structure :** Under electron microscope the plasma membrane appears three layered, *i.e.* trilaminar or tripertite. *One optically light layer* is of lipid and on *both sides two optically dense protein layers* are present.

Generally the plasma membrane is 75 Å thick (75 – 100Å), light layer is 35 Å while dark layers are 20 Å + 20 Å in thickness.

(4) **Molecular structure and different models :** Several models have been proposed to explain the structure and function of the plasma membrane.

(i) **Overton's model :** It suggests that the plasma membrane is composed of a thin lipid bilayer.

(ii) **Sandwich model :** It was proposed by *Davson* and *Danielli* (1935). According to this model the light biomolecular lipid layer is sandwiched between two dense protein layers. This model was also said to be unit membrane hypothesis.

(iii) **Robertson's unit membrane model :** It states that all cytoplasmic membranes have a similar structure of three layers with and electron transparent phospholipid bilayer being sandwiched between two electron dense layer of proteins. All biomembranes are either made of a unit membrane or a multiple of unit membrane. Its thickness is about 75 Å with a central *lipid layer of 35 Å thick* and two peripheral *protein layers of 20 Å thick*.

(iv) **Fluid mosaic model :** The most important and *widely accepted latest model* for plasma membrane was given by *Singer and Nicolson in* 1972. According to them it is "protein iceberg in a sea of lipids."