Chapter

1

Scope of Biochemistry and Clinical Pathology in Pharmacy, Cell and Biochemical Organization

Biochemistry, these days is treated to be the most important subject of life sciences whether medical sciences or plant sciences. Nearly 80 years ago, this subject was in infancy but now it is deep rooted and possesses a unique position because it is said to be the foundation of the modern medicine on which it exists.

The word Biochemistry is very broad in its meaning which may be defined as the study of different chemical processes going on in the body at molecular level, no matter it is a plant or animal body.

Besides, it also deals with the nature of the chemical constituents of the living organisms; the functions and transformations of such chemical entities in the biological systems and also with the chemical and energetic changes associated with such transformations during the course of activity.

Day by day, its applicability in the field of life sciences is going on increasing and it is being utilized for the benefit of mankind and plant kingdom as well.

The history of biochemistry, as it is in the present form is not very old. Most of the work in the field of biochemistry has been carried out in the last half century. In this period of development, it is being increasingly recognized as an essential discipline among the life sciences. In some of the advanced Western countries, there are full-fledged independent institutes of biochemistry, but unfortunately, there is even not a single separate full-fledged independent institute of biochemistry in our country because of which our country is lagging behind, as far as biochemical sciences are concerned. Unfortunately, our country has neither got a separate 'institute of biochemistry' nor 'biochemical engineering'; whereas, now the time is fully matured to establish such an independent institute in India, so that we could keep pace with the Western world. Although, we have some good institutes of science and technology like Central Drug Research Institute at Lucknow; Indian Institute of Toxicology Research (IITR) at Lucknow; National Institute of Immunology at New Delhi; National Institute of Nutrition at Hyderabad; Centre for Food and Technological Research Institute at Mysore; Indian Institute of Science at Bengaluru; Centre for Biochemical Technology at New Delhi; National Chemical Laboratory at Pune; Indian Institute of Chemical Technology at Hyderabad; Bhabha Atomic Research Centre at Trombay (Mumbai); Tata Institute of Fundamental Research at Mumbai; All India Institute of Medical Sciences at New Delhi; Centre for Cellular and Molecular Biology at Hyderabad, Centre for DNA Fingerprinting and Diagnostics at Hyderabad; National Brain

Research Centre at Manesar (Haryana); Institute of Genomics and Integrative Biology at New Delhi; International Centre for Genetic Engineering and Biotechnology at New Delhi, etc. which have got very good, well-equipped biochemistry sections. Scientists and doctors are working wholeheartedly round the clock at these centres to make 'science' more approachable and meaningful to the mankind. We have had some eminent biochemists in India like Prof GP Talwar, Padam Shree Awardee (Ex-Professor and Head of Department, Department of Biochemistry, AIIMS, New Delhi and Ex-Director, National Institute of Immunology, New Delhi), and at present Director, Talwar Research Foundation, New Delhi. Late Dr Bires Chandra Guha is said to be 'father of modern biochemistry' in India. Late Dr Lalji Singh (former Director, Centre for Cellular and Molecular Biology, Hyderabad), is said to be 'father of DNA fingerprinting technology in India', etc.

Various institutes having excellent sections of biochemistry in India are:

- CBT New Delhi
- CDRI Lucknow
- CFTRI Mysore
- CCMB Hyderabad
- IITR Lucknow
- ICGEB New Delhi
- IGIB New Delhi
- IISc Bangalore
- IICT Hyderabad
- NCL Pune
- NII New Delhi
- NIN Hyderabad
- TIFR Mumbai
- CDFD Hyderabad
- NBRC Manesar (Haryana)

A lot of work is being done both on basic and applied fields of biochemistry in order to make it more useful and viable for the benefit of mankind and understanding the secrets of life. The discipline has so far established a unique position in the field of medicine because of the fact that it is forming a major tool in explaining the development of disorders and diseases in the body; that is why, one finds sophisticated latest update on biochemical instruments like PCR, gas liquid chromatography, atomic absorption spectrophotometer, autoanalysers, radioisotope laboratory, tissue culture laboratory, etc. in the well-established good departments/sections/ units of biochemistry. The knowledge of biochemistry has been able to pinpoint the exact site of the disorders and to give a clue towards the line of treatment in most of the diseases. The new offshoot of biochemistry, i.e. genetic engineering is flourishing like anything in the Western advanced countries like USA, UK, Germany, France, Japan, etc. which may bring solution of several disorders like those of hereditary disorders and other incurable diseases, the treatment of which is not possible at the moment. Scientists and doctors are working day and night in these countries to synthesize genes responsible for various genetic defects, but unfortunately, in our own country, this science is still lagging behind. It is a cause of serious concern to the Government of India.

Hereditary diseases, which are considered to be incurable at present, might be easily controlled or treated satisfactorily in the near future by simply changing the nature of the particular gene(s) responsible for the causation of such disease(s). Attempts are being made to synthesize genes biochemically. Success in this field is bound to bring revolutionary, miraculous changes in the world of life sciences. Genes are the carriers of hereditary characters. After the synthesis of newer genes, it might be quite possible to produce off-springs of ones own choice. Suppose, then, if someone wishes a wrestler, or an athlete or a gymnast or a tall fellow, or a very intellectual one, it would be quite possible to have it. This also means that the usage of genes for the betterment of mankind may also be misused by someone, hence, care must be taken for its 'use' and not 'misuse'; therefore, the Governments must enact some law(s) in future for the use of 'genes' and the power of its usage should never be vested in a single hand (single doctor), rather in a board comprising at least 5–7 highly qualified specialists of that field.

Dr Har Gobind Khorana (9th January, 1922–9th November, 2011) was an Indian American biochemist. While on the faculty of the University of Wisconsin–Madison, he shared the 1968 Nobel Prize for Physiology or Medicine with Marshall W Nirenberg and Robert W. He is known for the contribution of the first synthetic gene and a renowned research worker in the field of nucleic acids and proteins.

The knowledge of biochemistry is also being extensively used in the field of diagnosis of diseases. Estimations of the levels of biogenic compounds and enzymes in the circulating blood/urine have been proved to be of valuable guidance to the physicians/surgeons in making the diagnosis of diseases, for instance, blood sugar level gets elevated in diabetes mellitus, blood urea and creatinine levels get raised in nephritis, serum calcium level gets elevated in hyperthyroidism and gets decreased in infantile tetany, serum inorganic phosphorus gets decreased in rickets, serum calcium level gets elevated in nephrosis, diabetes mellitus, obstructive jaundice, myxedema and xanthomatosis and may be decreased in hyperthyroidism, level of enzyme acid phosphatase gets increased in the carcinoma of prostate glands, serum glutamic oxaloacetic transaminase (GOT) and creatine phosphokinase (CPK or CK) levels get elevated in myocardial infarction, and so on.

Certain qualitative tests in urine, e.g. for the detection of sugar, protein, bile pigments, bile salts, blood, chyle, etc. are of great importance to the physicians/surgeons in making proper diagnosis. Besides qualitative tests, certain quantitative tests in urine like that of the estimation of total proteins in a 24-hr urine sample is of diagnostic value in cases of nephritic syndrome.

Estimation of hormones in serum is equally of great diagnostic value, e.g. the estimation of T_3 and T_4 is the most reliable means of confirming the diagnosis of hyperthyroidism or hypothyroidism. Highly specific and sensitive radioimmunoassays are used to measure serum T_3 and T_4 concentrations. In thyrotoxic states, serum TSH concentration is almost always low or undetectable. This is of little diagnostic value, since most assays cannot distinguish between normal and subnormal values. Measurement of serum TSH is the best means of distinguishing between untreated hypothyroidism of thyroid origin, in which the values are invariably increased, and pituitary or hypothalamic hypothyroidism, in which the values are usually undetectable or within the normal range.

Normal range

T4	4–12 mg/dl
T3	80–100 ng/dl
TSH	Less than 5 mU/ml

Certain diseases are merely controlled by using specific enzyme inhibitors or activators. Recent studies have proved that the medicines mostly act by influencing certain enzyme or enzyme systems. The same also holds true for hormones. Structure–activity relationships have been established in most of the cases. Such studies are covered in the field of biochemical pharmacology. After ascertaining the site of action and the structure responsible for a particular activity, it has now become possible to synthesize newer medicines having lesser toxicity and greater beneficial effects.

Biochemistry plays an important role for 'pharmacists'; unless the students pursuing such course are not well versed with the topics, such as: (i) carbohydrates, (ii) lipids (fats), (iii) proteins (immunoglobulins), (iv) pH, buffers, acids, (v) enzymes and isoenzymes, (vi) pH, buffers, acids, bases, (vii) vitamins, (viii) nutritional biochemistry, (ix) detoxification, (x) muscle physiology and biochemistry, (xi) biochemistry of nervous system, (xii) detoxification, (xiii) muscle physiology and biochemistry, (xiv) biochemistry of nervous tissue, (xv) hormonal biochemistry, (xvi) metabolism, (xvii) biophysical chemistry, etc. It shall not be possible for them to correlate properly with the diseases, understand their causes, means to cure them by dietary habitat, etc. Therefore, to understand the 'human biochemistry' for them is very essential and to the point.

Moreover, as one knows that the action of a drug almost always involves some change in the biochemical processes taking place in the body. As such, *pharmacists* must also be acquainted with the biochemical aspects of the human body. In pharmacy, biochemical testing provides indispensable insights into a drug's mode of action. Knowledge of biochemistry gives pharmacists an idea of the constitution of the drug, its chances of degradation with varying temperature, etc.

Role of Biochemistry in Preventive Medicine

Knowledge of biochemistry to such 'Pharmacy' students may be utilized to prevent certain very important diseases of infancy like kwashiorkor, marasmus (malnutrition disorders), vitamin 'A' deficiency disorders, minerals deficiency disorders (bone development, etc.), anaemia in boys and girls, diabetes in adults, etc.

Several common genetic diseases/disorders like Down syndrome, thalassemia, cystic fibrosis, Tay-Sachs disease, sickle cell anemia, glucose-6-phosphate dehydrogenase deficiency, etc. may be easily identified by the usage of clinical biochemistry.

Knowledge of biochemistry is extremely useful in designing many drugs useful in a number of pathological conditions. A sound knowledge of biochemistry is very important to pharmacy students from the point of view of nutrition in order to make our countrymen strong enough by making them aware of the importance of taking nutritious diet—its role in enhancing immunity. Role of nutritious diet for the senior citizens.

Understanding of detoxification may prove to be of great importance for them as to how drugs taken are detoxified and how are they metabolized. Advances in molecular biochemistry are very useful in the field of recombinant DNA technology in synthesizing many bioactive polypeptides, like human insulin.

Introduction to Pathology of Blood and Urine and its Scope

Knowledge of pathological/biochemical investigations and qualitative tests of various biomolecules present in fluids like blood and urine to such students shall prove to be a boon.

Such training/exposure to such students is meaningful for them in the following ways:

- They shall be able to do themselves certain qualitative tests of carbohydrates, proteins/amino acids and lipids in any fluid.
- They shall be able to perform qualitative analysis of various normal (like urea, uric acid, creatinine, allantoin, calcium, phosphate, etc.) and abnormal (like sugar, protein, acetone bodies, bile salts, bile pigments etc.) constituents present in one's urine and their sound interpretation which is a very essential component for such students. Their sound knowledge on such investigations is a counter-checking before dispensing medicines prescribed by the physicians/surgeons.
- They shall be able to perform certain quantitative determinations like glucose, creatinine, chlorides, etc. in urine samples and their interpretation.
- They shall be able to have some exposure by certain 'simulated' quantitative determinations like glucose, urea,

calcium, creatinine, cholesterol, SGOT, SGPT, etc. in blood samples and their interpretation which is the need of the day.

- Their exposure to hematology knowledge, i.e. total leucocytes count (TLC), differential leucocytes count [DLC—polymorphs (neutrophils), lymphocytes count, monocyte count, eosinophil count and basophil count, etc. is another important tool and a 'counter-checking' via media while dispensing medicines prescribed by physicians/surgeons.

In nutshell, knowledge of pathology of blood and urine is a 'must' for diploma-in-pharmacy students as they are partial doctors/physicians and the value of such pharmacists is not less than any doctor/physician abroad.

CELLS

The entire organism consists of cells. All multicellular organisms contain billions or trillions of cells organized into complex structures, but many organisms consist of a single cell. Even simple unicellular organisms exhibit all the characteristic properties of life, representing that the cell is the fundamental unit of life.

In general, two types of cells exist in nature. They are:

1. Prokaryotic cells (unicellular, i.e. bacteria).
2. Eukaryotic cells (multicellular, i.e. animals, plants and fungi).

Prokaryotic Cells

Prokaryotic cells (Greek *pro*—primitive or primary, and *karyon*—nucleus) do not have a well-defined nucleus or have nucleic acid but nuclear membrane is absent, e.g. bacteria. Eukaryote cells have a membrane-bound well-defined nucleus and other cell organelles. The two groups of prokaryotes, bacteria and archaea, contain organisms that exist as single cells, being simple in structure. As you recall, prokaryotes do not have a nucleus or other membrane-bound organelles. Fig. 1.1 shows a stylized prokaryotic cell, illustrating what a typical bacterium would look like (*E. coli*, here).

Structure of a Prokaryotic Cell

Prokaryotic structural components consist of macromolecules such as DNA, RNA,

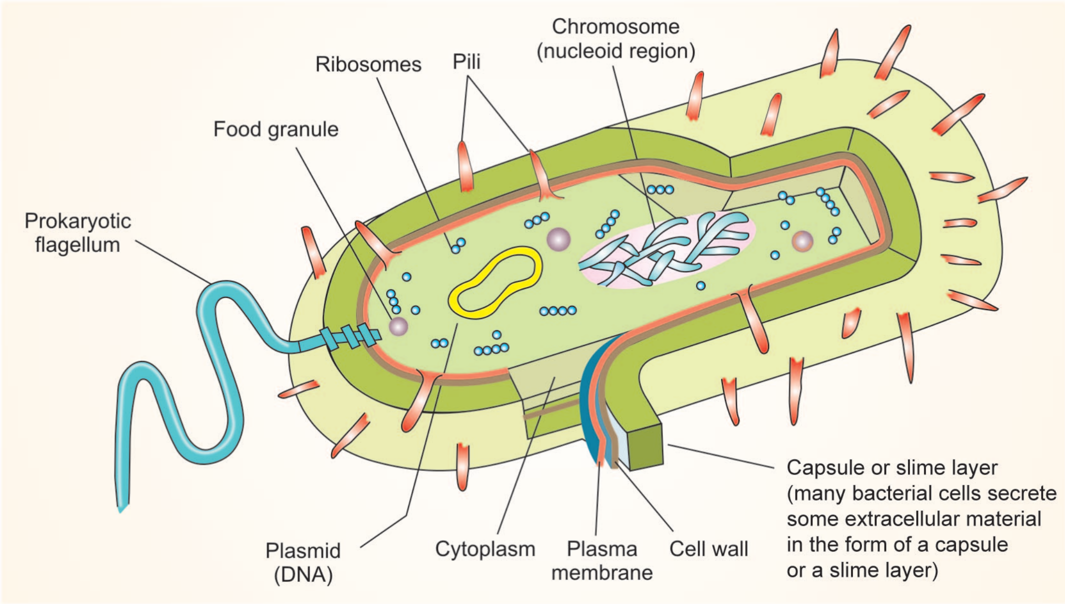

Fig. 1.1: Structure of a prokaryotic cell

Table 1.1: Macromolecules that make-up cell material

Macromolecules	*Primary subunits*	*Place in cell*
Proteins	Amino acids	Flagella, pili, cell walls, cytoplasmic membranes, ribosomes, cytoplasm
Polysaccharides	Sugars (carbohydrates)	Capsules, inclusions (storage), cell walls
Phospholipids	Fatty acids	Membranes
Nucleic acids (DNA/RNA)	Nucleotides	DNA: Nucleoid (chromosome), plasmids rRNA: Ribosomes; mRNA; tRNA: Cytoplasm

proteins, polysaccharides, phospholipids and other. The macromolecules that make-up cell material are made-up of primary subunits such as nucleotides, amino acids and sugar (Table 1.1). It is the sequence in which the subunits are put together in the macromolecule, called the primary structure.

A prokaryotic cell has five essential structural components—a nucleoid (DNA), ribosomes, cell membrane, cell wall, and some sort of surface layer, which may or may not be an inherent part of the wall.

Characteristics and Functions of Typical Bacterial Cell Structures

1. Flagella help in swimming movement, they consist of protein.
2. Sex pilus stabilizes mating bacteria during DNA transfer by conjugation made-up of protein.
3. Common pili or fimbriae attachment to surface; protection against phagotrophic engulfment of protein.
4. Capsule (includes slime layers and glycocalys) attachment to surface; protection against phagocytic engulfment, occasionally killing or digestion; reserve of nutrient or protection against desiccation of polysaccharide or occasionally polypeptide.
5. A gram-positive bacterium prevents osmotic lysis of cell protoplast and confers rigidity and shape on cells.
6. Gram-negative bacteria peptidoglycan prevents osmotic lysis and confers rigidity and shape; outer membrane is permeability barrier and has various functions.
7. Chromosome and plasmid's extra-chromosomal genetic material is DNA.

Eukaryotic Cells

Eukaryotic (Greek *eu*—true, *karyon*—nucleus) cells (Fig. 1.2) are the type of living cells that form the organisms of all of the life kingdoms, except monera. They are generally larger than bacterial cells. Eukaryotic cells have membrane-bound organelles. Their DNA is organized into linear threads called chromosomes which are located within a membrane, and the entire unit is called a nucleus. Organelles that might be found in a cell include ribosomes (80S), mitochondria, chloroplast and Golgi bodies. The cell surface membrane and the membranes which form organelles in eukaryotic cells, all have the same basic structure, known as the fluid mosaic model. Such membranes provide control of the entry and exit of substances into cells and organelles and such control is a result of the phospholipid bilayer and membrane proteins.

Functions

- Cell body contains:
  - Nucleus, which provides the genetic code for the production of neurotransmitter substances, e.g. acetylcholine and enzymes, e.g. cholinesterase.
  - Dense groups of ribosomes and endoplasmic reticulum called Nissl granules for production and transport of proteins and neurotransmitters.

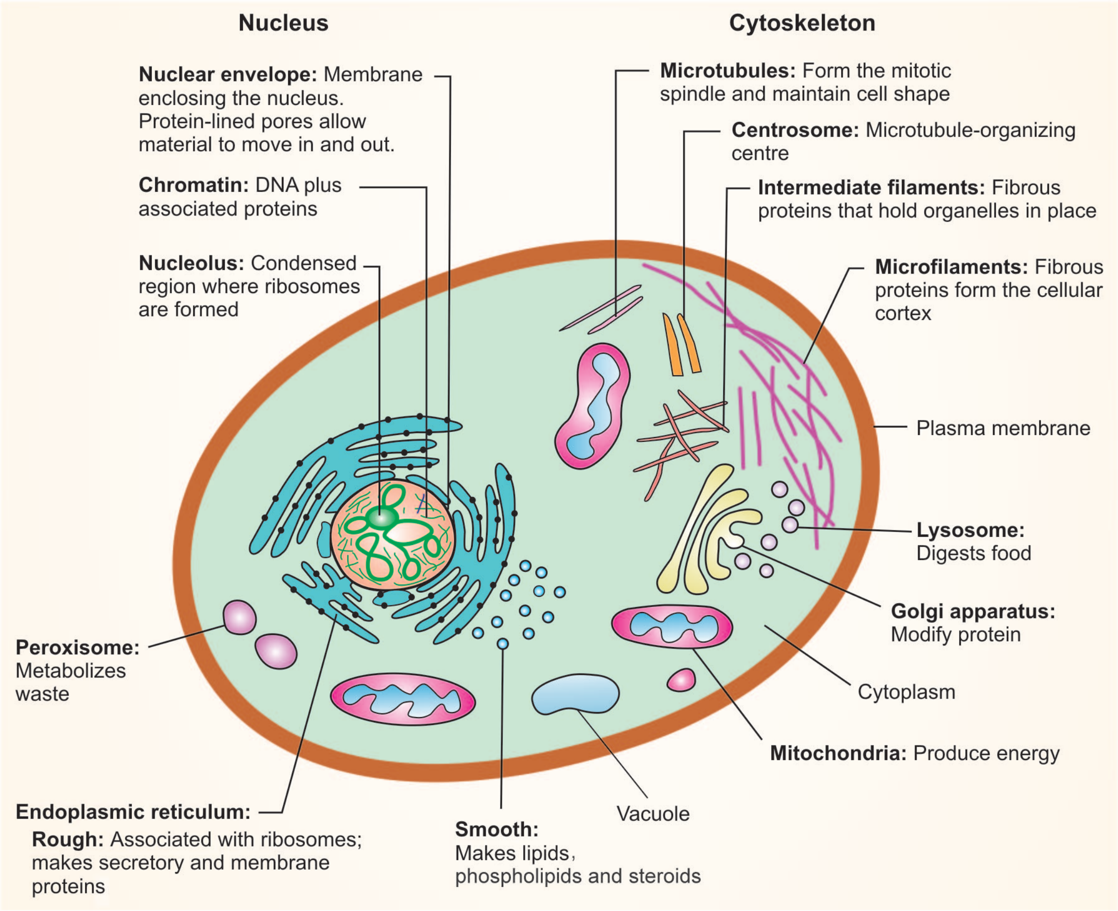

Fig. 1.2: Eukaryotic cell

- Synaptic knob at end of dendrite contains:
  - Many mitochondria to provide ATP for active refilling of synaptic vesicles.
  - Numerous vesicles for modification and release of chemical transmitters across the synapse.
- Double nuclear envelope to enclose and protect DNA (normally visible as chromatin granules).
- Nuclear pores allow entry of substances such as nucleotides for DNA replication and exit of molecules such as mRNA during protein synthesis.
- Normally, the nuclear pores are plugged by an RNA/protein complex. Small molecules pass through the pores by diffusion, whereas large molecules such as partly complete ribosomes pass through actively.
  - The matrix contains 70S ribosomes for protein manufacture, e.g. enzymes.
  - DNA codes for proteins.
  - Enzymes: An average mammalian cell generally contains about 3000 enzymes.
- Endoplasmic reticulum is of two types:
  - Rough endoplasmic reticulum: Synthesizes proteins.
  - Smooth endoplasmic reticulum: Synthesizes lipids, phospholipids and steroids.
- Golgi body: A Golgi body, also known as a Golgi apparatus, is a cell organelle that helps in process and package proteins and lipid molecules, especially proteins destined to be exported from the cell. Named after its discoverer, Camillo Golgi, the Golgi body appears as a series of stacked (heap) membranes.

- Ribosomes provide sites for the binding of mRNA which allows translation of the DNA code. There are two sites for the binding of 2 tRNA molecules. Ribosomes recognise the initiation and termination codons on mRNA. Ribosomes are capable of moving along with mRNA strand. This allows decoding of the mRNA and synthesis of a polypeptide chain.
- Lysosomes are vesicles which contain hydrolytic enzyme, collectively known as lysozymes. When released, these enzymes can break down old organelles, storage molecules or, indeed, the whole cell, when it dies.

All bacterial cells and archaea have a plasma membrane to regulate what enters and leaves the cell. The membrane is a phospholipid bilayer with proteins embedded in it. Most bacteria and archaea also have a cell wall exterior to the plasma membrane. Their DNA, though in a discrete region in the cell, is not separated by a membrane. Bacterial DNA is a circular molecule loose within the cell. It may be concentrated toward the middle and will attach to the plasma membrane before the cell divides. Though, there are few organelles, ribosomes are the sites of protein synthesis. Prokaryotic cells are able to carry out all functions, we recognize as fundamental to life, such as reproduction and energy transduction but are much more simple in structure than eukaryotic cells.

Differences between prokaryotic and eukaryotic cells have been summarised in Table 1.2.

STRUCTURE OF CELL

Cells vary in shape and size. Usually, they are microscopic, but some of them are also visible to naked eyes. They range from 0.2 to 0.5 nm in diameter. Cells can be of any shape. Their shape usually reflects the functions they carry out in an organism, e.g. nerve cells which have to transmit impulses to long distances; muscle cells are elongated cells may be flat, spindle, cuboidal-shaped and of other shapes as well. All cells have certain common features. Presence of nucleus in cells is one such main feature. Nucleus in cells is control centre. Bacteria

Table 1.2: Differences between prokaryotic and eukaryotic cells

Prokaryotic cells	*Eukaryotic cells*
The size is 0.1–5.0 nm	The size is 5–100 nm
Cell wall, if present, contains mucopeptide or peptidoglycan	Cell wall, if present, contains cellulose, peptidoglycan is absent
A typical nucleus is absent	A typical nucleus made of nuclear envelope, chromatin and nucleoplasm
DNA content is low	DNA lies inside the nucleus, mitochondria and plastids
DNA is naked or without any association with histone proteins	DNA is associated with histones
Introns are commonly absent in DNA, RNA, therefore, does not require splicing	Introns are quite common. RNA, therefore, requires splicing before becoming operational
Sexual reproduction is absent	Sexual reproduction is commonly present
Cell division does not show distinction of interphase and M phase	A distinction of interphase and mitotic phase occurs during cell cycle
Endocytosis and exocytosis are absent	They occur in eukaryotic cells
Flagella are smaller. A distinction of axoneme and sheath is absent in the flagellum	Flagella are longer. A flagellum shows distinction of axoneme and sheath.
Cyclosis is absent	Cyclosis or cytoplasmic streaming is common
Ribosomes are of small size (70S)	Ribosomes are large in size; smaller size (70S) in organelles

and blue-green algae are devoid of a typical nucleus. Viruses have no recognisable structure. They are so small that usually they are not visible without the help of an electron microscope. They do not have cytoplasm; however, they are crystals of nucleoprotein.

Cells can grow duplicate, process in sequence, respond to stimuli, and carry out an array of chemical reactions. These abilities define life. The living matter, i.e. protoplasm is composed of mainly six elements—carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur.

So, each cell is a small container of chemicals and water wrapped in a membrane. There are 100 trillion cells in a human, and each contains all of the genetic information necessary to manufacture a human being or any living organism. This information is encoded within the nucleus of cell in the form of DNA.

Cell Organelles

1. Nucleus
2. Endoplasmic reticulum
3. Golgi apparatus
4. Mitochondria
5. Lysosomes
6. Peroxisomes
7. Plasma membrane

Nucleus

Structure

Nucleus (Fig. 1.3) is a largest, double-membrane-bound cell organelle found in eukaryotic cells. The nucleus appears to be a dense, spherical organelle. It occupies about 10% of the total volume of the cell. In mammalian cells, the average diameter of the nucleus is approximately 6 micrometers. Nuclear membrane is made-up of two membranes, the outer membrane and the inner membrane. The outer membrane of the nucleus is continuous with the membrane of the rough endoplasmic reticulum. The space between these layers is known as the perinuclear space. The nuclear envelope separates the genetic material of the cell from the cytoplasm. It also serves as a barrier to prevent passage of macromolecules freely between the nucleoplasm and the cytoplasm. The nuclear envelope is perforated with numerous pores called nuclear pores. The nuclear pores are composed of many proteins known as nucleoproteins. The nuclear pores regulate the passage of the molecules between the nucleus and cytoplasm. The pores allow the passage of molecules of only about 9 nm wide. The genes are arranged on the chromosomes.

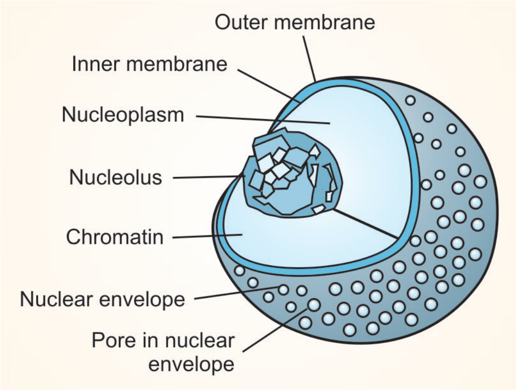

Fig. 1.3: Structure of nucleus

A human cell has nearly 6 feet of DNA, which is divided into 46 individual molecules. The nucleus constitutes the genetic material of the cell and maintains the integrity of the genes which regulate the gene expression, in turn, regulates the activities of the cell. Therefore, the nucleus is known as the control center of the cell. The nucleolus is not surrounded by a membrane, it is a densely-stained structure found in the nucleus. During cell division, the nucleolus disappears. The number of nucleoli is different from species to species but within a species the number is fixed. Studies suggest that nucleolus may be involved in cellular aging and senescence. The larger molecules are transferred through active transport. A fluid filled in nucleus is called nucleoplasm which is a viscous semi-solid fluid and is similar to the composition of the cytoplasm. DNA and RNA are found in the nucleus. The DNA molecules are in complex with a large variety of proteins (histones) constitutes chromatin. During cell division, the chromatin forms well-defined chromosomes.

Functions

1. It is responsible for protein synthesis, cell division, growth and differentiation.
2. The nucleus provides a site for genetic transcription that is segregated from the location of translation in the cytoplasm, allowing levels of gene regulation that are not available to prokaryotes.
3. Nucleolus produces ribosomes and is known as protein factory. It also regulates the integrity of genes and gene expression.
4. The main function of the cell nucleus is to control gene expression and mediate the replication of DNA during the cell cycle.

Endoplasmic Reticulum

Structure

The endoplasmic reticulum (ER) (Fig. 1.4) is a series of interconnected membranous sacs and tubules that collectively modifies proteins and synthesizes lipids. The membrane of the ER, which is a phospholipid bilayer embedded with proteins, is continuous with the nuclear envelope. However, these two functions are performed in separate areas of the ER—the rough ER and the smooth ER.

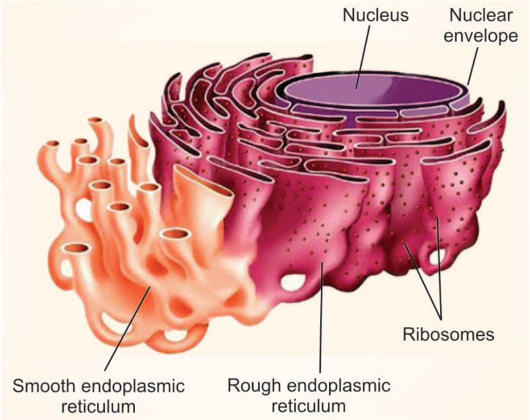

Fig. 1.4: Structure of endoplasmic reticulum

- Smooth endoplasmic reticulum, ribosomes are not attached with its surface and differ in function with respect to rough ER.
- Rough endoplasmic reticulum has ribosomes attached throughout the surface. These are present in cells, which are active in protein synthesis.

Biomedical importance of ER

A malfunction of the ER stress response caused by aging, genetic mutations, or environmental factors can result in various diseases, such as diabetes, inflammation, and neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, and bipolar disorder. Further mitochondrial DNA can be damaged by free radicals. Age-related degenerative disorders such as Parkinson's disease; cardiomyopathy may have a component of mitochondrial damage.

Functions

a. Common to both ER (smooth and rough)
   1. Forms the skeletal framework
   2. Active transport of cellular materials
   3. Metabolic activities due to presence of different enzymes
   4. Provides increased surface area for cellular reactions
   5. Formation of nuclear membrane during cell division
   6. Detoxification of various drugs is an important function of ER. Microsomal cytochrome P-450 hydroxylates drugs such as benzpyrine, aminopyrine, aniline, morphine, phenobarbitone, etc.

b. Functions of smooth ER
   1. Lipid synthesis,
   2. Glycogen synthesis, and

3. Steroid synthesis like cholesterol, progesterone, testosterone, etc.

c. Functions of rough ER
1. It provides site for protein synthesis, and
2. It helps in transport of proteins.

Golgi Complex (Apparatus)

Structure

Golgi apparatus (Fig. 1.5) was discovered by an Italian biologist Camillo Golgi in the year 1898. They are also called dictyosomes. The Golgi organelle is a network of flattened smooth membranes and vesicles. It may be considered as the converging area of endoplasmic reticulum. The Golgi complex has a proximal or *cis* compartment, a medial compartment and a distal or trans compartment.

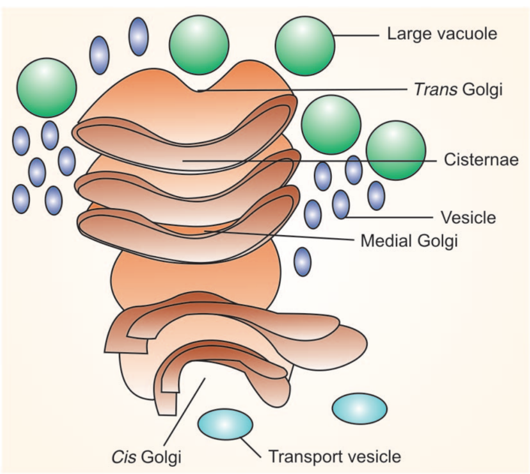

Fig. 1.5: Structure of Golgi apparatus

Functions

1. The main function of the Golgi apparatus is to modify, sort and package the macromolecules that are synthesized by the cells for secretion purposes within the cell.
2. It mainly modifies the proteins that are prepared by the rough endoplasmic reticulum.
3. They are also involved in the transport of lipid molecules around the cell.
4. The Golgi complex is, thus, referred as post office where the molecules are packaged, labelled and sent to different parts of the cell.
5. It imports substances like nucleotides from the cytosol of the cell. The modifications brought about by the Golgi body might form a signal sequence. This determines the final destination of the protein.
6. The Golgi complex also plays an important role in the production of proteoglycans. The proteoglycans are molecules that are present in the extracellular matrix of the animal cells.
7. It is also a major site of synthesis of carbohydrates. These carbohydrates include the synthesis of glycosaminoglycans, Golgi body attaches to these polysaccharides which then attaches to a protein produced in the endoplasmic reticulum to form proteoglycans.
8. The Golgi complex involves in the sulfation process of certain molecules. The process of phosphorylation of molecules by the Golgi complex requires the import of ATP into its lumen.

Mitochondria

Mitochondria are the powerhouse of the cell, where energy released from oxidation of foodstuffs is trapped as chemical energy in the form of ATP.

Structure

The mitochondria (Fig. 1.6) (Greek *mitos*—thread, *chondros*—granule) are the centers for the cellular respiration and energy metabolism. Mitochondria are typically round-to-oval in shape and range in size from 0.5 to 10 μm. In addition to producing energy, they also store calcium for cell signaling activities, generate heat, and mediate cell growth and death. They are bounded by a double membrane. This membrane is made-up of lipoprotein. The outer membrane is smooth, but the inner membrane is convoluted or folded internally to form cristae. Mitochondria are filled with a fluid known as matrix. Mitochondria

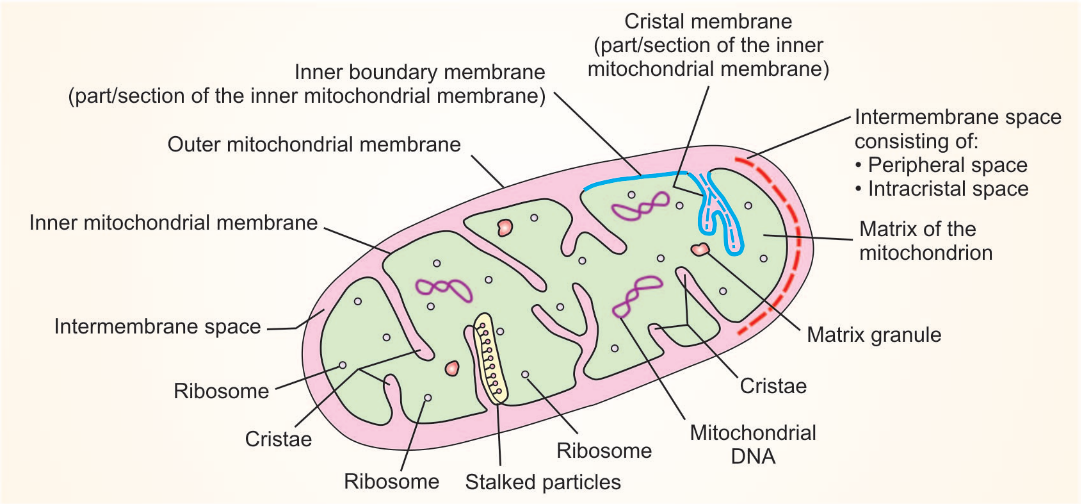

Fig. 1.6: Structure of mitochondria

contain cytochromes, dehydrogenase enzymes, respiratory pigment, flavin and some other enzymes that participate in lipid metabolism and the Krebs cycle. They usually occur in large number in nerve cells, muscle cells and secretory cells. Thus, mitochondria are actually the powerhouse or batteries of a cell, and therefore, of the organism as a whole.

Various enzymes found in mitochondria along with their locations have been summarised in Table 1.3.

Table 1.3: Location of enzymes in mitochondria

Mitochondria, outer membrane	Monoamine oxidase, Acyl-CoA synthesis
In between outer and inner membrane	Adenylase kinase
Inner membrane, outer surface	Glycerol-3-phosphate dehydrogenase
Inner membrane, inner surface	Succinate dehydrogenase
Soluble matrix	Enzymes of citric acid cycle

Functions

- Mitochondria act as high energy storage center taking active part in metabolism.
- Mitochondria convert the potential energy of different food materials into a kind of energy that can be used to carry out different activities of cell like reabsorption, growth reproduction, respiration, etc.
- This occurs by a process of cellular respiration, also known as aerobic respiration, which is dependent on the presence of oxygen.
- The two separate biochemical pathways are called the Krebs citric acid and electron transfer chain.
- The synthesis of ATP by the mitochondria is brought about by the conjugation of two separate biochemical pathways, each involving many enzymes.
- In Krebs cycle, the products are exposed to successive dehydrogenation. Cytochrome provides a mechanism whereby the electrons resulting from the oxidation of hydrogen are transported to oxygen functioning as acceptor for hydrogen ions to form water.
- The citric acid is a collection of about 10 enzymes which works together to remove energy from pyruvic acid molecules in a series of easy stages. This might be through a process of slow and controlled burning, in which the heat energy is trapped in ATP molecules.
- The major function of mitochondria is the production of energy during the production of adenosine triphosphate (ATP) through the citric acid cycle.

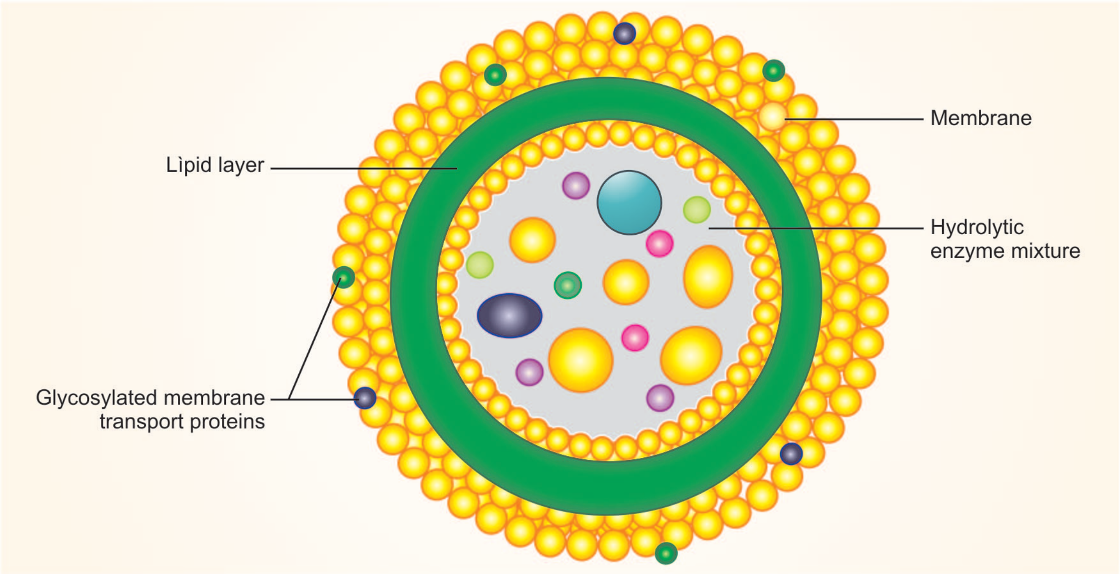

Fig. 1.7: Lysosome

Lysosomes

- Lysosomes (Fig. 1.7) are membrane-bound, dense granular structures containing hydrolytic enzymes responsible mainly for intracellular and extracellular digestion.
- Lysosomes are formed by budding of the Golgi apparatus, and the hydrolytic enzymes within them are formed in the endoplasmic reticulum.
- The word 'lysosome' is made-up of two words: '*lysis*' meaning breakdown, and '*soma*' meaning body.
- It is an important cell organelle responsible for the inter- and extracellular breakdown of substances.
- They are more commonly found in animal cells, while only in some lower plant groups (slime molds and saprophytic fungi).
- Lysosomes occur freely in the cytoplasm. In animals, they are found in all cells except in the RBCs.
- They are found in abundance in cells related to enzymatic reaction such as cells of liver, pancreas, kidney, spleen, leucocytes, macrophages, etc.

Structure

- Lysosomes are without any characteristic shape or structure, i.e. they are pheomorphic.
- They are mostly globular or granular in appearance.
- Each lysosome is 0.2–0.5 μm in size and is surrounded by a single lipoprotein membrane unique in composition.
- Membrane contains highly glycosylated lysosome-associated membrane proteins (LAMP) and lysosomal integral membrane proteins (LIMP).
- LAMPs and LIMPs form a coat on the inner surface of the membrane.
- They protect the membrane from attack by the numerous hydrolytic enzymes retained inside.
- The lysosomal membrane has a hydrogen proton pump which is responsible for maintaining pH conditions of the enzyme, i.e. acidic medium maintained by the proton pump that pumps H^+ inside the lumen, ensuring the functionality of the lysosomal enzymes.
- Inside the membrane, the organelle contains the enzymes in the crystalline form.

Lysosomal enzymes

For degradation of extra- and intracellular material, lysosomes fitted with enzymes called hydrolases. It contains about 40 varieties of enzymes which are classified into the following main types, namely:

- Proteases: Digest proteins
- Lipases: Digest lipids
- Amylases: Digest carbohydrates
- Nucleases: Digest nucleic acids
- Phosphomonoesterases: Catalyse the hydrolysis of phosphoryl (P=O) bonds

Collectively, the group of enzymes is called hydrolases, which cause breakdown of substrates by the addition of water molecules. Most of the lysosomal enzymes act in acidic medium.

Types of lysosomes

Lysosomes are of two types:

- Primary
  - Small sac-like structures enclosing enzymes synthesized by the rough endoplasmic reticulum.
  - Simply called storage granules storing enzymes.
- Secondary
  - Formed by the fusion of primary lysosome with phagosomes.
  - Contain engulfed material plus enzymes
  - Materials are progressively engulfed.

Functions of lysosomes

These serve two major functions:

1. Intracellular digestion:
   - To digest food, the lysosome membrane fuses with the membrane of food vacuole and transmits the enzymes inside.
   - The digested food then diffuses through the vacuole membrane and enters the cell to be used for energy and growth.
2. Autolytic action:
   - Cell organelles that need to be got ridden are covered by vesicles on vacuoles by the process of autophagy to form autophagosome.
   - The autophagosome is then destroyed by the action of lysosomal enzymes.

Processes in which lysosomes play very important roles are:

a. Heterophagy: The taking into the cell of exogenous material by phagocytosis or pinocytosis and the digestion of the ingested material after fusion of the newly formed vacuole with a lysosome.

b. Autophagy: A normal physiological process that deals with the destruction of cells in the body. It is essential for maintaining the homeostasis, for normal functioning by protein degradation, turnover of destroyed cell organelles for new cell formation.

Yoshinori Ohsumi, a Japanese cell biologist, was awarded the Nobel Prize in Physiology or Medicine in the year 2016 for his discoveries on how cells recycle their content, a process known as autophagy, a Greek term for 'self-eating'.

c. Extracellular digestion: Primary lysosomes secrete hydrolases outside by exocytosis resulting in degradation of extracellular materials, e.g. saphrophytic fungi.

d. Autolysis: It refers to the killing of an entire set of cells by the breakdown of the lysosomal membrane. It occurs during amphibian and insect metamorphosis.

e. Fertilization: The acrosome of the sperm head is a giant lysosome that ruptures and releases enzymes on the surface of the egg. This provides the way for sperm entry into the egg by digesting the egg membrane.

f. As janitors (sweepers) of the cell: Lysosomes remove 'junk' that may accumulate into the cell to prevent diseases.

Peroxisomes

Peroxisomes contain enzymes that oxidize certain molecules normally found in the cell, notably fatty acids and amino acids. Those oxidation reactions produce H_2O_2, which is the basis of the name peroxisome. The peroxisomes are single membrane, found in eukaryotic cell. They are approximately 0.1–1 μm in diameter. They are rich in leukocytes and platelets. They also have antioxidant enzymes, e.g. catalase and peroxidase. The free radicals damage molecules, cell membranes, tissues and genes. Catalase protects the cell from the toxic effects of H_2O_2, by converting it into H_2O and O_2. Peroxidase destroys the unwanted peroxides and other free radicals. Now, it has been shown that liver peroxisomes have an uncommonly active α-oxidative system capable of oxidizing long chain fatty acids (C 16 to 18 or >C 18). The α-oxidation enzymes of peroxisomes are rather unique in that, the first step of the oxidation is catalyzed by a flavoprotein, an 'acyl-CoA oxidase'.

$$\text{Acyl-CoA} + O_2 \rightarrow \alpha,\beta\text{-unsaturated acyl-CoA} + H_2O_2$$

Malfunctioning of peroxisomes causes 'Zellweger syndrome', a rare inherited metabolic disorder. Children with this syndrome usually do not survive beyond the first year of life. It is caused by mutations in any one of at least 12 genes; mutations in the PEX1 gene are the most common cause. Peroxisomes are organelles found in almost every cell of the body and are needed for the formation of organs like liver, kidneys and brain.

IMPORTANT QUESTIONS

1. What is the importance of biochemistry?
2. What are the various applications of biochemistry? Justify it.
3. Name various institutes with their places where researches pertaining to 'biochemistry' are carried out.
4. Explain the role of teaching of biochemistry in pharmacy course.
5. Write in detail about the cell structure and its organization with the help of a diagram.
6. Write short notes on:
   a. Mitochondria
   b. Lysosomes
   c. Peroxisomes
   d. Golgi apparatus
   e. Nucleus

MULTIPLE CHOICE QUESTIONS

Read the following questions carefully and put a tick (☑) mark in the box against the correct option.

1. Who discovered cell in 1665?
   (a) Robert Hook ☐
   (b) Robert Crook ☐
   (c) David Thomson ☐
   (d) Marie Francois ☐
2. Name the outermost boundary of the cell.
   (a) Plasma membrane ☐
   (b) Cytoplasm ☐
   (c) Nuclear membrane ☐
   (d) None of the above ☐
3. The jelly-like substance present inside the cell is known as:
   (a) Cytoplasm ☐
   (b) Ectoplasm ☐
   (c) Nucleoplasm ☐
   (d) None of the above ☐
4. Name the process in which the ingestion of material by the cells is done through the plasma membrane.
   (a) Egestion ☐
   (b) Diffusion ☐
   (c) Osmosis ☐
   (d) Endocytosis ☐

ANSWERS

1. a   2. a   3. a   4. d