Biochemistry

Author

Nidhi Sharma

Assistant Professor, Department of Pharmacy, AKTU (IEC College of Engineering & Technology, Greater Noida, Uttar Pradesh, India)

Publication Month and Year: September 2021

Pages: 61

E-BOOK ISBN: 978-81-952994-9-2

Academic Publications
C-11, 169, Sector-3, Rohini, Delhi, India
Website: www.publishbookonline.com
Email: publishbookonline@gmail.com

Contents

S. No.	Chapter	Page No.
1.	Introduction to Biochemistry	01-04
2.	Protein	05-14
3.	Carbohydrate	15-18
4.	Lipids	19-22
5.	Vitamin	23-28
6.	Role of Minerals and Water in Life Process	29-31
7.	Enzymes	32-35
8.	Metabolism	36-53
9.	Lymphocytes	54-61

Chapter - 1

Introduction to Biochemistry

What is biochemistry?

Biochemistry is also known as biological chemistry. It is the study of chemical processes within and relating to living organisms. It forms a bridge between biology and chemistry by studying how complex chemical reactions and chemical structures give rise to life and life's processes. Biochemical processes give rise to the complexity of life.

In other words, Biochemistry is the study of chemical processes in living organisms but it is not limited to, living matter.

OR

Biochemistry is the study of the chemistry of living things. This includes organic molecules and their chemical reactions. Most people consider biochemistry to be synonymous with molecular biology.

The aim of biochemists is to describe in molecular terms the structures, mechanisms and chemical processes shared by all organisms, providing organizing principles that underlie life in all its diverse forms.

It is closely related to molecular biology, the study of the molecular mechanisms by which genetic information encoded in DNA is able to result in the processes of life.

Biochemistry deals with the study of structures, functions and interactions of biological macromolecules, such as proteins, nucleic acids, carbohydrates and lipids, which provide the structure of cells and perform many of the functions associated with life.

Biomolecule

The four main classes of molecules (also called as biomolecule) in biochemistry are:

- Carbohydrates
- Lipids
- Proteins
- Nucleic acids

Carbohydrates

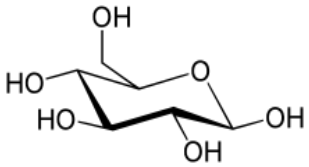
Carbohydrates are the molecule made up of Carbon & Hydrogen. And they are having the molecular formula $(CH_2O)_x$. All Sugars are carbohydrates, but not all carbohydrates are sugars.

Functions

- Energy storage and providing structure.
- Play important roles in cell to cell interactions and communications.

1) **Monosaccharide:** They are also known as simple sugars. They are the simple form of sugars and more basic. They can't be further hydrolyzed due to having single monomer unit. They having the formula $C_nH_{2n}O_n$.

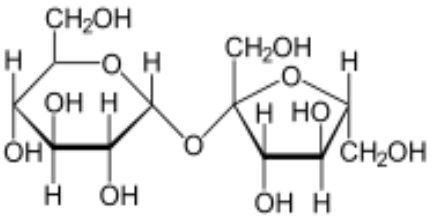
E.g.: Glucose, Fructose and Galactose.



Glucose

2) **Disaccharides:** They are made up of Two monosaccharides unit. In which two monosaccharides unit joined together by glycosidic linkage.

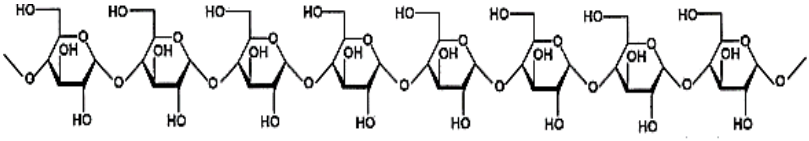
E.g.: Sucrose (glucose + fructose), Lactose and Maltose.



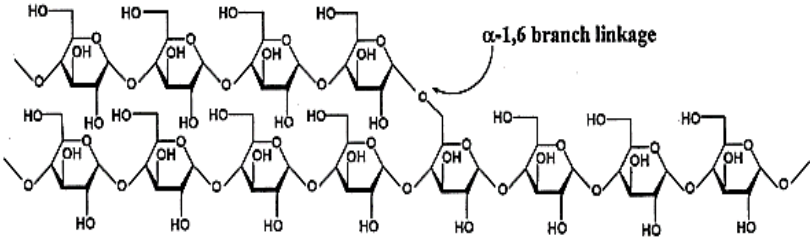
Sucrose

3) **Polysaccharides:** They are made up of more than two monosaccharides' units and they are joined together by glycosidic linkage.

E.g.: Starch, Glycogen, Chitin, Cellulose etc.



Segment of an amylose molecule



Segment of an amylopectin molecule, showing one α-1,6 branch linkage

Starch

Lipids

A lipid is chemically defined as a substance that is insoluble in water and soluble in alcohol, ether, and chloroform. Lipids are an important component of living cells. Like carbohydrates and proteins, lipids are also the main constituents of plant and animal cells. Cholesterol and triglycerides are lipids.

Lipids are usually made up from one molecule of glycerol combined with other molecules. In triglycerides, the main group of bulky lipids, in which one molecule of glycerol and three fatty acids are joined together.

Fatty acids are considered the monomer, and they may be Saturated (no double bonds in the carbon chain) or Unsaturated (one or more double bonds in the carbon chain).

Proteins

Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. They perform many functions within organisms, including:

- They act as an enzymes that catalyze biochemical reactions and are vital to metabolism.
- Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that maintains cell shape.

- Some proteins are important in cell signaling, immune responses, cell adhesion, and the cell cycle.
- In animals, proteins are needed in the diet to provide the essential amino acids that cannot be synthesized.
- Digestion breaks the proteins down for use in the metabolism.

Proteins differ from one another mainly in their sequence of amino acids, which is denoted by the nucleotide sequence of their genes, and which mainly results in protein folding into a specific three-dimensional structure that determines its activity.

A linear chain of amino acid residues is called a polypeptide. A protein contains at least one long polypeptide. Short polypeptides, containing less than 20-30 residues, are rarely known to be proteins and are commonly called peptides, or sometimes oligopeptides.

The individual amino acid residues are bonded together by peptide bonds and adjacent amino acid residues. Sometimes proteins have non-peptide groups attached, which can be called prosthetic groups or cofactors. Proteins can also work together to achieve a particular function, and they often associate to form stable protein complexes.

Uses of biochemistry

- Biochemistry is used to learn about the biological processes which take place in cells and organisms.
- Biochemistry may be used to study the properties of biological molecules, for a variety of purposes. For example, a biochemist may study the characteristics of the keratin in hair so that a shampoo may be developed that enhances curliness or softness.
- Biochemists find uses for biomolecules. For example, a biochemist may use a certain lipid as a food additive.
- Alternatively, a biochemist might find a substitute for a usual biomolecule. For example, biochemists help to develop artificial sweeteners.
- Biochemists can help cells to produce new products. Gene therapy is within the realm of biochemistry. The development of biological machinery falls within the realm of biochemistry.

Chapter - 2

Protein

Proteins are large biomolecules or macromolecules consisting of one or more long chains of amino acid residues. They perform many functions in the body including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another.

Proteins differ from one another primarily in their sequence of amino acids, which is described by the nucleotide sequence of their genes.

A linear chain of amino acid residues is called a polypeptide. A protein contains at least one long polypeptide. Short polypeptides, containing less than 20-30 residues and they are rarely considered to be proteins and are commonly called peptides, or sometimes oligopeptides. The individual amino acid residues are bonded together by peptide bonds and adjacent amino acid residues. The sequence of amino acid residues in a protein is defined by the sequence of a gene, which is encoded in the genetic code. In general, the genetic code specifies 20 standard amino acids.

Sometimes proteins have non-peptide groups attached, which can be called prosthetic groups or cofactors. Proteins can also work together to achieve a particular function, and they often associate to form stable protein complexes.

Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that maintains cell shape. Other proteins are important in cell signaling, immune responses, cell adhesion, and the cell cycle. In animals, proteins are needed in the diet to provide the essential amino acids that cannot be synthesized. Digestion breaks the proteins down for use in the metabolism.

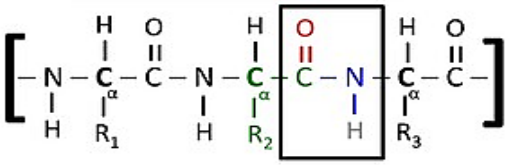


Fig: Most proteins consist of linear polymers built from series of up to 20 different L-α-amino acids. All proteinogenic amino acids possess common structural features, including an α-carbon to which an amino group, a carboxyl group, and a variable side chain are bonded.

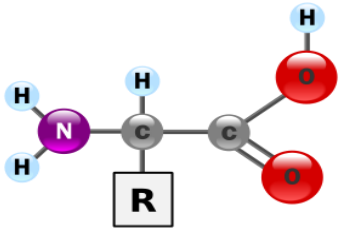


Fig: Basic structure of amino acids. Only proline differs from this structure as it contains an unusual ring to the N-end amine group, which forces the CO–NH amide moiety into a fixed conformation.

Classification

Proteins have wide structural and functional diversity. It is difficult to classify these on the basis of a single property or a characteristic. However, following are some common basis of classifying them.

- Shape and structure.
- Products of hydrolysis.
- Biological functions.

1. Classification on the basis of shape and size

On the basis of their composition, proteins get different shapes and size which give an indication of their various functions. These can be broadly put into two classes on the basis of their overall shape. These are as follows:

- **Globular proteins:** These contain compactly folded coils of polypeptide chains giving them shape of spheroids or ellipsoids. Examples of this type are albumins, globulins, histones, protamine's etc.

- **Fibrous proteins:** This class of proteins looks like fibers or threads. These are insoluble in water and aqueous solutions of acids and bases. These have high mechanical strength. Keratins in hair, actin and myosin in muscles and collagen are examples of this type of protein.

2. Classification on the basis of products of hydrolysis

On the basis of the products obtained on hydrolysis, proteins can be classified into three categories viz., simple proteins, conjugated proteins and derived proteins.

- **Simple proteins:** Simple proteins are those which are made of amino acid units, each joined by a peptide bond. Upon hydrolysis they yield only a mixture of amino acids. Following is some of the types of simple proteins.

Table 1: List of simple proteins.

S. No.	Type	Examples
1.	Albumins	Egg albumin, serum albumin, lactalbumin.
2.	Gliadin	Tissue globulin, serum globulin.
3.	Gliadins	Wheat gliadin, hordein (barley) etc.
4.	Albuminoids	Keratin of hairs, skin, egg shell and bones, elastin, collagen of tendons, ligaments and bones.
5.	Histones	Globin of hemoglobin.
6.	Protamine	Tissue globulin, serum globulin.

- **Conjugated proteins:** Conjugated proteins are composed of simple proteins combined with non-protein substances. The non-protein substance is called **prosthetic group** or **cofactor**. Following is some of the types of conjugated proteins.

Table 2: List of conjugated protein.

S. No.	Type	Examples
1.	Chromoproteins	Hemoglobin, in which the prosthetic group is iron.
2.	Phosphoproteins	Casein in milk and vitellin in egg yolk containing phosphoric acid as Prosthetic group.
3.	Lipoproteins	HDL (high density lipoprotein), LDL (low density lipoprotein) and VLDL (very low-density lipoprotein), have lipids as the prosthetic groups.
4.	Glycoprotein	Ovomucoid of egg white containing a carbohydrate moiety.
5.	Nucleoproteins	Ribosomes and viruses contain nucleic acids.

6.	Metalloproteins	Alcohol dehydrogenase - a Zn containing enzyme.
7.	Mucoproteins	Follicle stimulating hormone, ovomucoid.

- **Derived Proteins:** These are not naturally occurring proteins and are obtained from simple proteins or conjugated proteins by the action of enzymes and chemical agents, heat, mechanical shaking, UV or X-rays. It includes the following types:
    - **Primary** e.g., myosin, fibrin.
    - **Secondary** e.g., peptones, peptides, proteoses etc.

3. Classification on the basis of biological functions

The involvement of proteins in different functions also makes it a basis for their classification.

Table 3: Summary of the different classes along with the functions performed and examples.

S. No.	Type	Function	Example
1.	Enzymes	Catalytic activity	Kinases, Dehydrogenases
2.	Storage proteins	Store amino acids	Myoglobin, ferritin
3.	Regulatory proteins	Coordinate body activities	Insulin, glucagons
4.	Structural proteins	Give support and structure	Keratin, collagen
5.	Defensive/Protective Proteins	Protect against diseases	Immunoglobulins, Antibodies
6.	Transport proteins	Facilitate import of nutrient into cells or Release of toxic products into surrounding medium	Haemoglobin
7.	Contractile and Mobile proteins	Participate in contractile processes e.g., movement of Muscles	Actin, myosin

Role of proteins

Proteins are among the fundamental molecules of biology. They are common to all life present on Earth today, and are responsible for most of the complex functions that make life possible. They are also the major structural constituent of living beings.

According to the Central Dogma of Molecular Biology (proposed by Francis Crick in 1958), information is transferred from DNA to RNA to proteins. DNA functions as a storage medium for the information necessary to synthesize proteins, and RNA is responsible for (among other things) the

translation of this information into protein molecules, as part of the ribosome.

All the complex chemical functions of the living cell are performed by protein-based catalysts called enzymes. Specifically, enzymes either make or break chemical bonds. Protein enzymes should not be confused with RNA-based enzymes (also called ribozymes), a group of macromolecules that perform functions similar to protein enzymes. Further, most of the scaffolding that holds cells and organelles together is made of proteins.

In addition to their catalytic functions, proteins can transmit and reduce signals from the extracellular environment, duplicate genetic information, help in transforming the energy in light and chemicals with extremely surprising efficiency, convert chemical energy into mechanical work, and carry molecules between cell compartments.

Qualitative test of proteins

1) **Biuret test:** The biuret test (Piotrowski's test) is a chemical test used for detecting the presence of peptide bonds. In this test an aqueous sample is treated with an equal volume of 1% strong base (Sod. and Pot. Hydroxide) followed by few drops of aqueous copper (II) Sulphate. If the solution turns purple, protein is present. The biuret reagent is made of Sodium hydroxide (NaOH) & hydrated copper (II) sulphate with potassium & sodium titrate.
2) **Xanthoproteic test:** It is an identification test of protein and it gives a positive result with those proteins with amino acid carrying aromatic group. When protein is treated with hot concentrated nitric acid, a yellow color substance is formed. The yellow color is due to xanthoproteic acid which is formed by the nitration of certain amino acids present in protein such as tyrosine and tryptophan.
3) **Ninhydrin test:** This is a test for amino acids and proteins with free –NH2 group. When such an –NH2 group reacts with Ninhydrin, an intense blue color complex is formed.
4) **Millon's test:** When egg albumin is treated with Millon's reagent, it first gives a white colored precipitate which then changes to brick red on boiling. Gelatin does not give this test.

Deficiency disease associated with proteins

- Marasmus is a disease caused by a severe deficiency of protein & calories.

- Kwashiorkor is a disease caused by a sever deficiency of protein in diets that contain mostly from carbohydrates such as yams, rice & bananas.
- Deficiency of protein C & protein S are inherited condition cause abnormal blood clotting.
- Cachexia is a condition that involves protein deficiency, depletion of skeletal muscle & an increased rate of protein degradation.

Biological value of proteins

Biological value (BV) is a measure of the proportion of absorbed protein from a food which becomes incorporated into the proteins of the organism's body. It captures how readily the digested protein can be used in protein synthesis in the cells of the organism. Proteins are the major source of nitrogen in food.

Proteins are composed of 21 biological amino acids. 9 of these are "essential amino acids", which means our bodies cannot produce them, and they must be derived from food sources. The essential amino acids are Phenylalanine (25 milligrams per kg of body weight), Leucine (39), Lysine (30), Valine (26), Threonine (15), Methionine (15), Isoleucine (20), Histidine (10), and Tryptophan (4). When we digest a food with protein, it breaks down into its amino acids, & each is used by the body for slightly different purposes.

When a protein contains the essential amino acids in a proportion similar to that required by the body, it has a high Biological Value. When one or more of the essential amino acids are missing or present in low numbers, the protein has a low biological value.

The biological value of a protein is a number from 100 down to 0 that describes how well it is absorbed by the body. More precisely, it is a measure of the percentage of the protein that is actually incorporated into the proteins of the human body.

Polypeptides

Polypeptides are chains of amino acids and essential portions of proteins in cells. A polypeptide is a single linear chain of many amino acids, held together by amide bonds. A protein consists of one or more polypeptides (more than about 50 amino acids long). An oligopeptide consists of only a few amino acids (between two and twenty).

Polypeptides help make up proteins by bonding numerous amino acids together. Proteins are created by the bonding of two or more polypeptides. These are then folded into a specific shape for that particular protein.

Chemistry: Their structure is similar to their particular amino acid groups, except that they are interconnected by covalent (Electron sharing bond) bonds. They can become very diverse and very complex as they build together to form proteins. At one end of the polypeptide is the carboxyl group that is attached to it and called the C-terminal. On the opposite end is the amino terminal, or N-terminal.

Amino acids

Amino acids are biologically important organic compounds containing amine ($-NH_2$) and carboxyl (-COOH) functional groups, along with a side-chain (R group) specific to each amino acid. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen, though other elements are found in the side-chains of certain amino acids. Amino acids play a critical role in processes such as neurotransmitter transport & biosynthesis.

Chemistry: All peptides and polypeptides are polymers of α-amino acids. There are 20 α-amino acids that are relevant to the make-up of mammalian proteins. Several other amino acids are found in the body free or in combined states (e.g., not associated with peptides or proteins). This non-protein associated amino acids perform specialized functions such as citrulline and ornithine in the disposal of waste nitrogen via the urea cycle.

Several of the amino acids found in proteins also serve functions distinct from the formation of peptides and proteins, E.g.- Tyrosine in the formation of thyroid hormones or glutamate acting as a neurotransmitter.

The α-amino acids in peptides and proteins (excluding proline) consist of a carboxylic acid (–COOH) and an amino ($–NH_2$) functional group attached to the same tetrahedral carbon atom. This carbon is the α-carbon. Distinct R-groups, that distinguish one amino acid from another, also are attached to the alpha-carbon (except in the case of glycine where the R-group is hydrogen). The fourth substitution on the tetrahedral α-carbon of amino acids is hydrogen.

Classification of amino acids based on chemical structure: The properties of each amino acid are dependent on its side chain (-R), the side chains are the functional groups that are responsible for the structure and function of proteins, as well as the electrical charge of the molecule. Amino acids with charged, polar, or hydrophilic side chains are usually exposed on

the surface of proteins. The nonpolar hydrophobic residues are usually enfolding in the hydrophobic interior or core of a protein and are out of contact with water. The 20 amino acids in proteins encoded by DNA and are classified according to their side-chain functional groups.

There are 9 essential amino acids and 11 nonessential amino acids.

Table 4: List of essential & non-essential amino acids.

Essential amino acids	Non-essential amino acids
Histidine	Alanine
Leucine	Asparagine
Isoleucine	Arginine
Lysine	Aspartic acid
Methionine	Glutamic acid
Phenylalanine	Cysteine
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Serine
	Tyrosine

Qualitative test

- **Ninhydrin test:** In the pH range of 4-8, all α- amino acids react with ninhydrin (oxidizing agent) to give a purple-colored product (diketohydrin). All primary amines and ammonia react similarly but without the liberation of carbon dioxide. The amino acids proline and hydroxyproline also react with ninhydrin, but they give a yellow-colored complex instead of a purple one. Besides amino acids, other complex structures such as peptides, peptones and proteins also react positively with ninhydrin.
- **Xanthoproteic acid test:** Aromatic amino acids, such as Phenyl alanine, tyrosine and tryptophan, respond to this test. In the presence of concentrated nitric acid, the aromatic phenyl ring is nitrated to give yellow colored nitro-derivatives. At alkaline pH, the color changes to orange due to the ionization of the phenolic group.
- **Pauly's diazo test:** This test is specific for the detection of Tryptophan or Histidine. The reagent used for this test contains sulphanilic acid dissolved in hydrochloric acid. Sulphanilic acid upon diazotization in the presence of sodium nitrite and hydrochloric acid results in the formation a diazonium salt. The

diazonium salt formed couples with either tyrosine or histidine in alkaline medium to give a red-colored chromogen (azo dye).

- **Millon's test:** Compounds containing hydroxyl benzene radical E.g.- Tyrosine react with Millon's reagent to form a red colored complex. Millon's reagent is a solution of mercuric sulphate in sulphuric acid.
- **Histidine test:** This reaction involves bromination of histidine in acid solution, followed by neutralization of the acid with excess of ammonia. Heating of alkaline solution develops a blue or violet coloration.
- **Hopkins Cole test:** This test is specific test for detecting tryptophan. The indole moiety of tryptophan reacts with Glyoxylic acid (oxo acetic acid) in the presence of concentrated sulphuric acid to give a purple-colored product. Glyoxylic acid (oxo acetic acid) is prepared from glacial acetic acid by being exposed to sunlight.
- **Sakaguchi test:** Under alkaline condition, α- naphthol (1-hydroxy naphthalene) reacts with a mono-substituted guanidine compound like arginine, which on treatment with hypobromite or hypochlorite produces a characteristic red color.
- **Lead sulphide test:** Sulphur containing amino acids, such as cysteine and cystine on boiling with sodium hydroxide (hot alkali), yield sodium sulphide. This reaction is due to partial conversion of the organic sulphur to inorganic sulphide, which can be detected by precipitating it to lead sulphide, using lead acetate solution.

Role of amino acids

Amino acids are small organic molecules that play several important roles in living organisms.

- An amino acid is a chemical unit that enables the cell to maintain their structure by providing them with all the necessary building material.
- These are also essential for the repair, growth & maintenance of the cells. Amino acids are a source of energy, like proteins, they can provide about 4 calories per gram.
- In the body some amino acids behave like precursors for the neurotransmitters or amino acid-based hormones, Tyrosine is a precursor for dopamine, epinephrine, norepinephrine, thyroxine etc.

- They serve as precursors for many biologically active molecules, such as neurotransmitters (e.g., dopamine, serotonin, GABA, epinephrine), local mediators (e.g., the allergy mediator histamine), energy-related metabolites (e.g., creatine, citrulline, carnitine), the oxygen-binding molecule ‘heme ‘, and DNA bases called purines.
- They serve as an energy source during prolonged fasting, diabetes, and when the diet is rich in proteins.
- Some act as regulators of gene expression and cellular signaling. This multiple the physiological processes that are related to growth, maintenance, reproduction and immunity.

Deficiency disease

Possible symptoms of amino acid deficiencies and imbalances are:

- Low energy levels (even chronic fatigue)
- Depression
- Anxiety
- Memory and concentration problems
- Low thyroid function (which affects everything!)
- Allergic symptoms
- Digestive symptoms
- Inability to detoxify properly
- Loss of muscle mass
- Increased body fat

Chapter - 3

Carbohydrate

A carbohydrate is a biological molecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen-oxygen atom ratio of 2:1 (as in water), In other words, with the empirical formula $Cm(H_2O)n$ (where m could be different from n). Some exceptions exist, for example, deoxyribose, a sugar component of DNA has the empirical formula $C_5H_{10}O_4$. Carbohydrates are hydrates of Carbon, structurally they are also known as polyhydroxy aldehydes and ketones.

Foods containing carbohydrates come mainly from plants and are a good source of energy for the body. There are three main groups of carbohydrates:

1. **Monosaccharide or single sugar:** There are many single sugars or monosaccharide. The most common single sugar are the sugars with six carbon atoms - glucose, fructose, and galactose.

    - Glucose is found in all animals, at least in small quantities.
    - Fructose is found in plants, but rare in animals.
    - Galactose is found in milk sugar from mammals.

    Glucose, fructose, and galactose have the same general formula $C_6H_{12}O_6$, but their atoms are arranged differently within the molecules. These different arrangements give the sugars different chemical and biological properties.

2. **Disaccharides or double sugars:** Double sugars are so called because each molecule of a double sugar is made up of two molecules of single sugars bonded together. Double sugars are also called disaccharides. Disaccharides have the general formula $C_{12}H_{22}O_{11}$. However, their atoms have different arrangements within the molecules.

    Common disaccharides are:

    - Maltose
    - Sucrose
    - Lactose

3. **Polysaccharides or complex carbohydrates:** Polysaccharides consist of many monosaccharide molecules joined together. Starch, glycogen, and cellulose are complex carbohydrates. Plants store glucose in the form of starch. In animals and fungi, the main store of glucose is glycogen. Cellulose forms the main parts of the cell walls of plants. Starch, glycogen, and cellulose are each made up of numerous glucose molecules condensed together, but the glucose molecules are linked together and arranged in different ways. Hence, starch, glycogen, and cellulose have different properties.

Functions of carbohydrates

- As a substrate for respiration, to provide energy for cell activities.
- To form supporting structures, For example-cell walls in plants.
- To be converted into other organic compounds such as amino acids and fats.
- For the formation of nucleic acids, For example-DNA.
- To synthesize lubricants, for example, mucus which consists of a carbohydrate and a protein.
- To synthesize the nectar in some flowers. Nectar is a sweet liquid that plants produce to attract insects.

Qualitative test

1. **Molisch's test:** This is a common test for all carbohydrates larger than tetroses. In a test tube, add 2 ml of the test carbohydrate solution and 2 drops of α-naphthol solution. Carefully incline the tube and pour drop-wise conc. H_2SO_4, using a dropper, along the sides of the tube. Observe the violet color at the junction of the two liquids.
2. **Fehling's test:** This forms the reduction test of carbohydrates. Fehling's solution contains blue alkaline cupric hydroxide solution, heated with reducing sugars gets reduced to yellow or red cuprous oxide and is precipitated. In a test tube, add 2 ml of the test carbohydrate solution and add equal volumes of Fehling A & Fehling B and place it in a boiling water bath for few minutes, mix them together and observe any change in color or precipitate formation. The production of yellow 'or brownish-red precipitate of cuprous oxide indicates the presence of reducing sugars in the given sample.

3. **Benedict's test:** In the test tube with 2 ml of Benedict's reagent, add 5-6 drops of carbohydrate solution and mix well. Place the test tube in a boiling water bath for 5 minutes and observe any change in color or precipitate formation. Cool the solution. Observe the color change from blue to green, yellow, orange or red depending upon the amount of reducing sugar present in the test sample.

4. **Barford's test:** Barford's test is used to detect the presence of monosaccharide (reducing) sugars in solution. Barford's reagent is a mixture of ethanoic (acetic) acid and copper (II) acetate. To 2 mL of the test solution add about 2-3 mL of Barford's reagent. Mix it well and boil it for one minute in the water bath and allow standing for a few minutes. Formation of a red precipitate of cuprous oxide in the bottom and along the sides of the test tube immediately caused. Since Barford's reagent is slightly acidic, this test is specific for monosaccharides.

5. **Seliwanoff's test:** It is a color reaction specific for ketoses. To 2 mL of Seliwanoff's reagent, add two drops of test solution. The mixture is heated to just boiling. A cherry red condensation product will be observed indicating the presence of ketoses in the test sample. There will be no significant change in color produced for aldose sugar.

6. **Bial's test:** Bial's test is used to distinguish between pentoses and hexoses. To 5 mL of Bial's reagent add 2-3 mL of test solution and warm gently in a hot water bath for 2 minutes. The formation of a bluish green product is indicative of pentoses. Hexoses generally react to form muddy brown products.

7. **Iodine test:** This test is used for the detection of starch in the solution. Add 2 drops of iodine solution to about 2 mL of the carbohydrate containing test solution. A blue-black color is observed which is indicative of presence of polysaccharides.

8. **Osazone test:** To 0.5 g of phenyl hydrazine hydrochloride add 0.1 gram of sodium acetate and 10 drops of glacial acetic acid. Add 5 mL of test solution to this mixture and heat under boiling water bath for about half an hour. Cool the solution slowly and examine the crystals under a microscope. Needle-shaped yellow osazone crystals will be observed for glucose and fructose, whereas lactosazone shows mushroom shaped and maltose produces flower-shaped crystals.

Deficiency disease of carbohydrate metabolism

1. **Diabetes mellitus (DM):** It is a group of metabolic disease in which there are high blood sugar levels over a prolonged period.

   Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. There are **two main types** of diabetes mellitus:

   a) **Type 1 DM** results from the pancreas's failure to produce enough insulin. This form was previously referred to as **"Insulin-Dependent Diabetes Mellitus" (IDDM) or "Juvenile Diabetes"**. The cause is unknown.

   b) **Type 2 DM** begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. This form was previously referred to as **"Non-Insulin Dependent Diabetes Mellitus" (NIDDM) Or "Adult-Onset Diabetes"**. The most common cause is excessive body weight and not enough exercise.

      The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 DM, while they usually develop much more slowly and may be subtle or absent in type 2 DM.

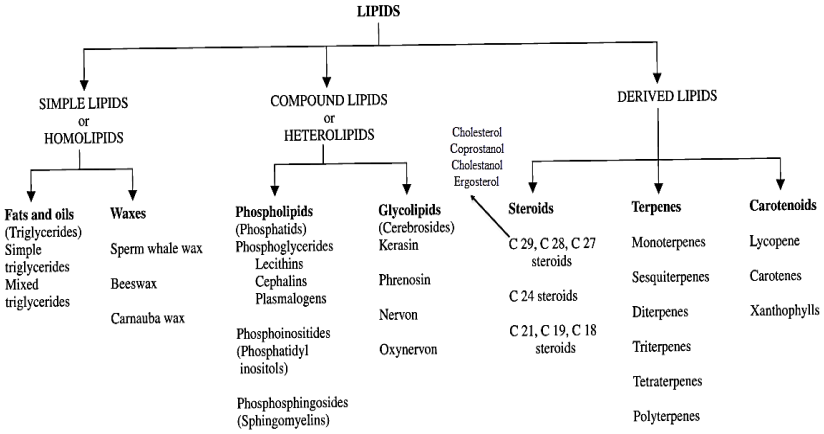
2. **Glycosuria:** When glucose is excreted in urine, the condition is called as glycosuria. Glycosuria occurs due to the elevated blood glucose level.
3. **Galactosemia:** Galactosemia caused due to the deficiency of enzyme galactose-1-phosphate uridyltransferase & galactokinase. Galactose cannot be converted into glucose, which leads to a condition called a galactosemia.
4. **Glycogen storage disease** (**GSD**, also **glycogenosis** and **dextrinosis**)**:** It is the result of defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types.
5. **Fructose-intolerance:** One of the very normal hexose sugars of fruits (i.e., fructose) gets normally metabolized to give CO_2 & Energy but defective metabolism of fructose develops in high concentration of this sugar in blood, disorder known as fructose-intolerance.

Chapter - 4

Lipids

Lipids are defined as organic molecules that are poorly soluble in water but dissolve well in organic (non-polar) solvents such as chloroform. It includes fatty acids and their esterified forms (phospholipids, triglyceride) and sterols and their esters. Fatty acids are carboxylic acids. Their detergent nature is exemplified by soap, which is made from triglyceride saponified by caustic soda. Free or un-esterified forms are therefore generally not found in significant concentrations in the body. In plasma, fatty acids are bound to albumin. Biologically important fatty acids are 16-22 carbons long. Their carbon-carbon bonds may be saturated, mono-unsaturated or polyunsaturated.

Lipoproteins are associations of proteins and lipids that undergo changes in their passage through the circulation. The slightly polar lipids and non-polar lipids associate into spherical structures that are suspended in the plasma and whose fate is dependent on the associated proteins.



Flow chart of classification of lipids

Classification

1. **Simple lipids**

   - **Waxes**

     Structure: Esters of long-chain fatty acids and long-chain alcohols.

     Function: Coatings, protection against environment.

     Example: Carnauba wax (palm leaves): **CH_3 $(CH_2)_{30}$-COO-$(CH_2)_{33}CH_3$.**

   - **Fatty acids and Triacyl glycerol (Fats and Oils)**

     Structure of fats & oils: Glycerol backbone esterified with three fatty acids.

     Function: Fatty acid storage, long-term source of energy, layer of insulation.

2. **Compound's lipids**

   - **Phospholipids:** The term phospholipid is used to describe any Lipid containing phosphorus. They contain phosphoric acid, fatty acid, alcohol & generally a nitrogenous base.
   - **Glycolipids:** Combination of Carbohydrate & lipid are glycolipids. Basically, they are amphipathic carbohydrate glyceride derivative but do not contain phosphate.

     Example: Cerebrosides, Gangliosides.
   - **Lipoproteins:** Biomolecule containing lipids & proteins found in mitochondria, endoplasmic reticulum & nuclei. Lipid part consists of triacylglycerol, phospholipids & cholesterol.
   - **Lipopolysaccharides:** These are found in gram negative micro-organisms which are macro- polymer, very complex in structure, when these are released to blood stream produces untoward reaction & are toxics. They are also known as endotoxins.

3. **Derived lipids**

This class of Lipids consists of fatty acids, steroids, fat soluble vitamins like Vit. A & Vit. D, eicosanoids like prostaglandins, ketone bodies, glycerol & other alcohols.

   - **Steroids:** These are chemical compounds which contain a steroid nucleus called as cyclopentanoperhydrophenanthrene. It consists of phenanthrene ring fused with a cyclopentane ring.

Example: Cholesterol, Bile acids, Vitamin D, Sex hormones etc.

Chemistry

Fatty acids: Some key points about fatty acid structure & properties:

- The number of C in the chain is always **even -** biosynthesis by condensation of decarboxylated malonyl esters adds 2 C pieces to growing chain.
- **Saturated** fatty acids of 12 - 20 C are common.
- Unsaturated fatty acids in nature are always **cis (Z) isomers**.
- As the number of double bonds increases (polyunsaturated) melting points decrease

Types

1. Saturated fatty acids

- Palmitic acid **$CH_3(CH2)_{14}COOH$**
- Stearic acid **$CH_3(CH_2)_{16}COOH$**

2. Unsaturated fatty acids

Table 5: List of unsaturated fatty acids.

Name of fatty acid	No. of carbon atoms	No. of double bonds	Position of double bonds
Palmitoleic acid	16	1	9
Linoleic acid	18	2	9, 12
Linolenic acid	18	3	9, 12, 15
Arachidonic acid	20	4	5, 8, 11, 14

Qualitative test

1) **Solubility:** Solubility of the lipids in organic solvents depends on length of hydrocarbon chain of fatty acid attached to glycerol. Lipids are soluble in solvents like chloroform, ether, alcohol, hexane etc.
2) **Formation of translucent spot-on paper:** Ordinary writing paper becomes semitransparent when a drop of oil is applied to paper.
3) **Formation of acrolein:** Glycerol from fat gets dehydrated with the help of potassium bisulphate and acrylic aldehyde or acrolein is produced.
4) **Emulsification:** When oil or lipid fat is shaken with water, it is finely divided & is dispersed in the water to form emulsion. Shake a

drop of oil with little water in a test tube. The oil becomes finely divided forming an emulsion.

5) **Iodine absorption test:** This test is for unsaturated fatty acids. A drop of iodine is added to fat solution (fat solution is prepared in chloroform) and shaken. The solution will decolorize if unsaturated fatty acid is present.

Disease related to lipids

- **Obesity:** It is a disorder which is caused due to the accumulation of body fat.
- **Lipidosis:** It indicates the abnormal lipoproteins in blood or specific blood in tissues.
- **Hyperlipidemia:** It is a condition in which plasma cholesterol or plasma triglyceride level is increased. This condition occurs due to inherent genetic defect.
- **Hyperlipoproteinemia:** An ailment in which cholesterol or triglycerides containing lipoprotein are in high concentration in the blood.
- Excessive excretion of fat in the faeces is known as steatorrhea but it is either maldigestion of fats due to inadequate secretion of pancreatic lipase or bile salt or even may be defective absorption due to intestinal disease.

Chapter - 5

Vitamin

A vitamin is an organic compound and a vital nutrient that an organism requires in limited amounts. An organic chemical compound (or related set of compounds) is called a vitamin when the organism cannot synthesize the compound in sufficient quantities, and it must be obtained through the diet. They perform different function in the body. They act as redox agent, as Coenzyme or Isoenzyme in the metabolic processes. The two organizations that create guidelines for vitamin intake are by the Food and Nutrition Board of the National Academy of Sciences and the Food and Drug Administration (FDA).

Classification

On the basis of solubility, vitamins are generally classified as water soluble vitamins and fat-soluble vitamins.

1. Fat soluble vitamins

Vitamins that are soluble in only fat or lipids and are absorbed into the body through the intestinal tract or more specifically the small intestines.

Some fat-soluble vitamins are Vitamins A, D, E, and K. These vitamins are stored in the liver and adipose tissues. Fat-soluble vitamins except for vitamin K, are stored for long periods of time and then excreted after this long duration of time. For this reason, overdosing on fat-soluble vitamins is highly feasible if ingested at high, toxic levels and it could possibly lead to hypervitaminosis.

- **Vitamin A:** It is used for growing healthy new cells like skin, bones, and hair. Also, is used for surface lining upkeep of the eyes, urinary tract, intestinal tract, and respiratory system. Vitamin A helps vision by keeping cells which are used for transduction of light into nerve signals healthy. Vitamin A also performs other major functions in the body. It is required for reproductive functions such as normal growth and development of sperm and ovaries. The main sources of Vitamin A are egg yolk, whole milk, and butter. In plants Vit. A is found in the precursor form, as provitamin A. All

the provitamin A belongs to the family of pigmented hydrocarbons are called carotenoids. It is also known as Retinol.

- **Vitamin D:** Ergocalciferol (Vit. D2) & cholecalciferol (Vit. D3) are precursors of active form of vit. D, in plants and animals respectively. It is needed for the body to properly use calcium and phosphorous. It is also used in the formation of some RNA, maintain a normal heart, and keep a stable nervous system and blood clotting. Along with absorbing calcium, Vitamin D can also help regulate the amount of calcium and phosphorus that is present in the blood. Vitamin D can be found in dairy products, fish.

  Vitamin D deficiency caused severe growth retardation. The lack of calcium in the bones resulted in deformities of the skeleton. Vitamin D deficiency is also associated with a low-normal blood calcium, low or low-normal fasting blood phosphorus, and elevated parathyroid hormone (PTH) levels that cause a mineralization defect in the skeleton.

- **Vitamin E:** It refers to a group of compounds that include both tocopherols and tocotrienols. Of the many different forms of vitamin E, γ-tocopherol is the most commonly found. γ-Tocopherol can be found in corn oil, soybean oil etc. α-tocopherol, the most biologically active form of vitamin E, is the second-most common form of vitamin E in the diet. It is an antioxidant that helps the body get rid of free radicals to keep tissues healthy. It is also used in the creation of red blood cells. The use of vitamin A, C and K are assisted by Vitamin E. Although the role of Vitamin E is not completely understood but it is clear that it performs antioxidant properties in the body. They get rid of the free radicals in the body by preventing the oxidation of lipid-based cell membranes. Free radicals are very reactive and can steal electrons from membranes which could ultimately damage DNA. Good sources of Vitamin E are almonds, spinach, wheat.

- **Vitamin K:** It is required for normal blood coagulation. It is needed for the formation of prothrombin. Vitamin K1 (phylloquinone) is found in plants, while vitamin K2 (menaquinone) is formed by intestinal bacteria in animals.

  It helps in creating proteins in the body like those that create blood clots. It also allows for calcium regulation within the body. Vitamin K has ability to help the clotting of blood is important for healing.

The clotting ability could help in slowing or stopping bleeding in injured patients. During surgery, Vitamin K is often given to patients to reduce bleeding. Sources of Vitamin K include spinach, Brussels sprouts, asparagus, and broccoli.

2. Water soluble vitamins

Water Soluble vitamins are those that easily dissolvable in water and easily excreted out of the body via urine. Some water-soluble vitamins are Vitamins B complex (B1, B2, B3, B6, B7, B12) and Vitamin C.

- **Vitamin B:** They are essential for creating dopamine, epinephrine, serotonin, and myelin. They also help the hemoglobin hold oxygen and lower cholesterol. Vitamin B is essential to good health. It is also used for energy production in the human cells. B vitamins help convert food often consumed as carbohydrates into fuel. They also help the nervous system function properly. Good sources of Vitamin B are bananas, potatoes, whole grains, and chili peppers.
- **Vitamin C:** It helps to regulate the immune system and relieve pain caused by tired muscles. It is also needed in the manufacture of collagen and norepinephrine. Vitamin C is also an antioxidant which can enhance the immune system by stimulating white blood cells in the body. Vitamin C also helps to benefit the skin, teeth, and bones. Vitamin C is often in citrus fruits such as papayas, oranges, and lemons.
- **Vitamin B complex**
  - **Vitamin B1:** Thiamin is another name for vitamin B1. It helps to convert blood sugar into energy for your body. It also helps the mucous membranes of the muscular, cardiovascular, and nervous systems in good shape. Some good sources of Vitamin B1 are whole grain cereals, pork, navy beans, and wheat germs.
  - **Vitamin B2:** Riboflavin is another name for vitamin B2. There are two coenzymes of Vit. B2, Flavin mononucleotide (FMN) & Flavin adenine dinucleotide (FAD). FMN & FAD are prosthetic groups of oxidation reduction enzymes.

    It works with the other vitamin B complexes to process the carbohydrates, proteins, and fats into calories for energy in body. The body also needs this for healthy skin, good vision, growth, and red blood cell creation. Some good sources of Vitamin B2 are Dairy, red meats, and leafy green vegetables.

- **Vitamin B3:** Niacin is another name for vitamin B3. It is required for the formation of NAD^+ & $NADP^+$ and also required for the biological redox reactions.

  It also works with other Vitamin B complexes to process the carbohydrates, proteins, and fats into calories for energy in the body. The difference is that it helps the digestive systems functions along with promoting a healthy appetite and healthy nerves. Some good sources of Vitamin B3 are yeasts, meat, and peanuts.

- **Vitamin B5:** Pantothenic acid is another name for vitamin B5. It is required for the formation of coenzymes A. Coenzyme A is required for almost all acetate & fatty acid metabolism in the body. Like B3 and B2 it helps break down carbohydrates, proteins, and fats for energy. Some good sources of Vitamin B5 are from meats, peas, and whole grain cereals.

- **Vitamin B6:** Pyridoxine is another name for Vitamin B6. It includes pyridoxal & pyridoxamine also. The active form of this vitamin is pyridoxal phosphate.

  Vitamin B6 working along with B12 and B9 helps prevent heart attacks. It also helps the body process proteins, carbohydrates, and fats into energy. Some good sources of Vitamin B6 are from meats, eggs, soybeans, whole grains, and nuts.

- **Vitamin B7:** Vitamin H or Biotin is other names for Vitamin B7. Vitamin B7 helps the formation of fatty acids and glucose to be used as fuel for the body. Some Good sources of Vitamin B7 are from bananas, yeast, cereal, and liver.

- **Vitamin B9:** Folic Acid is another name for vitamin B9. It is made from a pteridine ring, glutamic acid & p-amino benzoic acid (PABA) in most bacteria. The active form of folic acid is tetrahydrofolate (THF). This act as a coenzyme for the transferase enzyme.

  It is very important during pregnancy since it is used for making and maintaining new cells. Vitamin B9 prevents anemia by keeping up the production of red blood cells and prevent low birth weight and prematurity in births. Some good sources of Vitamin B9 are from mushrooms, leafy greens, peas, and broccoli.

- **Vitamin B12:** Vitamin B12 is also known as cyanocobalamin. It works with Vitamin B9 in keeping red blood cells healthy and also helps to keep the central nervous system healthy. Some good sources of Vitamin B12 are meat, eggs, and dairy.

Disease related to vitamins

Based on their role in biological processes and their affect different vitamins have different functions, their function can be best understood by knowing about their deficiency diseases.

Table 6: Given below is the list of vitamins and their deficiency diseases.

S. No.	Vitamin	Deficiency disease
1.	Vitamin A	Hardening of cornea in eye, night blindness.
2.	Vitamin B1	Beriberi, dwarfism.
3.	Vitamin B2	Digestive system, skin burning sensations, cheilosis.
4.	Vitamin B6	Convulsions, conjunctivitis, and sometimes neurological disorders.
5.	Vitamin B12	Pernicious anemia and decrease in red blood cells in hemoglobin.
6.	Vitamin C	Bleeding in gums and scurvy.
7.	Vitamin D	Improper growth of bones, soft bones in kids, rickets.
8.	Vitamin E	Weakness in muscles and increases the fragility of red blood cells.
9.	Vitamin K	Increases the time taken by blood to clot. Severe deficiency may cause death due to excessive blood loss in case of a cut or an injury.

Coenzymes

Cofactors or Coenzymes are small molecules or compounds that bind to enzymes and allow the enzymes to function. Cofactors are not proteins or amino acids. Enzymes catalyze so many types of reactions. However, most of the enzymes require the presence of cofactors in order to be active. The cofactor's role and mechanism vary with different enzymes.

The non-protein, low molecular weight and organic substances associated with functions of enzymes are called as coenzymes. Vitamins are necessary for the cell growth. They are needed for the action of certain enzymes. Mostly water-soluble vitamins are converted to their active form in the body. These active forms of vitamins are coenzymes.

The cenzymes are derived from vitamins.

Table 7: The coenzymes derived from vitamins are listed in table.

S. No.	Vitamins	Active form or Co-enzymes
1.	Thiamine	Thiamine pyrophosphate
2.	Lipoic acid	Lipoic acid
3.	Riboflavin	FMN & FAD
4.	Nicotinic acid	NAD & NADP
5.	Pantothenic acid	Co-enzyme A
6.	Pyridoxine	Pyridoxal Phosphate Pyridoxine phosphate Pyridoxamine phosphate
7.	Biotin	Biotin
8.	Folic acid	Tetra hydrofolate THF
9.	Cyanocobalamin	Deoxyadenosine cobalamins
10.	Ascorbic acid	Ascorbic acid

Chapter - 6

Role of Minerals and Water in Life Process

Role of mineral in life process

Minerals are inorganic substances that are found in soil and rocks. They are essential nutrients that the body needs to survive and carry out daily functions and processes. You receive minerals by eating plants that absorb them from the earth and by eating meat from animals, which graze on plants. Minerals keep you healthy and have key roles in several body functions. You require these important nutrients from your daily diet.

1) **Bone and teeth health:** Your skeleton provides motility, protection and support for the body. It also stores minerals and other nutrients. Though they appear hard and unyielding, your bones are actually constantly being reabsorbed and reformed by your body. Several minerals make up the lattice architecture of your bones. Calcium is the most abundant mineral in your body and is found in your bones and blood. Along with the mineral's phosphorus and magnesium, calcium gives your bones strength and density. This mineral also builds and maintains strong, healthy teeth. BBC Health reports that a calcium deficiency due to poor nutrition or illness can lead to osteoporosis, a condition in which the bones become brittle and less dense, increasing the risk of fractures. Kids Health notes that foods that are rich in calcium include milk and other dairy products, green, leafy vegetables and canned fish with bones.

2) **Energy production:** You require oxygen to produce energy that is necessary for every bodily function and process. Red blood cells or Erythrocytes carry oxygen to each of your infinite cells, where it is used to generate energy. Red blood cells contain a heme or iron component that binds to oxygen so that it can be transported. The University of Maryland Medical Center notes that without the iron molecules, oxygen could not be attached to the blood cells and the body would not be able to produce the energy necessary for life. Iron is an essential mineral, and failing to get enough from your diet can lead to a condition called anemia, which causes weakness and

fatigue. This mineral is primarily found in the blood, and it is also stored in your liver, spleen, bone marrow and muscles.

3) **Nerve and muscle function:** Potassium is found in bananas, dates, tomatoes, green leafy vegetables, citrus fruits and legumes such as peas and lentils. According to Kids Health, this nutrient is important to keep muscles and the nervous system functioning normally. Potassium helps to maintain the correct water balance in the cells of your nerves and muscles. Without this essential mineral, your nerves could not generate an impulse to signal your body to move, and the muscles in your heart, organs and body would not be able to contract and flex.

4) **Immune health:** Some minerals such as calcium are needed in large quantities, while others such as zinc are only needed in trace amounts. Zinc is an essential mineral that is important for keeping your immune system strong and helps your body fight infections, heal wounds and repair cells. The mineral selenium is also needed in small amounts for immune health. A deficiency of selenium has been linked to an increased risk of heart disease and even some types of cancers.

Role of water in the body

Water is one of very few vital needs for human beings. An adult person should drink at least 1.5 liters of water per day. This level of water intake balances water loss and helps keeping the body properly hydrated. The water you consume through food and drinks follows a very precise route to arrive in your cells, of which it is a vital constituent. After passing through the stomach, water enters the small intestine, where it is largely absorbed in the first sections, the duodenum and jejunum. The rest passes into the colon. It crosses the intestinal mucous membrane into the bloodstream, then into the interstitial tissues that make up the framework of every organ, to arrive in the cells. Blood brings nutritional elements to cells (minerals, vitamins, protein components, lipids and carbohydrates). Waste products are then removing through urines. Water plays also an essential function in helping the regulation of temperature.

Human body is made up largely of water. It serves vital functions:

1) **Cell life:** Water is essential for cells to function properly. It enters into the composition of the cells.

2) **Chemical and metabolic reactions:** By enabling hydrolysis reactions, water participates in the biochemical breakdown of what we eat (proteins, lipids and carbohydrates). This is one of many reactions in which water is involved.
3) **Transport of nutrients and removal of waste:** Water as a main constituent of blood contributes to the transport of nutrients to the cells. Water, as a carrier, also helps removing waste products through urines.
4) **Body temperature regulation:** Water has a large heat capacity which helps limit changes in body temperature in a warm or a cold environment. Water enables the body to release heat when ambient temperature is higher than body temperature. We begin to sweat, and the evaporation of water from the skin surface cools the body very efficiently.

Water is the heart of life. This is why a human being can survive no longer than few days without water. Drinking water every day (approximately 1.5 liters), and at regular intervals, 8 times a day (before, during and in-between meals), without waiting until you're thirsty, is important as part of a healthy lifestyle, at every stage of life.

Chapter - 7

Enzymes

Enzymes are protein molecules in cells which work as catalysts. Enzymes speed up chemical reactions in the body, but do not get used up in the process.

Almost all biochemical reactions in living things need enzymes. With an enzyme, reactions work very much faster than they would without.

The substances at the start of the reaction are called substrates. The substances at the end of the reaction are the products. Enzymes work on the substrates, and turn them into products.

Anselme Payen found the first enzyme in 1833.

Enzymes are large molecules made from many amino acids. The amino acids link together in a long chain, which is folded up into a complex structure. Enzymes have a part which holds the substrate: a "claw, cleft, hollow or knob to grasp, hold, stretch and bend the molecule it acts on, the substrate".

There are thousands of different enzymes. Enzymes have names which show what they do. Enzyme names usually end in –ase to show that they are enzymes. Examples of this include ATP synthase.

Allosteric enzyme: These are regulatory enzymes. The catalytic activity regulates by itself. The regulation is mediated by specific metabolites. These metabolites are called as allosteric modulators. Allosteric enzymes have allosteric sites, in addition to active site, to which the allosteric modulators bind.

For a given allosteric enzyme, there are two types of allosteric modulators. Modulators are called as positive allosteric modulator & Negative allosteric modulators. If they increase the activity of an enzyme they are known as **Positive Modulator** & if they reduce the activity of an enzyme they are known as **Negative Modulators**.

Co-factors: Cofactors are derived from the vitamins and they are also known as coenzymes. They are low molecular weight organic substances.

Classification

Enzymes have been classified by the **International Union of Biochemistry**. Their Commission on Enzymes has grouped all known enzymes into six classes:

- **Oxido-reductases:** Catalyze transfer of electrons.
- **Transferases:** Transfer functional group from one molecule to another.
- **Hydrolases:** Add –OH (Hydroxyl) group.
- **Lyases:** Split chemical bonds and often add double bond or ring structure.
- **Isomerases:** A –> B where B is an isomer of A.
- **Ligases:** Join two large molecules: Ab + C –> A–C + b.

Factor affecting enzymes

- **Temperature:** As temperature increases, initially the rate of reaction will increase, because of increased Kinetic Energy. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease. The temperature at which the maximum rate of reaction occurs is called the enzyme's Optimum Temperature. This is different for different enzymes. Most enzymes in the human body have an Optimum Temperature of around 37.0 °C.
- **pH:** As the pH increases, initially the rate of the reaction also increases, it reaches at the optimum pH then the rate of reaction begins to decreases as the pH increases.
- **Concentration:** Changing the Enzyme and Substrate concentrations affect the rate of reaction of an enzyme-catalyzed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so it’s Metabolism. Changing the concentration of a substance only affects the rate of reaction if it is the limiting factor: that is, it the factor that is stopping a reaction from proceeding at a higher rate. If it is the limiting factor, increasing concentration will increase the rate of reaction up to a point, after which any increase will not affect the rate of reaction. This is because it will no longer be the limiting factor and another factor will be limiting the maximum rate of reaction. As a reaction proceeds, the rate of reaction will decrease, since the Substrate will get used up.

- **Substrate concentration:** Increasing Substrate Concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed. However, after a certain concentration, any increase will have no effect on the rate of reaction, since Substrate Concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.
- **Enzyme concentration:** Increasing Enzyme Concentration will increase the rate of reaction, as more enzymes will be colliding with substrate molecules. However, this too will only have an effect up to a certain concentration, where the Enzyme Concentration is no longer the limiting factor.

Mechanism of action

The confirmation of the active site may be explained by two methods:

1) Lock & Key model.
2) Induced Fit theory.

The process of enzyme action is as follows:

- The substrate contacts the active site.
- The enzyme-substrate complex is formed.
- The substrate molecule is altered (atoms are rearranged, or the substrate is broken into smaller parts, or the substrate is combined with another molecule).
- Product (s) is/are released from the active site.
- The enzyme is unchanged and can catalyse a new reaction.

Therapeutic and pharmaceutical importance of enzymes

1) **Medicinal significance of enzyme:** A study of the mode of the action of enzymes, explain the action of certain drugs, which are enzyme inhibitors. These drugs belong to the class of antimetabolites.

   Many drugs mediate their action by inhibiting certain enzymes.

   - Sulphanilamide selectively kills pathogenic organism by inhibiting folic acid synthetase enzyme.
   - Allopurinol is used in the treatment of gout, which prevents the

formation of uric acid. Allopurinol is a competitive inhibitor of Xanthin Oxidase enzyme. This enzyme is needed for the formation of xanthine & hypoxanthine to urea.

2) **Enzyme therapy:** It has been found that enzymes can be used in the treatment of disease. For e.g.: Asparagine used in the treatment of tumors. Galactosidase can be used for the treatment of lactose intolerance in children.

3) **Manufacturing of bulk drugs:** Some enzymes are used in bulk drug manufacturing. For e.g.: Penicillin Acylase is used for the production of 6-amino penicillanic acid from penicillin G. Amino penicillanic acid is needed for almost all semisynthetic Beta-lactum antibiotic. Papain, Pepsin and Tyrosine are used as digestant. Hyaluronidase is used in orthopedic practice. Urokinase in cardiac disease, Streptokinase in the treatment of thrombosis.

4) **Diagnostic uses of enzyme:** The enzyme pattern is different for different tissues in higher animals. E.g. aspartate aminotransferase and lactate dehydrogenase are seen in cardiac and hepatic tissues, while its proportion is very less in others. Such enzymes are called marker enzymes. Creatin-kinase is seen in skeletal or cardiac muscles.

Marker enzymes	Diagnostic uses
Lactate dehydrogenase	Myocardial infarction
Aspartate aminotransferase	Hepatitis

Chapter - 8

Metabolism

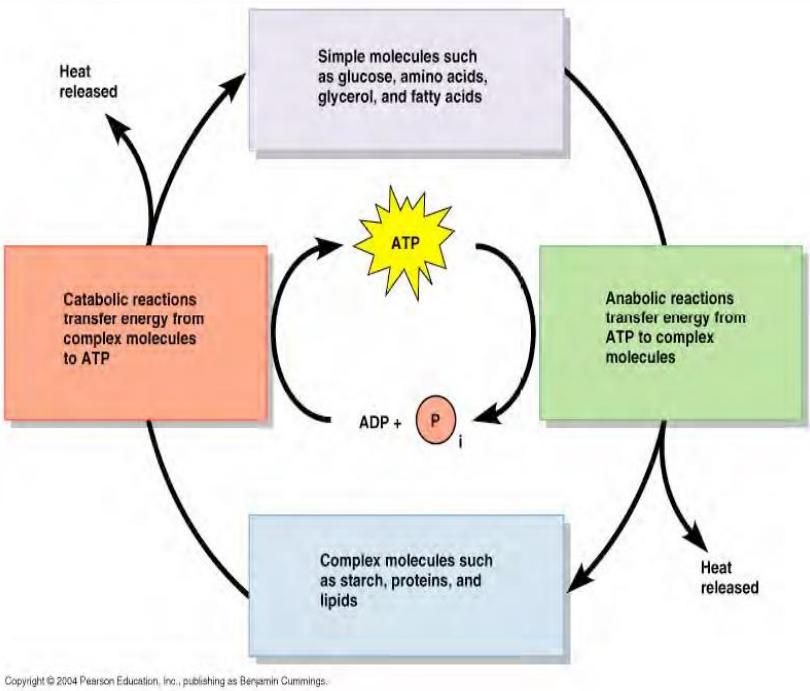
Metabolism is chemical transformations within the cells of living organisms. The three main purposes of metabolism are

- The conversion of food/fuel to energy to run cellular processes.
- The conversion of food/fuel to building blocks for proteins, lipids, nucleic acids.
- Some carbohydrates and the elimination of nitrogenous wastes.

These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures and respond to their environments. The word metabolism can also refer to the sum of all chemical reactions that occur in living organisms, including digestion and the transport of substances into and between different cells, in which case the set of reactions within the cells is called intermediary metabolism or intermediate metabolism.

Metabolism is usually divided into two categories

1) **Catabolism,** the breaking down of organic matter, for example, by cellular respiration.
2) **Anabolism,** the building up of components of cells such as proteins and nucleic acids. Usually, breaking down releases energy and building up consumes energy.



Carbohydrate metabolism

It denotes the various biochemical processes responsible for the formation, breakdown and inter-conversion of carbohydrates in living organisms.

The most important carbohydrate is glucose, a simple sugar (monosaccharide) that is metabolized by nearly all known organisms. Carbohydrates can be chemically divided into two types: complex and simple.

Simple carbohydrates consist of single or double sugar units (monosaccharides and disaccharides, respectively). Sucrose or table sugar (a disaccharide) is a common example of a simple carbohydrate.

Complex carbohydrates contain three or more sugar units linked in a chain, with most containing hundreds to thousands of sugar units. They are digested by enzymes to release the simple sugars. Starch, for example, is a polymer of glucose units and is typically broken down to glucose. Cellulose is also a polymer of glucose but it cannot be digested by most organisms.

There is a list of cycles involve in the metabolism of Carbohydrate:

1) **Glycolysis:** The oxidation metabolism of glucose molecules to obtain ATP and pyruvate.
2) **Citric acid cycle:** Pyruvate from glycolysis enters the Krebs cycle, also known as the citric acid cycle, in aerobic organisms after moving through pyruvate dehydrogenase complex.
3) **Pentose phosphate pathway:** Which acts in the conversion of hexoses into pentoses and in NADPH regeneration. NADPH is an essential antioxidant in cells which prevents oxidative damage and acts as precursor for production of many biomolecules.
4) **Glycogenesis:** The conversion of excess glucose into glycogen as a cellular storage mechanism; this prevents excessive osmotic pressure buildup inside the cell.
5) **Glycogenolysis:** The breakdown of glycogen into glucose, which provides glucose supply for glucose-dependent tissues.
6) **Gluconeogenesis:** De-Novo synthesis of glucose molecules from simple organic compounds. An example in humans is the conversion of a few amino acids in cellular protein to glucose.

Metabolic use of glucose is highly important as an energy source for muscle cells and in the brain, and red blood cells.

Normal metabolism of carbohydrate

Glycolysis

It is also known as Embden-Myerhoff Pathway. Glycolysis is an anaerobic process (does not involve oxygen) consisting of a 10-step metabolic pathway that converts 1 glucose molecule into 2 pyruvic acid molecules and generates 2 molecules of ATP by substrate level phosphorylation. Oxidation of glucose to pyruvic acid with some ATP and NADH produced.

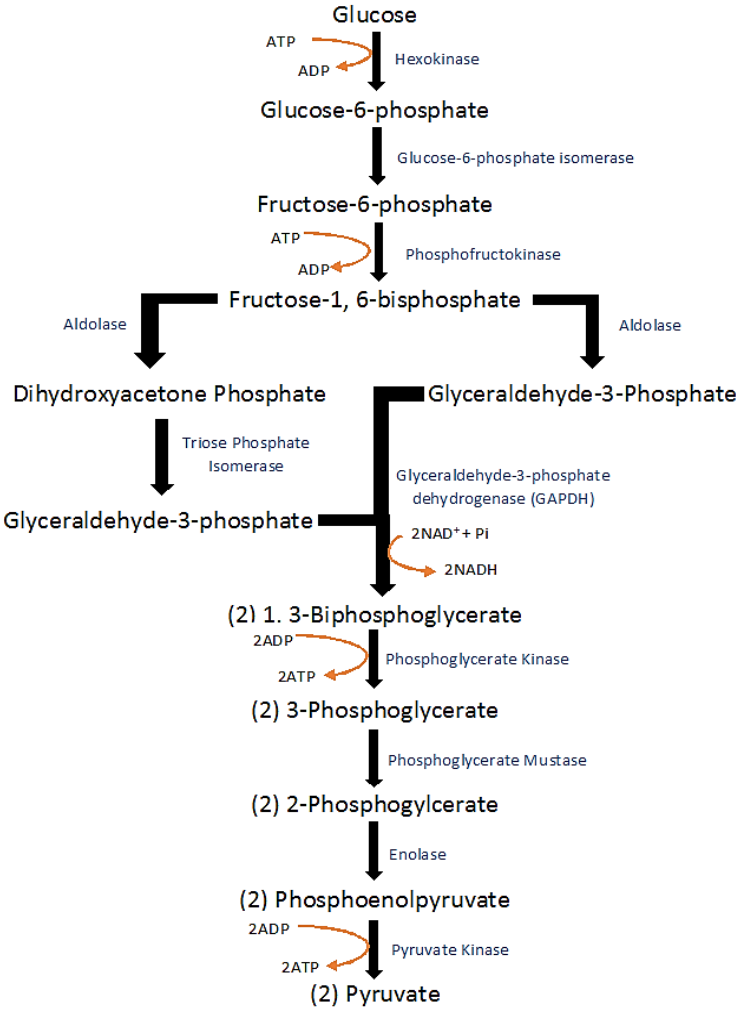
Two stages:

- **Preparatory stage:** The two ATP molecules are used to phosphorylate One 6-carbon glucose and catabolize it into two 3-carbon molecules.
- **Energy conservation stage:** The two 3-carbon molecules are oxidized to generate two 3-carbon pyruvic acid molecules. At the same time two NAD^+ molecules are reduced to two NADH

molecules and four ATP molecules are produced by substrate level phosphorylation.

Summary of glycolysis

Glucose + 2 NAD^+ + 2 ADP + 2 P →2 Pyruvic acid + 2 NADH + 2 H+ + 2 ATP



Citric acid cycle

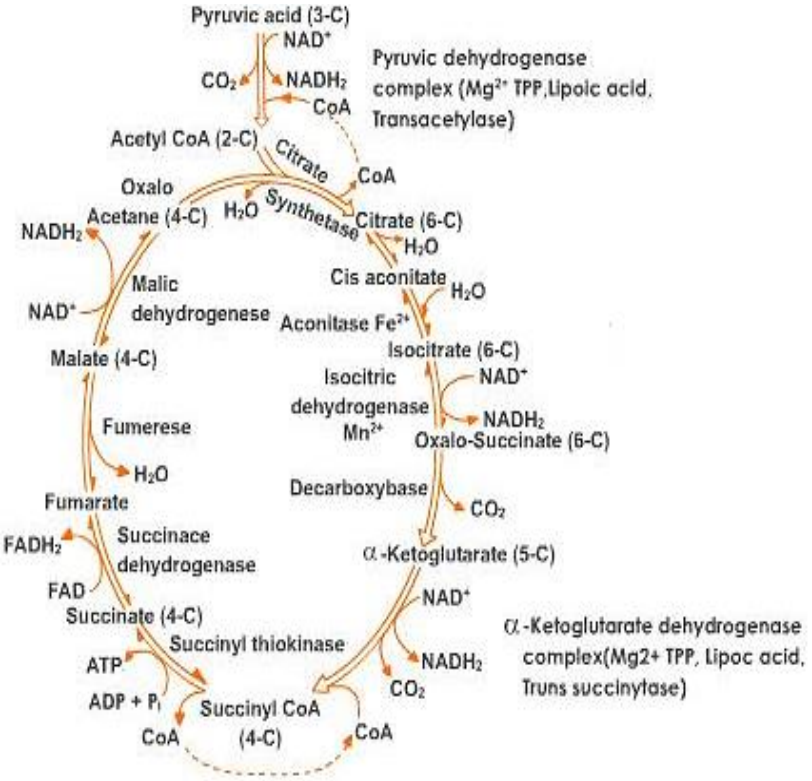
It is also known as Kreb's Cycle, Citric Acid Cycle and Tricarboxylic Acid Cycle. It involves oxidation of acetyl to carbon dioxide with some ATP, NADH and $FADH_2$ produced.

This part of the aerobic metabolism of glucose involves 8 enzymatic reactions that function to reduce the coenzymes NAD^+ and FAD. The 2-carbon acetyl is attached to a 4-carbon oxaloacetic acid creating a 6-carbon citric acid. Oxidation and decarboxylation reactions occur which catabolize the 6-carbon citric acid back into a 4-carbon oxaloacetic acid and two carbon dioxide molecules. At the same time three NAD^+ and one FAD are reduced into three NADH and one $FADH_2$ respectively, and one ATP is produced by substrate level phosphorylation (remember that for each glucose there are two acetyl molecules so this will run twice).

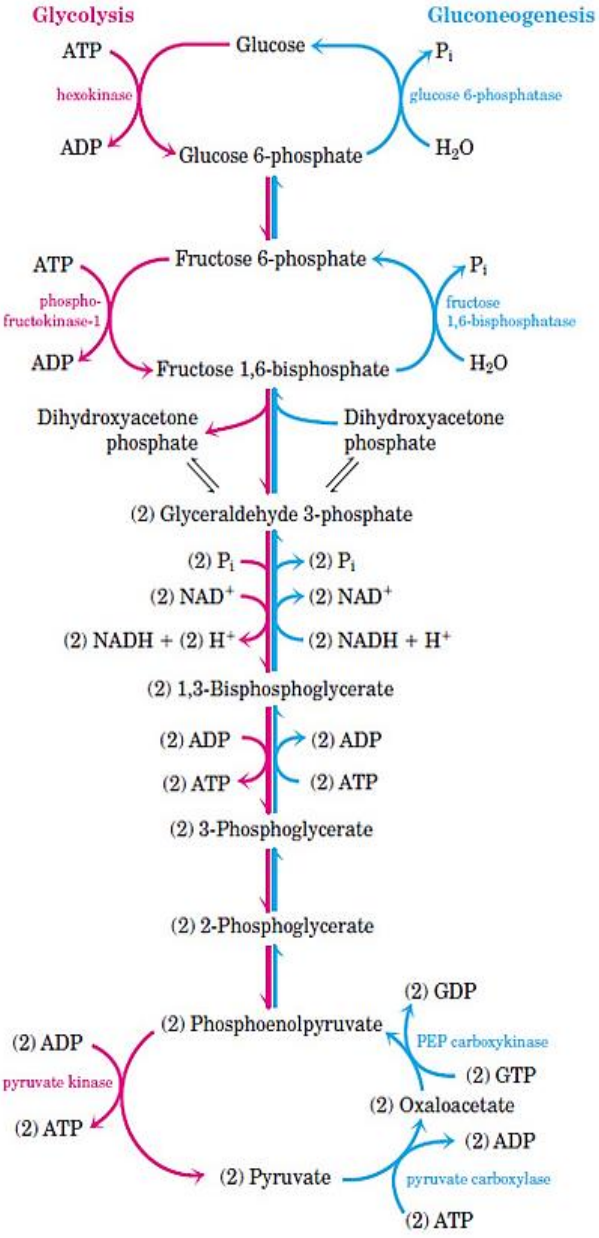
Summary of the Kerb's cycle

$$2 \text{ Acetyl Co A} + 6 NAD^+ + 2 FAD + 2 ADP + 2 P + 4 H_2O \rightarrow$$

$$2 \text{ CoA} + 4 CO_2 + 6 NADH + 4 H^+ + 2 FADH_2 + 2 ATP$$



Gluconeogenesis



Electron transport chain

NADH and $FADH_2$ are oxidized providing electrons for redox reactions to generate ATP. The majority of the ATP is produced at this step. NAD (Nicotinamide adenine dinucleotide) and FAD (Flavin adenine dinucleotide) are coenzymes that function to transport electrons in the form of hydrogen: NAD^+ carries 2 electrons but only one proton, FAD carries 2 complete hydrogen atoms.

This is the aerobic part of the aerobic metabolism of glucose. Oxidative phosphorylation occurs on a membrane to generate most of the ATP produced from glucose. Coenzymes from the previous reactions pass electrons to a series of electron carrier molecules, which carry out redox reactions resulting in the chemiosmotic generation of ATP.

There are three classes of carrier molecules:

1) **Flavoproteins:** Protein + Flavin coenzyme.
2) **Cytochromes:** Protein + an Iron group.
3) **Ubiquinone's** or **Coenzyme Q (Nonprotein).**

Events of the electron transport chain

1) NAD^+ and FAD collected hydrogens (electrons) from organic molecules during Glycolysis, Decarboxylation, and the Kerb's Cycle becoming NADH and $FADH_2$.
2) NADH and $FADH_2$ pass hydrogens (electrons and protons) to the electron transport chain consisting of flavoproteins, cytochromes, and coenzyme Q. As electrons are passed along the chain, protons are pushed out through the membrane. This sets up a concentration gradient with protons (positive charge) on the outside and electrons (negative charge) on the inside.
3) At the end of the chain the electrons are accepted by oxygen (or another inorganic if anaerobic) creating an anion (O^-) inside, which has a strong affinity for the cation (H^+) outside.
4) **Chemiosmosis generates ATP:** H^+ from the outside moves toward O- on the inside through special membrane channels that are coupled to ATP synthase and the high energy diffusion of H+ drives the reaction ADP + P →ATP. The energy from 1NADH can generate 3 ATP, and that from 1 $FADH_2$ can generate 2 ATP.
5) H^+ combines with O^- inside the membrane creating water (H_2O).

Summary of the ETC

2 NADH from Glycolysis + 2 NADH from Decarboxylation + 6 NADH from Kreb's cycle + 2 $FADH_2$ from Kreb's Cycle + 6 O_2 + 34 ADP + 34 P → 12 H_2O + 34 ATP + 10 NAD^+ + 2 FAD.

Abnormal metabolism of carbohydrates

Though good number of diseases are reported to abnormal metabolism of carbohydrates, important of them are diabetes mellitus, pentosuria, galactosemia & fructosemia.

These diseases are as follows:

1) **Diabetes mellitus** (**DM):** It is a group of metabolic disease in which there are high blood sugar levels over a prolonged period.

   Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. There are three main types of diabetes mellitus:

   a) **Type 1 DM** results from the pancreas's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM). The cause is unknown.

   b) **Type 2 DM** begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. This form was previously referred to as "non-insulin dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The most common cause is excessive body weight and not enough exercise.

   The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 DM, while they usually develop much more slowly and may be absent in type 2 DM.

2) **Glycosuria:** When glucose is excreted in urine, the condition is called as glycosuria. Glycosuria occurs due to the elevated blood glucose level.

3) **Galactosemia:** It is caused due to the deficiency of enzyme galactose-1-phosphate uridyltransferase & galactokinase. Galactose cannot be converted into glucose, which leads to a condition called a galactosemia.

4) **Glycogen storage disease (GSD, also known as glycogenosis and dextrinosis):** It is the result of defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types.

5) **Fructose intolerance:** One of the very normal hexose sugars of fruits (i.e. fructose) gets normally metabolized to give CO_2 & Energy but defective metabolism of fructose develops in high concentration of this sugar in blood, disorder known as fructose intolerance.

Metabolism of proteins

Proteins are high molecular weight compounds in which the building blocks are the amino acids.

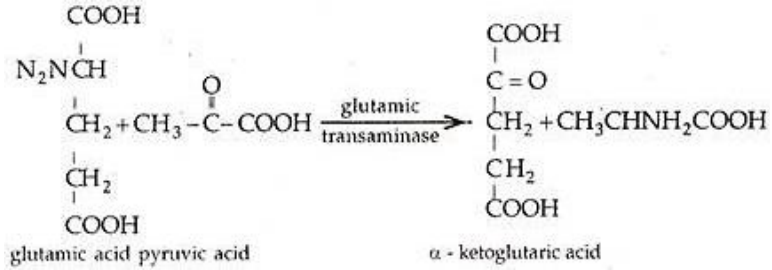
During digestion proteins are broken down by proteolytic enzymes (peptidases) to their respective amino acids units. These amino acids are absorbed by the blood stream and transported to different tissues of the body where they are either used in replacing the damaged tissues or in the synthesis of proteins.

Some amino acids are oxidized in the body to form CO_2 and H_2O, and others may be deaminized in the kidney or liver. The amino acid metabolism involves a number of enzymes, and amino acids may undergo the following metabolic fates:

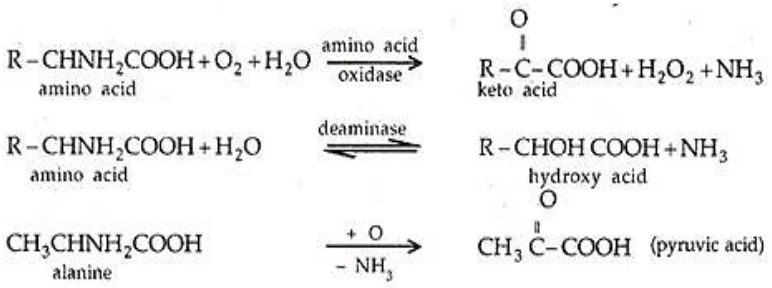
- Oxidation, transamination, deamination and decarboxylation reactions.
- Conversion to other nitrogen containing compounds such as certain vitamins.
- Protein biosynthesis.

Process of protein metabolism

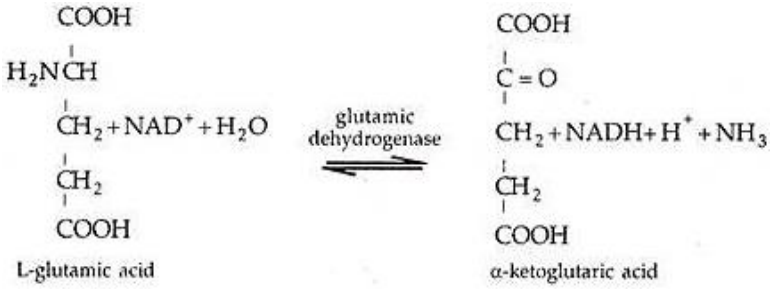
1. **Transamination:** This process involves the reversible transfer of an amino group to an organic acid, called keto acid. Thus, there is an interconversion of an amino acid to the corresponding keto acid and vice versa. These changes are catalyzed by transaminases. An important example of transamination is the conversion of glutamic acid in the presence of pyruvic acid to α-ketoglutaric acid and alanine as shown below with a few exceptions nearly all amino acids take part in transamination. These are of great importance as connecting links between carbohydrate and amino acid metabolism. Thus, carbohydrates may enter amino acid metabolism and vice versa.



2) Deamination: This process involves the removal of amino group by oxidation of a particular amino acid to produce the corresponding keto or hydroxy acid and free ammonia. The reaction is catalyzed by an oxidase, an enzyme specific for the deamination of a particular type of amino acid.



Thus, alanine is converted into pyruvic acid, glutamic acid into a-ketoglutaric acid and so on. The deamination of glutamic acid is catalyzed by the enzyme glutamic dehydrogenase and the coenzyme NAD or NADP.



The action of glutamic dehydrogenase is also an important link between carbohydrate and protein metabolism, since it converts α-ketoglutaric acid (an intermediate of Kreb's cycle) to an important amino acid, namely glutamic acid. The latter is used not only as amino acid component of many proteins but also takes part in the formation of other amino acids.

2. **Decarboxylation:** In the enzymatic reaction of amino acids, amino acid decarboxylases require pyridoxal phosphate as cofactor. Some of the amines formed as a result of decarboxylation have important physiological effects. Thus, histidine decarboxylase found in animal tissues produce histamine, a substance which among other effects stimulates gastric secretion.

Normal metabolism of protein

Urea cycle

It is also an important aspect of the protein metabolism. The urea is formed in the liver (to some extent in the kidney also) from ammonia, amino groups, and CO_2 in the presence of ATP and some enzymes. The amino groups separated in the deamination process combine with CO_2 to form urea.

Steps in the urea cycle

The urea cycle is a series of five reactions catalyzed by several key enzymes. The first two steps in the cycle take place in the mitochondrial matrix and the rest of the steps take place in the cytosol. Thus, the urea cycle spans two cellular compartments of the liver cell.

- In the first step of the Krebs-Hensley cycle, ammonia produced in the mitochondria is converted to carbamoyl phosphate by an enzyme called carbamoyl phosphate synthetase I. The reaction can be given as follows:

  NH_3 + CO_2 + 2ATP → Carbamoyl phosphate + 2ADP + Pi

- The second step involves the transfer of a carbamoyl group from carbamoyl phosphate to ornithine to form citrulline. This step is catalyzed by the enzyme ornithine transcarboxylase (OTC). The reaction is given as follows:

  Carbamoyl phosphate + Ornithine → Citrulline + Pi

- Citrulline thus formed is released into the cytosol for use in the rest of the steps of the cycle.

- The third step is catalyzed by an enzyme called arginine succinate synthetase, which uses citrulline and ATP to form a citrullyl-AMP intermediate, which reacts with an amino group from aspartate to produce arginosuccinate. This reaction can be given as follows:

  Citrulline + ATP + Aspartate → Arginosuccinate + AMP + PPi

- The fourth step involves the cleavage of arginosuccinate to form fumarate and arginine. Arginosuccinate lyase is the enzyme catalyzing this reaction, which can be represented as follows:

Arginosuccinate → Arginine + Fumarate

- In the fifth and last step of the urea cycle, arginine is hydrolyzed to form urea and ornithine. This is catalyzed by arginase and can be given as follows:

Arginine → urea + ornithine

Significance of the urea cycle

The main purpose of the urea cycle is to eliminate toxic ammonia from the body. About 10 to 20 g of ammonia is removed from the body of a healthy adult every day. A dysfunctional urea cycle would mean excess amount of ammonia in the body, which can lead to hyper ammonemia and related diseases. The deficiency of one or more of the key enzymes catalyzing various reactions in the urea cycle can cause disorders related to the cycle. Defects in the urea cycle can cause vomiting, coma and convulsions in new born babies. This is often misdiagnosed as septicemia and treated with antibiotics in vain. Even 1mm of excess ammonia can cause severe and irreversible damages.

Abnormal metabolism of protein

1. Protein in urine as a marker of disease

The kidneys act as filtration units for the blood. As blood passes through the kidneys, they filter out waste, leaving behind essential molecules which then recirculate through the body.

Most proteins are too large to filter through into the urine, hence in a healthy person protein is not present in urine. However, if the kidneys are damaged, proteins are able to filter through into urine. The protein most commonly found in urine is albumin, which is found in the blood, and helps to retain fluids.

In a healthy metabolic state, urine sometimes contains trace amounts of protein. For example, this can happen after a strenuous physical workout. Other factors such as extreme temperatures, fever, and emotional stress can temporarily increase protein levels in urine by a small amount. However, significant amounts of protein in the urine over the long-term can indicates the abnormal condition of the body.

When the kidneys become damaged typically through some sort of inflammation the resulting symptoms often include protein in urine. Many infections and diseases can cause this type of inflammation to occur including hypertension, diabetes, and kidney disease.

The presence of protein in urine is therefore a signal that the kidneys have been sufficiently damaged by inflammation to allow larger molecules to pass into the urine.

Causes of protein in urine

Many medical conditions can cause protein levels in urine to be consistently high. These include- Amyloidosis, Kidney failure, Diabetes, Heart disease and heart failure, High blood pressure, certain types of cancer including leukemia and Hodgkin's lymphoma, Lupus, Malaria, Pericarditis, Rheumatoid arthritis, Sarcoidosis, Sickle cell anemia.

Lipid metabolism

Lipids comprise a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides and others. The main biological functions of lipids include storing energy, signaling, and acting as structural components of cell membranes.

Fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28. Fatty acids are usually derived from triglycerides or phospholipids.

Essential fatty acids: Fatty Acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them.

Non-essential fatty acids: These fatty acids can be synthesized in the body. These include saturated fatty acids.

Normal metabolism of lipid

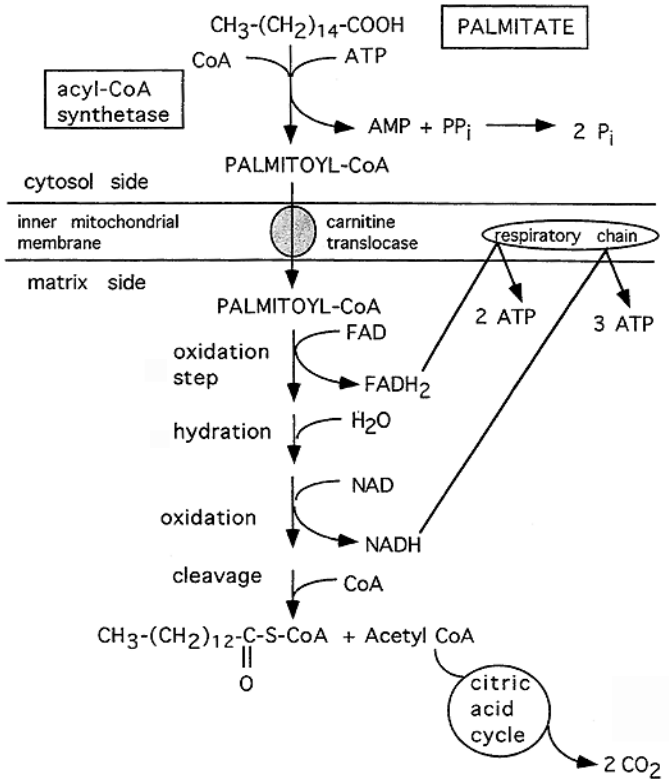
Lipid metabolism is the break down or storage of fats for energy, these facts are obtained from consuming food and absorbing them or they are synthesized by an animal's liver. Lipid metabolism does exist in plants, though the processes differ in some ways when compared to animals. Lipogenesis is the process of synthesizing these fats. Lipid metabolism often begins with hydrolysis, which occurs when a chemical break down as a reaction to coming in contact with water. Since lipids (fats) are hydrophobic,

hydrolysis in lipid metabolism occurs in the cytoplasm which ends up creating glycerol and fatty acids. Due to the hydrophobic nature of lipids, they require special transport proteins known as lipoproteins, which are hydrophilic. Lipoproteins are categorized by their density levels. The varying densities between the types of lipoproteins are characteristic to what type of fats they transport. A number of these lipoproteins are synthesized in the liver, but not all of them originate from this organ.

Oxidation of fatty acids

Once the fatty acid is inside the mitochondrial matrix, beta-oxidation occurs by cleaving two carbons every cycle to form acetyl-CoA. The process consists of 4 steps.

1) A long-chain fatty acid is dehydrogenated to create a trans double bond between C2 and C3. This is catalyzed by acyl CoA dehydrogenase to produce trans-delta 2-enoyl CoA. It uses FAD as an electron acceptor and it is reduced to $FADH_2$.
2) Trans-delta2-enoyl CoA is hydrated at the double bond to produce L-3-hydroxyacyl CoA by enoyl-CoA hydratase.
3) L-3-hydroxyacyl CoA is dehydrogenated again to create 3-ketoacyl CoA by 3-hydroxyacyl CoA dehydrogenase. This enzyme uses NAD as an electron acceptor.



4) Thiolysis occurs between C2 and C3 (alpha and beta carbons) of 3-ketoacyl CoA. Thiolase enzyme catalyzes the reaction when a new molecule of coenzyme A breaks the bond by nucleophilic attack on C3. This releases the first two carbon units, as acetyl CoA, and a fatty acyl CoA minus two carbons. The process continues until all of the carbons in the fatty acid are turned into acetyl CoA.

Fatty acids are oxidized by most of the tissues in the body. However some tissues such as the red blood cells (which do not contain mitochondria), and cells of the central nervous system (because fatty acids cannot cross the blood-brain barrier into the interstitial fluids that bathe these cells) do not use fatty acids for their energy requirements, but instead use carbohydrates.

Abnormal metabolism of fats

Fats (lipids) are an important source of energy for the body. The body's store of fat is constantly broken down and reassembled to balance the body's

energy needs with the food available. Groups of specific enzymes help the body break down and process fats. Certain abnormalities in these enzymes can lead to the buildup of specific fatty substances that normally would have been broken down by the enzymes. Over time, accumulations of these substances can be harmful to many organs of the body. Disorders caused by the accumulation of lipids are called lipidoses. Other enzyme abnormalities prevent the body from converting fats into energy normally. These abnormalities are called fatty acid oxidation disorders.

1. Gaucher's disease

Gaucher's disease is caused by a buildup of gluco-cerebrosides in tissues. Children who have the infantile form usually die within a year, but children and adults who develop the disease later in life may survive for many years.

In Gaucher's disease, gluco-cerebrosides, which are a product of fat metabolism, accumulate in tissues. Gaucher's disease is the most common lipidosis. The disease is most common among Ashkenazi (Eastern European) Jews. Gaucher's disease leads to an enlarged liver and spleen and a brownish pigmentation of the skin. Accumulations of gluco-cerebrosides in the eyes because yellow spots called pinguecula to appear. Accumulations in the bone marrow can cause pain and destroy bone.

Type 1, the chronic form of Gaucher's disease is the most common. It results in an enlarged liver and spleen and bone abnormalities. Most commonly diagnosed during adulthood, type 1 Gaucher's disease may lead to severe liver disease, including increased risk of bleeding from the stomach and esophagus and liver cancer. Neurologic problems can also occur.

Type 2, the infantile form usually causes death in the first year of life. Affected infants have an enlarged spleen and severe neurologic problems.

Type 3, the juvenile form can begin at any time during childhood. Children with type 3 disease have an enlarged liver and spleen, bone abnormalities, and slowly progressive neurologic problems. Children who survive to adolescence may live for many years.

Many people with Gaucher's disease can be treated with enzyme replacement therapy, in which enzymes are given by vein, usually every 2 weeks. Enzyme replacement therapy is most effective for people who do not have nervous system complications.

2. Tay-sachs disease

In Tay-Sachs disease, gangliosides, which are products of fat metabolism, accumulate in tissues. The disease is most common among families of Eastern European Jewish origin. At a very early age, children with this disease become progressively intellectually disabled and appear to have floppy muscle tone. Spasticity develops and is followed by paralysis, dementia, and blindness. These children usually die by age 3 or 4. The disease cannot be treated or cured.

Before conception, parents can find out whether they carry the gene that causes the disease. During pregnancy, Tay-Sachs disease can be identified in the fetus by chorionic villus sampling or amniocentesis.

3. Niemann-Pick disease

Niemann-Pick disease is caused by a buildup of sphingomyelin or cholesterol in the tissues. This disease causes many neurologic problems.

In Niemann-Pick disease, the deficiency of a specific enzyme results in the accumulation of sphingomyelin (a product of fat metabolism) or cholesterol. Niemann-Pick disease has several forms, depending on the severity of the enzyme deficiency, which determines how much sphingomyelin or cholesterol accumulates. The most severe forms tend to occur in Jewish people. The milder forms occur in all ethnic groups.

In the most severe form (type A), children fail to grow normally and have several neurologic problems. These children usually die by age 3. Children with type B disease develop fatty growths in the skin, areas of dark pigmentation, and an enlarged liver, spleen, and lymph nodes. They may be intellectually disabled. Children with type C disease develop symptoms during childhood, with seizures and neurologic deterioration.

Some forms of Niemann-Pick disease can be diagnosed in the fetus by chorionic villus sampling or amniocentesis. After birth, the diagnosis can be made by a liver biopsy (removal of a tissue specimen for examination under a microscope). None of the types of Niemann-Pick disease can be cured, and children tend to die of infection or progressive dysfunction of the central nervous system. Currently, some therapies that may slow or halt the progression of symptoms in types B and C are being studied.

4. Fabry's disease

Fabry's disease is caused by a buildup of glycolipid in tissues. This disease causes skin growths, pain in the extremities, poor vision, recurrent episodes of fever, and kidney or heart failure.

In Fabry's disease, glycolipid, which is a product of fat metabolism, accumulates in tissues. Because the defective gene for this rare disorder is carried on the X chromosome, the full-blown disease occurs only in males. The accumulation of glycolipid causes noncancerous skin growths (angiokeratomas) to form on the lower part of the trunk. The corneas become cloudy, resulting in poor vision. A burning pain may develop in the arms and legs, and children may have episodes of fever. Children with Fabry's disease eventually develop kidney failure and heart disease, although most often, they live into adulthood. Kidney failure may lead to high blood pressure, which may result in stroke.

Fabry's disease can be diagnosed in the fetus by chorionic villus sampling or amniocentesis. The disease cannot be cured or even treated directly, but researchers are investigating a treatment in which the deficient enzyme is replaced by transfusion. Treatment consists of taking analgesics to help relieve pain and fever or anticonvulsants. People with kidney failure may need a kidney transplant.

Chapter - 9

Lymphocytes

A lymphocyte is one of the subtypes of white blood cell in a vertebrate's immune system. Lymphocytes include Natural Killer Cells (NK cells) which function in cell mediated, cytotoxic innate immunity, T cells for cell-mediated, cytotoxic adaptive immunity, and B cells for humoral, antibody-driven adaptive immunity. They are the main type of cell found in lymph, so called as "lymphocyte". Lymphocytes can be identified by their large nucleus.

Types

A stained lymphocyte surrounded by red blood cells viewed using a light microscope. There are three major types of lymphocytes are:

- Thymus (T) cells.
- Bone marrow or Bursa derived (B) cells.
- Natural Killer (NK) cells.

T cells and B cells

T cells (thymus cells) and B cells (bone marrow cells) are the major cellular components of the adaptive immune response. T cells are involved in cell mediated immunity, whereas B cells are primarily responsible for humoral immunity (relating to antibodies). The function of T cells and B cells is to recognize specific “non-self” antigens, during a process known as antigen presentation. Once they have identified an invader, the cells generate specific responses that are tailored to maximally eliminate specific pathogens or pathogen-infected cells. B cells respond to pathogens by producing large quantities of antibodies which then neutralize foreign objects like bacteria and viruses. In response to pathogens some T cells, called *T Helper cells*, produce cytokines that direct the immune response, while other T cells, called *Cytotoxic T cells*, produce toxic granules that contain powerful enzymes which induce the death of pathogen-infected cells. Throughout the lifetime of an animal, these memory cells will remember each specific pathogen entered, and are able to mount a strong and rapid

response if the same pathogen is detected again; this is known as **Acquired Immunity**.

Natural killer cells

NK cells are a part of the innate immune system and play a major role in defending the host from both tumors and virally infected cells. NK cells distinguish infected cells and tumors from normal and uninfected cells by recognizing changes of a surface molecule called MHC (major histocompatibility complex) class I. NK cells are activated in response to a family of cytokines called interferons. Activated NK cells release cytotoxic (cell-killing) granules which then destroy the altered cells. They were named "natural killer cells" because of the notion that they do not require prior activation in order to kill cells which are missing MHC class I. This notion seemed doubtful at the time but turned out to be correct.

In the circulatory system, they move from lymph node to lymph node. This contrasts with macrophages, which are rather stationary in the nodes.

Lymphocytes and disease

A lymphocyte count is usually part of a peripheral complete blood cell count and is expressed as the percentage of lymphocytes to the total number of white blood cells counted. A general increase in the number of lymphocytes is known as lymphocytosis, whereas a decrease is known as lymphocytopenia.

An increase in lymphocyte concentration is usually a sign of a viral infection (in some rare case, leukemias are found through an abnormally raised lymphocyte count in an otherwise normal person). A high lymphocyte count with a low neutrophil count might be caused by lymphoma. Pertussis toxin (PTx) of *Bordetella pertussis*, formerly known as lymphocytosis-promoting factor, causes a decrease in the entry of lymphocytes into lymph nodes, which can lead to a condition known as lymphocytosis, with a complete lymphocyte count of over 4000 per μl in adults or over 8000 per μl in children. This is unique in that many bacterial infections illustrate neutrophil-predominance instead.

A low normal to low absolute lymphocyte concentration is associated with increased rates of infection after surgery or trauma.

One basis for low T cell lymphocytes occurs when the human immunodeficiency virus (HIV) infects and destroys T cells (specifically, the $CD4^+$ subgroup of T lymphocytes). Without the key defense that these T cells provide, the body becomes susceptible to opportunistic infections that

otherwise would not affect healthy people. The extent of HIV progression is typically determined by measuring the percentage of $CD4^+$ T cells in the patient's blood - HIV ultimately progresses to acquired immune deficiency syndrome (AIDS). The effects of other viruses or lymphocyte disorders can also often be estimated by counting the numbers of lymphocytes present in the blood.

Platelets

Platelets, also called **thrombocytes** (Thromb + cyte "blood clot cell"), are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clotting blood vessel injuries. Platelets have no cell nucleus: they are fragments of cytoplasm that are derived from the megakaryocytes of the bone marrow, and then enter the circulation. These un-activated platelets are biconvex discoid (lens-shaped) structures, 2-3 μm in greatest diameter. Platelets are found only in mammals, whereas in other animals (e.g., birds, amphibians) thrombocytes circulate as intact mononuclear cells.

Structure

Structurally the platelet can be divided into four zones, from peripheral to innermost:

- **Peripheral zone:** is rich in glycoproteins required for platelet adhesion, activation, and aggregation.
- **Sol-gel zone:** is rich in microtubules and microfilaments, allowing the platelets to maintain their discoid shape.
- **Organelle zone:** is rich in platelet granules. Alpha granules contain clotting mediators such as factor V, factor VIII, fibrinogen, fibronectin, platelet-derived growth factor, and chemotactic agents. Delta granules, or dense bodies, contain ADP, calcium, serotonin, which are platelet-activating mediators.
- **Membranous zone:** contains membranes derived from megakaryocytic smooth endoplasmic reticulum organized into a dense tubular system which is responsible for thromboxane A2 synthesis. This dense tubular system is connected to the surface platelet membrane to aid thromboxane A2 release.

Role in health and disease

Increased oxidative stress appears to be of fundamental importance in the pathogenesis and development of several disease processes. Indeed, it is

well known that reactive oxygen species (ROS) exert critical regulatory functions within the vascular wall, and it is, therefore, plausible that platelets represent a relevant target for their action.

Platelet activation cascade (including receptor-mediated tethering to the endothelium, rolling, firm adhesion, aggregation, and thrombus formation) is tightly regulated. In addition to already well-defined platelet regulatory factors, ROS may participate in the regulation of platelet activation. It is already established that enhanced ROS release from the vascular wall can indirectly affect platelet activity by scavenging nitric oxide (NO), thereby decreasing the antiplatelet properties of endothelium. On the other side, platelets themselves generate ROS, which may evoke pro-thrombotic responses, triggering many biological processes participating in atherosclerosis initiation, progression, and complication. That oxidative stress may alter platelet function is conceivable when considering that antioxidants play a role in the prevention of cardiovascular disease, although the precise mechanism accounting for changes attributable to antioxidants in atherosclerosis remains unknown.

It is possible that the effects of antioxidants may be a consequence of their enhancing or promoting the antiplatelet effects of NO derived from both endothelial cells and platelets. This review focuses on current knowledge regarding ROS-dependent regulation of platelet function in health and disease, and summarizes in vitro and in vivo evidence for their physiological and potential therapeutic relevance.

Erythrocytes

There are important cells in your body that travel in the blood. They are involved in a gas exchange that is essential to human life. **Red blood cells (RBCs)** are their most common name, but they are also called **erythrocytes**. In medical terminology, *erythro-* means red, and while *-cyte* means cell.

Erythrocytes have specific characteristics that all begin with the letter R.

- Erythrocytes are red and consist of a protein called hemoglobin, which contains red iron. This is why our blood is red in color.
- Erythrocytes are round. When these cells are normal, they can look like doughnuts with the holes in the center. Hemoglobin is responsible for the erythrocytes round shape; it increases their surface area, allowing them to carry more oxygen molecules.
- Finally, erythrocytes are like rubber, in that they're smooth and can easily bend. This gives them the ability to travel quickly in the

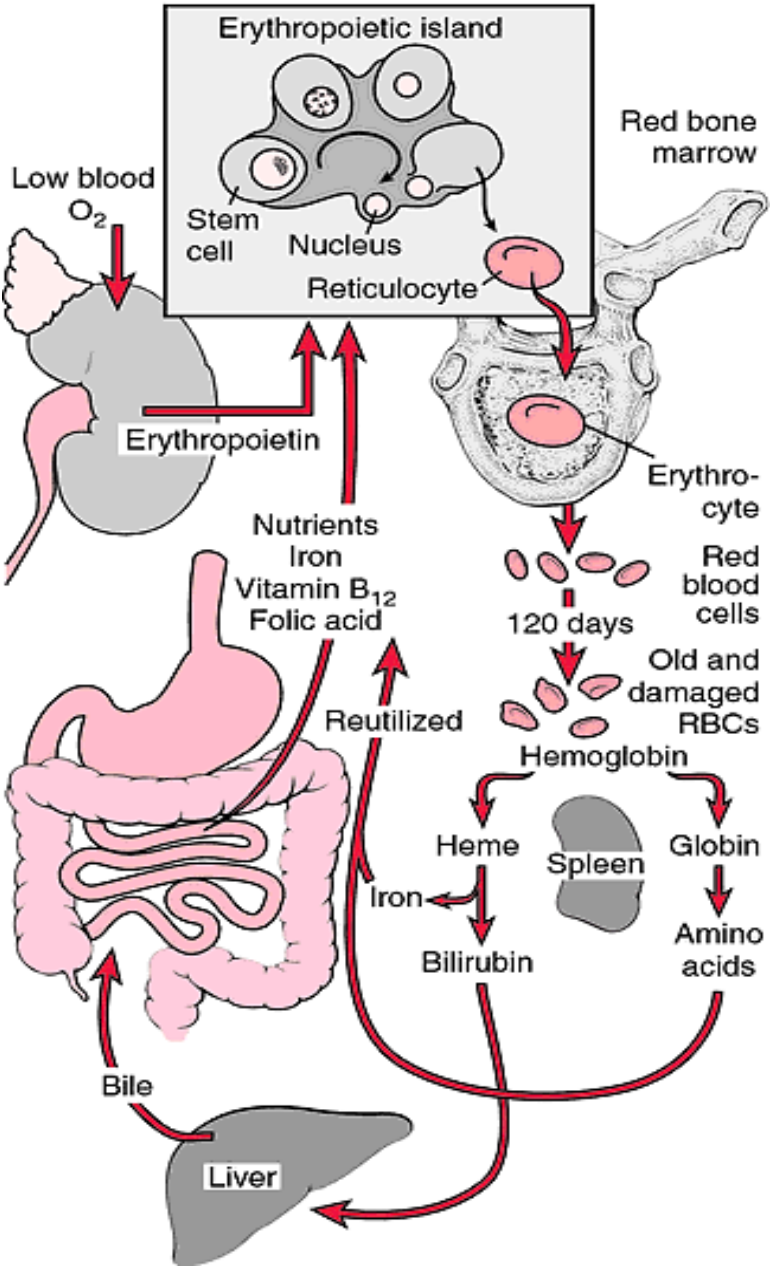
blood and squeeze through small vessels to get to various locations in the body

- One of the formed elements in the peripheral blood, constituting the great majority of the cells in the blood. In humans the normal mature erythrocyte is a biconcave disk without a nucleus, about 7.7 mm in diameter, consisting mainly of hemoglobulin & a supporting framework called the stroma.
- Erythrocyte formation takes place in the red bone marrow in the adult, and in the liver, spleen, and bone marrow of the fetus.
- It requires an ample supply of dietary elements such as iron, cobalt, copper, amino acids, and certain vitamins. They are also called as red cell or corpuscle and red blood cell or corpuscle.

Functions

1) **Transportation of oxygen and carbon dioxide:** They carry oxygen & carbon dioxide to blood containing hemoglobin a combination of an iron-containing prosthetic group *(heme)* with a protein *(globin).* Hemoglobin attracts and forms a loose connection with free oxygen, and its presence enables blood to absorb 60 times the amount of oxygen that the plasma by itself absorbs. Oxy-hemoglobin is red, which gives oxygenated blood its red color. Erythrocytes are stored in the spleen, which acts as a reservoir for the blood system and discharges the cells into the blood as required. The spleenmay discharge extra erythrocytes into the blood during e mergencies such as hemorrhage or shock.

2) Maintenance of normal acid-base balance:



The significance of erythrocyte antigen site density

The importance of antigen site density has been studied by means of a model passive hemolysis system using red cells coupled with sulfanilic acid groups. Relative site numbers were estimated from the covalent linkage of sulfanilic acid to red cell membrane protein, and the effective antigen site number was determined with ^{125}I-labeled rabbit IgG anti-sulfanilic acid (anti-S).

Immune hemolysis was demonstrated for red cells which had greater than a threshold number of antigen sites, the value of which was different for normal human cells (80,000 sites/cell), cells from a patient with paroxysmal nocturnal hemoglobinuria (PNH) (40,000 sites/cell), and sheep red blood cells (RBC) (15,000 sites/cell). Cells with antigen site densities below these values did not hemolyze when tested with 1 mg/ml purified rabbit IgM anti-S. 2-8 times greater antigen site densities were required to obtain hemolysis with IgG anti-S. Above the threshold value, hemolysis titers were proportional to the antigen site number until maximal values were obtained. The greater hemolytic efficiency of IgM antibody was demonstrated in this system, and it was established that the magnitude of the difference was related to the test cell antigen site density.

These data, taken with previously reported hemagglutination studies, have been used to develop a general classification of immune hemolysis and hemagglutination based on antigen site density and antibody class. It is suggested that the heterogeneity of blood group systems is caused by differences in the site separation of erythrocyte membrane antigens.

The significance of small erythrocytes

Small erythrocytes (mean corpuscular volume less than 80 mu-3 by the Coulter Model S) were found in 222 (2.75%) of 8,086 consecutive patients admitted to a large suburban general hospital. Forty-five (20.3%) of these 222 patients had laboratory findings consistent with thalassemia. Seventy-six (31.2%) were found to be iron deficient. Patients whose hemoglobin values were below 9.0 Gm. per 100 ml. were more likely to be iron deficient. The hemoglobin A2 values were significantly lower in iron-deficient than in non-iron-deficient patients. Although the mean corpuscular volume is much lower and the erythrocyte count is higher in thalassemia than in iron deficiency, hematologic values obtainable from the Counter S (such as MCV, erythrocyte count, and hemoglobin) alone are not valuable in differentiating thalassemia from iron deficiency.

Abnormal constituents of urine and their significance in disease

Urine is an excretion product by the kidney. Normal urine contains the waste products (formed in the body as a result of metabolic process) like urea, uric acid, creatinine and certain salts such as chloride & sulphates etc. It does not contain any substances which are required by the body or tissues. Such urine is described as the physiological or normal urine.

In certain physiological disorders, the substances essential to body or tissues may also be excreted in the urine. E.g.- Sugar excreted in the urine in the physiological disorders or in the pathological conditions. If such abnormal substances are excreted in the urine, then such a urine is described as the pathological or abnormal urine.

The substances which are not present in normal urine i. e. abnormal constituents, indicate disease or renal disorders.

Table 8: The abnormal constituents of urine and their significance in disease indicated in brief in the table.

S. No.	Abnormal constituents	Significance in disease
1.	Sugar (glucose)	In diabetes mellitus, the condition is described as glycosuria.
2.	Ketone bodies	It is called Ketosis. Diabetes mellitus, carbohydrate starvation, pregnancy & in anesthesia.
3.	Albumin	Condition is described as proteinuria. In sever exercise, high protein meal, pregnancy, nephritis & nephrosis.
4.	Bile pigments/ Salts	Present in jaundice.
5.	Blood	Condition is described as hematuria. Acute inflammation of any of the urinary organs, tuberculosis, cancer & renal stone. In malaria, typhoid, hemolytic jaundice, transfusion with incompatible blood, hemolytic poison & in burns covering the considerable area of the body.

References

1. Wikipedia