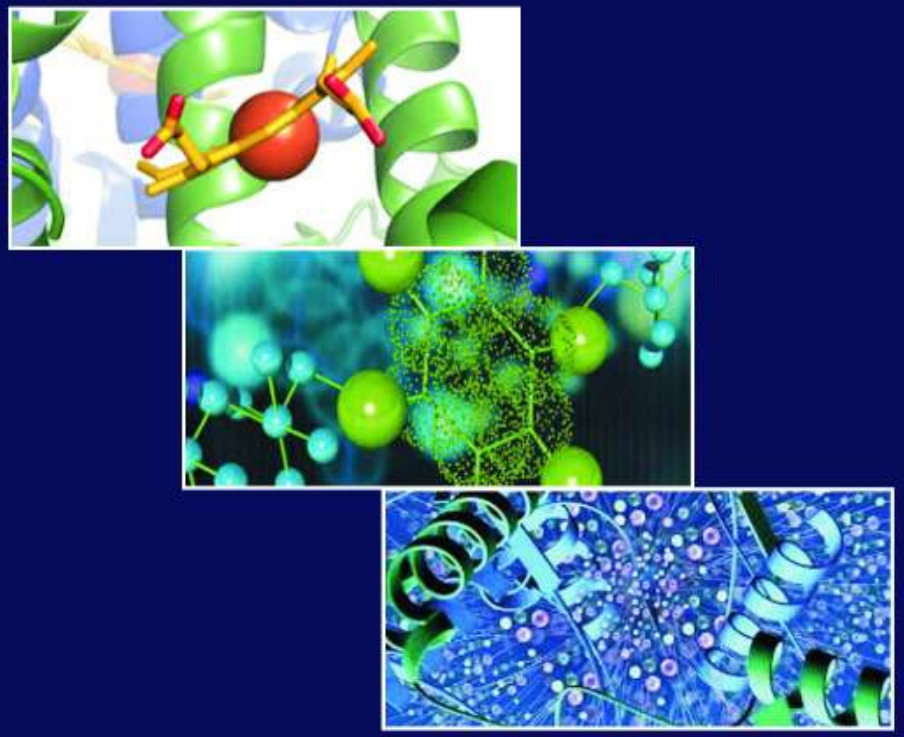
Biochemistry

Volume-II



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A. Arockia Jenecius Alphonse
G. Amala Jothi Grace
P. Subhavathy

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BIOCHEMISTRY

VOLUME - II

Dr. A. Arockia Jenecius Alphonse

Dr. G. Amala Jothi Grace

Dr. P. Subavathy

St. Mary's College (Autonomous),

Thoothukudi - 628 001.

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Authors: **Dr. A. Arockia Jenecius Alphonse, Dr. G. Amala Jothi Grace, Dr. P. Subavathy**

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Ph : 044-2247 0770, 94440 40272,
E-mail : cbapublisher@gmail.com

PREFACE

We feel pleasure to publish this book "***Biochemistry***" **Volume -II** as a continuation of our first volume to the Undergraduate and Postgraduate students of Science. This book includes the metabolism and mechanism of important biomolecules, Hormones, Biochemical Techniques, Cellular Respiration and Biological Oxidation. Comments and suggestions for this book from faculty and students for the improvement of this book are always welcome. We request the readers to send your valuable suggestions and comments to smcjasbooks@gmail.com

Authors

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We feel blessed and thank our almighty for giving us strength, courage and support to complete the second volume of Biochemistry book.

We voice our sincere thanks to our Secretary Dr. Sr. C.Shibana, Principal Dr. Sr. A.S.J. Lucia Rose for their continuous support to publish this second volume of Biochemistry. This team work is possible only by the courage from the Heads of Departments of Botany, Chemistry and Zoology We thank all the faculty members of Science Department for their enthusiastic support.

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Dr. Sr. A. Arockia Jenecius Alphonse
Dr. G. Amala Jothi Grace
Dr. P. Subavathy

Biochemistry - Volume II

Syllabus

Unit I Bioenergetics

Introduction to bioenergetics – Thermodynamic principles - System and it's types - Biological reactions – Exergonic reaction – Endergonic reaction – Energy and its forms - Energy Poor compounds Energy Rich compounds – Adenosine triphosphate – Guanosine triphosphate – Uridine triphosphate – Cytidine triphosphate – Acyl phosphate - Energy coupling.

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Unit IX Minerals

Introduction- Macrominerals- Microminerals- Role of Minerals in Human Life: Macrominerals - Calcium, Sodium, Potassium, Phosphorous, Magnesium, Chloride, Sulphur, Copper, Iodine. Microminerals - Iron, Boron, Zinc, Selenium, chromium, Manganese, Molybdenum.

Unit X General Biochemical Procedures

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CHAPTER

1 BIO–ENERGETICS

1.1 Introduction

Bioenergetics deals with energy flow in living organisms. Photosynthesis, Cellular respiration are bioenergetics process. Exergonic and Endergonic reactions are the types of bioenergetics reaction. Photosynthesis is the major biological process. The major source of energy for all the living beings is Sun. We need energy to do work. Some examples of biological work are nerve impulse, cellular functions, membrane functions, cellular growth and maintenance, muscle contraction. Bioenergetics tells us how to acquire and transform energy in living organism.

Example: Glycolysis, Gluconeogenesis, Citric acid cycle, ketosis, Oxidative phosphorylation, Photosynthesis.

Energy and work can be measured quantitatively in calorie. A calorie is the amount of heat required to raise the temperature of 1gram of water by °C

1 calorie = 4.18 Joules

1.2 Thermodynamic Principles

Thermodynamics is the study of the relations between heat, work, temperature, and energy. By learning the laws of thermodynamics, we can understand how the energy in a system changes and whether the system can perform useful work on its surroundings.

1.2.1 First Law of Thermodynamics

"Energy of the universe remains constant" Energy may be converted from one form into another but can never be created nor destroyed.

Examples:

- In photosynthesis, the radiant energy of light is transformed into the chemical energy.

- In muscles and nerves, chemical potential energy stored is transformed into kinetic and electric energy.

1.2.2 Second Law of Thermodynamics

"Matter tends to move disorderliness rather than orderliness, because orderliness in matter is always accompanied by a high degree of potential energy".

Laws of thermodynamics helps to understand the flow of energy through living systems. They help us to determine whether a biochemical reaction is possible or not.

1.3 System

The system consists of those molecules which are reacting. In biology, there are three types of systems namely

1. Isolated system
2. Closed system
3. Open system.

1.3.1 Isolated System

An isolated system exchanges neither matter nor energy with its surroundings.

Example: Earth system.

1.3.2 Closed System

A closed system exchanges only energy and not matter with its surroundings.

Example: Chlorophyll system.

1.3.3 Open System

An open system exchanges both energy and matter with its surroundings.

Example: Cell system.

Let us consider the following system

1.4 Biologic Reactions

Biological reactions takes place inside the living organism. In biologic system, there are two kinds of reactions.

1. Exergonic reactions
2. Endergonic reactions.

1.4.1 Exergonic Reactions

Exergonic reaction is a chemical reaction which involves the release of free energy and the free energy change is negative. The reaction is spontaneous and it provides energy for performing some work.

$$\Delta G = \text{-ve}$$

Example: Adenosine triphosphate (ATP) is hydrolysed to form Adenosine diphosphate (ADP) and Phosphoric acid.

$$ATP + H_2O \rightarrow ADP + H_3PO_4$$

$$\Delta G = -7300 \text{ calories/mole}$$

This spontaneous process provides 7300 calories/mole of free energy at pH 7. This energy is utilized to bring about various biochemical reactions.

1.4.2 Endergonic Reactions

Endergonic reaction is a chemical reaction in which the free energy is absorbed. In endergonic reaction, the free energy change is positive. The reaction is not spontaneous. Energy has to be supplied from outside.

$$\Delta G = + \text{ ve}$$

Example: Glucose is phosphorylated to form glucose 6-phosphate and water.

$$\text{Glucose} + H_3PO_4 \rightarrow \text{Glucose 6-phosphate} + H2O$$

$$\Delta G = +5500 \text{ calories/mole}$$

5500 calories has to be supplied from outside to get one mole of glucose 6-phosphate.

1.5 Energy and its Forms

Energy is the capacity to do work. Energy occurs in many forms. The important type of energy are

1. Potential energy
2. Kinetic energy
3. Internal energy

1.5.1 Potential Energy

Potential energy is the energy due to position The following are examples of potential energy.

1.5.2 Kinetic Energy

Kinetic energy is the energy associated due to its motion.

$$\text{Kinetic energy} = \tfrac{1}{2}\, mv^2$$

where, m = mass; v = velocity

When a body is moving, potential energy is converted in to kinetic energy. The conversion of potential energy into kinetic energy always involves the production of heat. So there is always some wastage of potential energy.

All living organism need energy to perform its activities. In biochemistry there are two types of energy containing compounds. They are Energy poor and energy rich compounds.

1.6 Energy-Poor Compounds

In energy-poor compounds, the phosphorous is attached to the compound by means of an ordinary co-valent bond. The structure may be represented as R-P. Removal of the phosphorous P from R-P releases a small amount of free energy. For example, glucose 6-phosphate upon hydrolysis produces glucose and phosphoric acid. Removal of phosphoric acid from glucose 6-phosphate releases about 3300 calories/mole of free energy at pH7.

$$\text{Glucose 6-phosphate} + H_2O \rightarrow \text{Glucose} + H_3PO_4$$

$$\Delta G = -3300 \text{ calories/mole}$$

1.7 Energy-Rich Compounds

Phosphate containing compounds are high energy compounds. In energy-rich compounds, the phosphorous is attached to the compound by means of a special high energy bond. Lipmann introduced the symbol "~" to indicate a high energy bond which possesses high levels of chemical energy. There is nothing special about the bonds themselves. They are high-energy bonds in the sense that much free energy is released when they are hydrolyzed. The structure may be represented as R~P. Removal of the phosphorous from R~P releases 7000-14000 calories/mole of free energy. These compounds are specialized for storage and transfer of free energy. Some high energy compounds found in cells with their standard free energy changes (ΔG).

1.7.1 Adenosine Triphosphate (ATP)

- ATP occurs in all living cells. ATP transports within the cells for metabolism.

ATP plays the role of energy currency in all living creature. ATP contains three phosphate groups.

- The main functions of ATP are transporting organic substances, synthesizing chemical compounds and supplying energy for cellular activities.

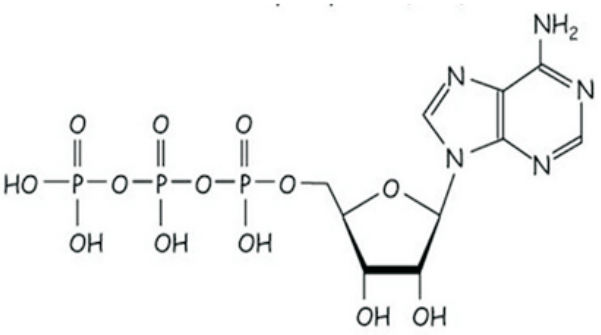


Figure 1.1 Structure of ATP

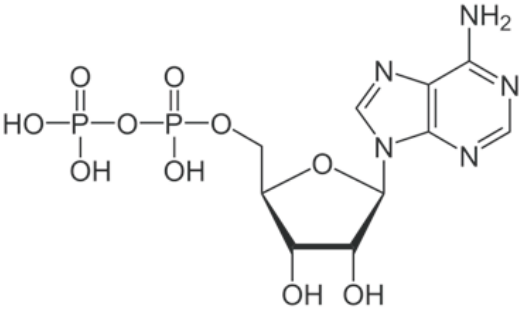


Figure 1.2 Structure of ADP

- The structure of ATP may be represented as A ~ P ~ P ~ P where A represents the nucleoside, adenosine which has adenine and ribose and P represents Phosphoric acid group. The terminal and second phosphoric acid groups are attached to the compound by means of high-energy bond. So, ATP has two high energy bonds.

$$A \sim P \sim P \sim P + H_2O \rightarrow A \sim P \sim P + H_3PO_4$$

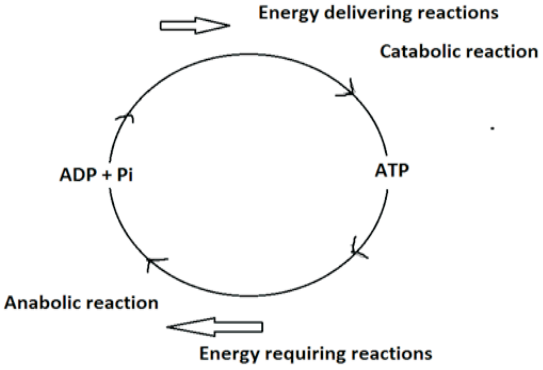
$$\Delta G = -7300 \text{ calories/mole}$$

$$A \sim P \sim P + H_2O \rightarrow A \sim P + H_3PO_4$$

$$\Delta G = -6500 \text{ calories/mole}$$

ADP has one high energy bond. The second phosphoric acid group can be removed by hydrolysis. The ADP becomes adenosine monophosphate (AMP) and phosphoric acid. The process release 6500 calories/mole of free energy at pH 7.

The hydrolysis of AMP to adenosine releases 2200 calories/mole of free energy at pH 7. ATP is the energy bank of the cell as it lends and also conserves the energy released by a system.



1.7.2 Guanosine Triphosphate (GTP)

- Guanosine is a purine nucleoside triphosphate. GTP acts as an activator in metabolic reactions. GTP is mainly employed in protein synthesis and gluconeogenesis.
- GTP is mainly involved in the energy transfer within the cell.GTP is also used in genetic translation and mitochondrial function.
- The structure of GTP may be represented as G ~ P ~ P ~ P. G represents guanosine which contains guanine and ribose. P stands for phosphoric acid group. GTP has two high-energy bonds. Upon hydrolysis, GTP becomes guanosine diphosphate (GDP), and phosphoric acid. The process releases 7300 calories/mole of free energy at pH 7.

$$G \sim P \sim P \sim P + H_2O \rightarrow G \sim P \sim P + H_3PO_4$$

$\Delta G = -7300$ calories/mole

$$G \sim P \sim P + H_2O \rightarrow G \sim P + H_3PO_4$$

$\Delta G = -6300$ calories/mole

GDP has a single high-energy bond. Upon hydrolysis it becomes guanosine monophosphate (GMP) and phosphoric acid. The process releases 6500 calories/mole of free energy at pH 7.

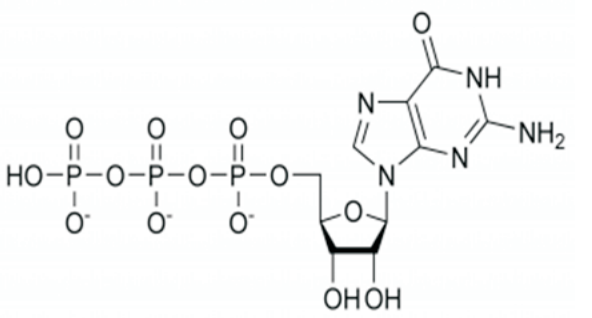


Figure 1.3 Structure of GTP

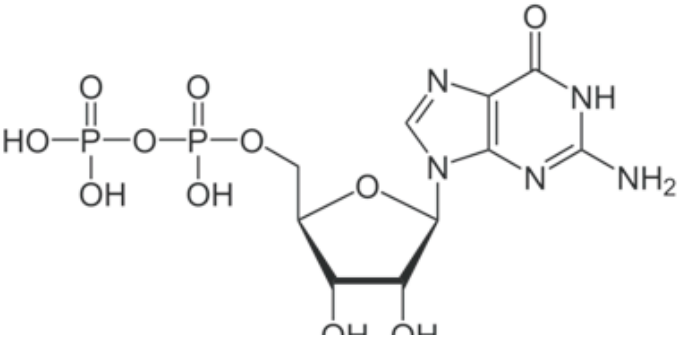


Figure 1.4 Structure of GDP

1.7.3 Uridine Triphosphate (UTP)

- Uridine triphosphate is a pyrimidine nucleoside triphosphate UTP is primarily used for the synthesis of polysaccharides. UTP and UDP are important metabolites in glycogenesis.

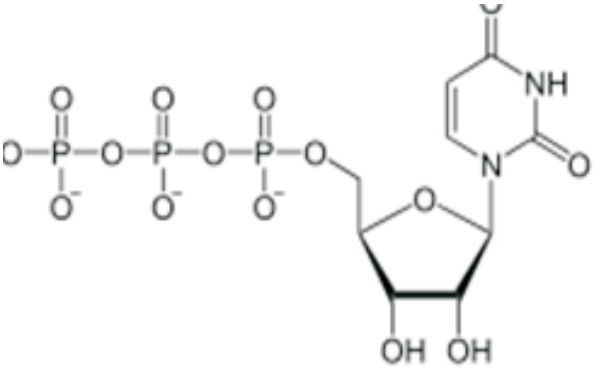


Figure 1.5 Structure of UTP

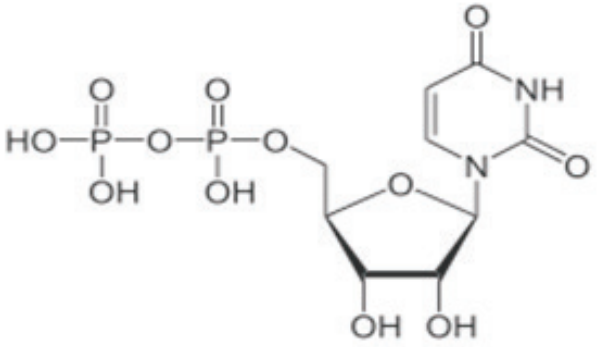


Figure 1.6 Structure of UDP

- The structure of UTP may be represented as U ~ P ~ P ~ P. G represents uridine which contains uracil and ribose. P stands for phosphoric acid group. It has two high-energy bonds.
- Upon hydrolysis UTP becomes uridinediphosphate (UDP) and phosphoric acid. The process releases a free energy of 7300 calories/mole at pH 7. UDP has a single high-energy bond upon hydrolysis it becomes uridine monophosphate (UMP) and phosphoric acid. The process releases 6500 calories/mole of free energy at pH 7.

$$U \sim P \sim P \sim P + H_2O \rightarrow U \sim P \sim P + H_3PO_4$$

$$\Delta G = -7300 \text{ calories/mole}$$

$$U \sim P \sim P + H_2O \rightarrow U \sim P + H_3PO_4$$

$$\Delta G = -6500 \text{ calories/mole}$$

1.7.4 Cytidine Triphosphate (CTP)

- CTP is a high energy molecule similar to ATP. CTP is primarily employed in lipid synthesis.
- CTP is a coenzyme in metabolic reactions. CTP also acts as the inhibitor of enzyme aspartate carbomyl transferase.
- The structure may be represented as C ~ P ~ P ~ P. C represents nucleoside cytidine which contains cytosine and ribose. P stands for phosphoric acid group. It has two high-energy bonds.
- Upon hydrolysis CTP becomes cytidine diphosphate (CDP) and phosphoric acid. The process releases a free energy of 7300 calories/mole at pH 7.

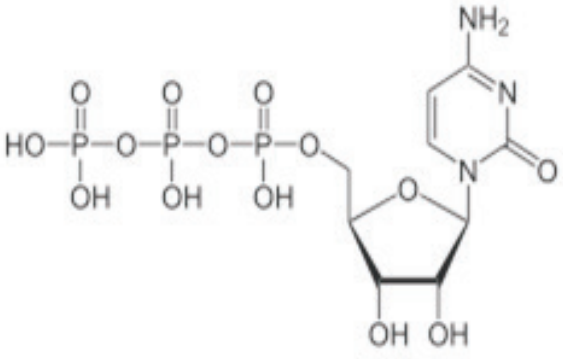


Figure 1.7 Structure of CTP

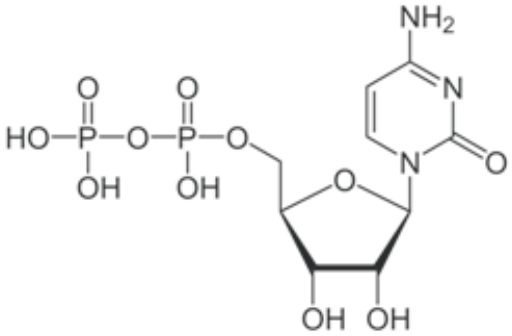


Figure 1.8 Structure of CDP

- CDP has a single high-energy bond upon hydrolysis it becomes cytidine monophosphate (CMP) and phosphoric acid. The process releases 6500 calories/mole of free energy at pH 7.

$$C \sim P \sim P \sim P + H_2O \rightarrow C \sim P \sim P + H_3PO_4$$

$$\Delta G = -7300 \text{ calories/mole}$$

$$C \sim P \sim P + H_2O \rightarrow C \sim P + H_3PO_4$$

$$\Delta G = -6500 \text{ calories/mole}$$

1.7.5 Acyl Phosphate

1,3 Diphosphoglyceric acid is an example of acyl phosphate. It has one high-energy phosphate bond. Upon hydrolysis it becomes 3-phosphoglyceric acid and phosphoric acid. The process releases free energy of 11800 calories/mole at pH 7.

1.8 Energy Coupling

Energy coupling occurs in an biological system. An exergonic reaction provides large amount of free energy. An endergonic reaction, requires energy from outside. Both exergonic and endergonic reaction can be combined in a suitable manner. This is known as coupling of chemical reactions.

The energy released by the exergonic reaction can be used to carry out the endergonic reaction. Consider, the following equations of pH 7.

Exergonic reaction

$$ATP + H_2O \rightarrow ADP + H_3PO_4 \qquad \Delta G = -7500 \text{ calories/mole}$$

The hydrolysis of ATP provides 7500 calories/mole of free energy.

Endergonic reaction

$$\text{Glucose} + H_3PO_4 \rightarrow \text{Glucose 6-phosphate} + H_2O$$

$$\Delta G = +5500 \text{ calories/mole}$$

About 5500 calories of free energy has to be supplied from outside to get one mole of glucose 6-phosphate. So, these two reactions can be coupled together. The free energy released by the first reaction is used by the second reaction.

Adding the above reactions,

$$ATP + \text{Glucose} \rightarrow ADP + \text{Glucose 6-phosphate}$$

$$\Delta G = -2000 \text{ calories/mole}$$

So the overall reaction is exergonic so, the reaction moves in forward direction.

Hence ATP plays a very important role in biological systems. So, the coupling of reactions using ATP is very important in bio-energetics.

CHAPTER

2 METABOLISM OF CARBOHYDRATES

2.1 Introduction

2.1.1 Metabolism

- Metabolism is defined as the enzymatic reaction taking place in the cell. It covers all the changes from the event of entry of a substance into the organisms to the discharge of the final end products into the outside environment.
- Through the metabolic process, energy obtained is utilized for the proper growth, maintenance of life and the performance of biological functions.

2.2 Carbohydrate Metabolism

Carbohydrate metabolism is a fundamental biochemical process that ensures a constant supply of energy to living cells. Carbohydrates form the major bulk of human diet and also one of the chief sources of energy.

During digestion, carbohydrates are broken down into simple, soluble sugars that can be transported across the intestinal wall into the circulatory system to be transported throughout the body.

Carbohydrates are mainly in the form of polysaccharides and disaccharides which are hydrolyzed into monosaccharide such as glucose, fructose and galactose by the enzymes of the digestive tract.

In the liver all the other hexoses are converted to glucose by the respective Isomerases. The glucose thus formed take-up any one of the following routes in the liver depending upon the necessity of the body.

1. ***Oxidation:*** At the time of physiological demand for energy, glucose is oxidized to carbon dioxide, water and energy in all the tissues.
2. ***Storage:*** In the absence of physiological demand for energy, excess glucose may be converted to glycogen and is stored in the liver, muscle and other tissues.

3. ***Conversion of fat:*** As the amount of glucose stores in the liver is limited, excess of this is converted to fatty acids and glycerol and are stored as triglycerides in the fat depots.
4. ***Conversion to other carbohydrates:*** A part of glucose may be converted to ribose and deoxyribose which are required for the synthesis of nucleic acids.

   Glucose may also form glucuronic acid which is involved in the formation of mucopolysaccharides and also in the detoxification reactions.

   It may also form galactose which is a component of glycolipids and lactose.

   It also forms mannose, glucosamine and galactosamine which form the components of mucopolysaccharides and glycoproteins.
5. ***Conversion to amino acids:*** Animals do not depend upon their diet for the non-essential amino acids and these amino acids are synthesized within the body itself either from glucose or their metabolites. Such amino acids can also be converted back into glucose, hence these amino acids are said to be glycogenic.
6. ***Glycolysis:*** Under special circumstances such as severe muscular concentration, glucose undergoes partial degradation namely glycolysis resulting in the formation of lactic acid which is largely disposed off by the liver.

2.3 Anabolic and Catabolic Pathways

The major metabolic pathways for carbohydrates may be as follows:

2.3.1 Anabolic Pathways

Anabolic pathways are those involved in the synthesis of the compounds which contains the body's structure and machinery. The free energy required for these processes comes from catabolism.

Ex - Glycogenesis

2.3.2 Catabolic Pathways

Catabolic pathways involve oxidative processes that release free energy, usually in the form of high-energy phosphate or reducing equivalents.

Ex - Glycogenolysis, Glycolysis and the oxidation of pyruvate, Citric acid cycle and Pentose Phosphate Pathway.

2.4 Intermediary Metabolism of Carbohydrates

The metabolism of carbohydrates occurs through the following pathways:

- Glycogenesis
- Glycogenolysis
- Glycolysis
- Citric acid cycle
- Hexose Monophosphate Shunt and
- Gluconeogenesis

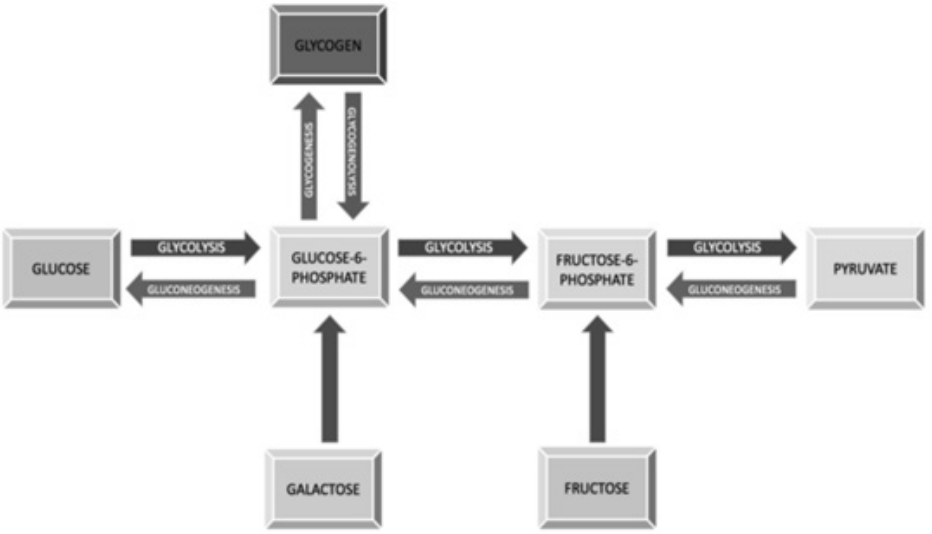


Figure 2.1 Metabolism of Carbohydrate

2.4.1 Glycogenesis

Glycogenesis is the process of glycogen synthesis from glucose. Glycogenesis takes place in the cytosol and requires ATP and UTP, besides glucose.

It occurs in all the tissues of the body but the major sites are liver and muscles. A considerable amount is synthesized in kidney also.

Glycogenesis is a very essential process. The excess glucose is converted and stored up as glycogen which could be utilized at the time of requirement. If this process does not take place the tissues are exposed to excess of glucose immediately after a meal.

The following are the various reactions of glycogenesis:

Step 1: Synthesis of UDP glucose – The enzymes hexokinase (in muscle) and glucokinase (in liver) convert glucose to glucose 6-phosphate. Glucose is first phosphorylated in the presence of ATP and the activation of Mg++ takes place.

Step 2: Glucose 6-phosphate is then reversibly converted to glucose-1-phosphate by the enzyme phosphoglucomutase in the presence of Mg++. Glucose 1,6-diphosphate act as coenzyme in this reaction.

Step 3: Hydrolysis of uridine triphosphate activates the glucose 1-phosphate in the presence of enzyme uridine diphosphate glucose pyrophosphorylase. It results in the formation of uridine diphosphoglucose. The inorganic pyrophosphate is eliminated in this reaction.

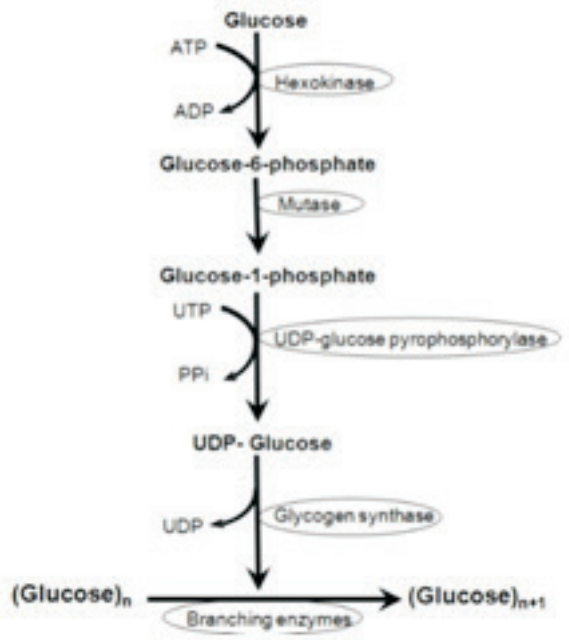


Figure 2.2 Glycogenesis

Step 4: In the end of an already existing glycogen chain the UDPG transfer the glucose molecule. The carbon 1(C1) of the activated glucose of UDPG forms a glycosidic bond with the carbon 4 (C4) of the terminal glucose residue of glycogen liberating UDP by the enzyme glycogen synthetase. As glucose molecules are added to the pre-existing glycogen chain by the successive α-1,4 linkages the chain becomes elongated.

Step 5: When the chain has become long with more than 8 glucose residues, a second enzyme namely the branching enzyme (amylo 1,4- 1,6 transglycosylase) acts on the glycogen and helps in joining of 1, 4 glycogen chain with a similar neighboring chain to form α-1, 6 linkage thus forming a branching point in the molecule.

This leads to the formation of a new non-reducing end, besides the existing one. Glycogen is further elongated and branched, by the enzymes glycogen synthase and glucosyl 4-6 transferase.

The glycogen thus formed may be stored in tissues mainly in liver and muscles and to some extend in the kidney also.

2.4.2 Glycogenolysis

Glycogenolysis means break down of glycogen into glucose (i.e. glucose-6-phosphate).

Glycogenolysis is the process of breaking down stored glycogen in the liver so that glucose may be produced for use in energy metabolism.

Glycogen may be broken down to glucose in liver and kidney or it may be broken down to glucose 6-phosphate in the muscles.

The following are the various reaction of glycogenolysis:

Step 1: The first step is the breakdown of glycogen catalyzed by two enzymes which act independently. Two key enzymes in glycogenolysis are glycogen phosphorylase and debranching enzyme.

The cleavage of terminal α-1, 4 bond of glycogen is catalyzed by the first enzyme namely glycogen phosphorylase with inorganic phosphate. It produces glycogen with one glucose molecule less and a molecule of glucose 1-phosphate.

The removal of glucose residues as glucose 1-phosphate continues until about 4 glucose residues remain on either side of the α-1,6 branch. Glycogen acts upon phosphorylase alone it results in the formation of a glycogen molecule. It contains each branch having 4 glucose units and this is called the limit dextrin.

The enzyme phosphorylase cannot cleave α-1, 6 linkages. This is carried out by another enzyme called the debranching enzyme which hydrolyses these bonds and thus make more α-1, 4 linkages accessible to the action of glycogen phosphorylase.

The combined action of glycogen phosphorylase and the debranching enzyme converts glycogen into glucose 1-phosphate.

The enzymes phosphorylase involved in the process of glycogenolysis, occurs in the liver and muscles tissues. In the liver, phosphorylase exists in two forms, namely an inactive form known as dephosphophosphorylase and an active form known as phosphosphorylase. The inactive forms can be converted into the active form in the presence of ATP and an enzyme dephosphophosphorylase kinase.

Muscle phosphorylase also exist in two distinct forms, namely phosphorylase a and phosphorylase b. Phosphorylase b can be converted into active phosphorylase a with the presence of the enzyme phosphorylase b kinase and ATP.

Step 2: The glucose 1-phosphatae is then reversibly converted to glucose 6-phosphate by the action of the enzyme phosphoglucomutase.

Step 3: The conversion of glucose 6-phosphate to glucose takes place in the liver and kidney by the action of the enzyme glucose 6-phosphatase. This enzyme removes phosphate from glucose 6-phosphate enabling the free glucose to diffuse from the cell into the extracellular spaces including the blood.

The reaction does not occur in the muscles because muscles lack the enzyme glucose 6-phosphate.

Glycogenolysis occurs in the hepatocytes and also in myocytes.

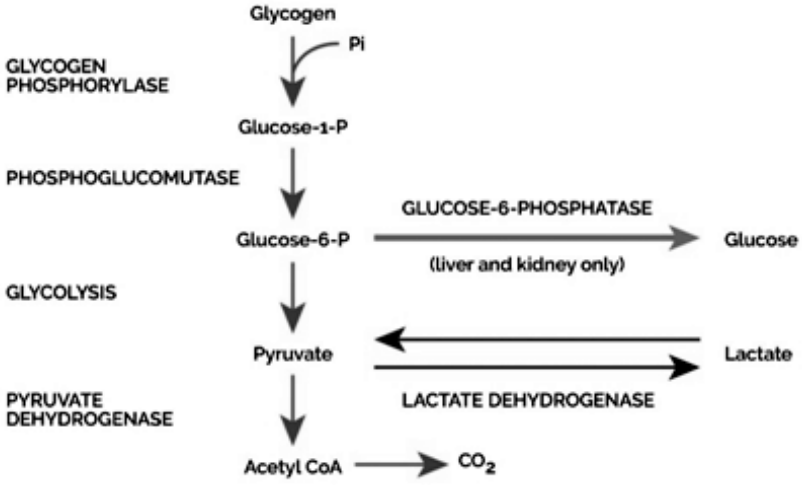


Figure 2.2 Glycogenolysis

2.4.3 Glycolysis

Glycolysis is the process of breaking down of a molecule of glucose to two molecules of pyruvic acid, two molecules of ATP, two molecules of NADH and two molecules of water.

The term glycolysis is derived from two Greek word "glycos" means sugar and "lysis" means dissolution and the term "glycolysis" means splitting of sugar.Glycolysis takes place in the cytoplasm. Glycolysis can occur with or without oxygen.

It is also known as the Embden – Meyerhof pathway.

Glycolysis is a primitive metabolic pathway since it operates in even the simplest and archaic cells. Glucose 6-phosphate is a principal compound in the metabolism of glucose.

There are 10 enzymes involved in the breaking down of sugar, where a molecule of glucose yields two molecules of pyruvic acid.

The enzyme involved in glycolysis are found in the extra mitochondrial soluble fraction of the cells and hence glycolysis occurs in the cytoplasm of the cells outside the mitochondria.

Step 1: Phosphorylation - Glucose is converted to glucose 6-phosphate with the presence of hexokinase. Glucose undergoes phosphorylation to produce glucose 6-phosphate. This is an irreversible reaction which requires ATP and Mg^{++}. ATP is the phosphate group donor and reacts as the Mg-ATP complex. It donates one high energy phosphate group and converted into ADP.

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{++}]{} \text{Glucose 6-phosphate} + \text{ADP}$$

Step 2: Isomerisation - Glucose 6-phosphate is converted to its isomeric form fructose 6-phosphate in the presence of the enzyme phosphohexose isomerase. It involves an aldose-ketose isomerisation.

$$\text{D-Glucose 6-phosphate} \xleftrightarrow{\text{Phosphohexose isomerase}} \text{D-Fructose 6-phosphate}$$

Step 3: SecondPhosphorylation -The second phosphorylation reaction is followed by the isomerization. Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-diphosphate and this reaction is catalyzed by the enzyme phosphofructokinase.

$$\text{D-fructose6-phosphate} + \text{ATP} \xleftrightarrow{\text{Phosphofructokinase}} \text{D-fructose 1,6-diphosphate} \quad (Mg^{++})$$

Step 4: Cleavage -Fructose 1,6-diphosphate is split by aldolase into two triose phosphates (3 C sugars), glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The splitting occurs between carbon atoms 3 and 4.

$$\text{D-fructose1,6-diphosphate} \xleftrightarrow{\text{Aldolase}} \text{D-glyceraldehyde 3-phosphate} + \text{Dihydroxyacetone phosphate}$$

Step 5: Isomerization - Glyceraldehyde 3-phosphate and dihydroxyacetone phosphate undergo inter conversion (isomerisation) in the presence of phosphotriose isomerase. Thus two molecules of glyceraldehyde 3-phosphate are formed from one molecule of fructose 1,6-diphosphate.

$$\text{D-Glyceraldehyde 3-phosphate} \xleftrightarrow{\text{Phospho triose isomerase}} \text{Dihydroxyacetone phosphate}$$

Step 6: Phosphorylation and Oxidative Dehydrogenation - Glyceraldehyde 3-phosphate is oxidized and phosphorylated simultaneously and converted into 1,3- diphosphoglyceric acid in the presence of NAD+, phosphoric acid and an enzyme phosphotriose dehydrogenase.

$$\text{D-Glyceraldehyde 3-phosphate} + NAD^{+} + \text{Pi} \xleftrightarrow{\text{Glyceraldehyde 3-dehydrogenase}} \text{1,3-diphosphoglyceric acid} + NADH + H^{+}$$

The coenzyme NAD in the oxidized form has a net positive charge and hence is written as NAD^{+}. When glyceraldehyde 3-phosphate is oxidized, NAD+ is reduced to NADH and one proton is released into the aqueous medium.

Step 7: Substrate level phosphorylation and ATP production - The high energy phosphate group in 1,3 – diphospho glycerate is transferred to ADP resulting in the formation of 3- phosphoglyceric acid and ATP. The reaction is catalyzed by phosphoglycerate kinase in the presence of Mg^{++}. Since 2-molecules of PGA are formed per molecule of glucose undergoing glycolysis, 2 molecules of ATP are generated.

The phosphorylation which occurs in this step is substrate level phosphorylation where a high energy phosphate bond is formed directly.

Phosphoglycerate kinase

1,3-diphosphoglyceric acid + ADP ⟷ 3-phosphoglyceric acid + ATP

Step 8: Isomerisation -The 3-phosphoglyceric acid arising from the above reaction undergoes internal rearrangement to form 2-phosphoglyceric acid in the presence of enzyme, phosphoglycerate mutase. The phosphate group is transferred from the third carbon atom to the second carbon atom.

Phosphoglycerate mutase

3-phosphoglyceric acid ⟷ 2-phosphoglyceric acid

Step 9: Dehydration -The 2-phosphoglyceric acid undergoes dehydration and redistribution of energy within the molecule, raising the phosphate on position 2 to the high energy state, thus forming phosphoenol pyruvic acid. The reaction is catalysed by enolase, whose activity can be inhibited by fluoride. Enolase requires Mg^{++} or $Mn^{++}$for the activity.

Enolase

2-phosphoglyceric acid ⟷ Phosphoenolpyruvic acid + H2O

Step 10: Substrate level phosphorylation and ATP generation - In the last step, there is a transfer of high energy phosphate from phosphoenolpyruvic acid to ADP by the enzyme pyruvate kinase. Thus, a molecule of ATP is directly synthesized and pyruvic acid is also formed. Pyruvic acid is accompanied by considerable loss of free energy as heat and must be regarded as physiologically irreversible.

Pyruvate kinase

Phosphoenolpyruvic acid + ADP ⟷ Pyruvic acid + ATP

At the end of glycolysis 2-molecules of pyruvic acid are produced per glucose molecule.

Under anaerobic conditions, the reoxidation of NADH by transfer of reducing equivalents through the respiratory chain to oxygen is prevented. Pyruvic acid is reduced by the NADH to lactic acid in the presence of enzyme, lactate dehydrogenase.

Lactate dehydrogenase

Pyruvic acid + NADH + H^+ ⟷ Lactic acid + NAD^+

2.4.3.1 Energy yield in glycolysis

The two molecules of ATP are generated in the conversation of glucose to pyruvic acid the reduced NAD ($NAD+H^+$) enters transport chain, where the reoxidation of NADH to NAD^+ occur with the liberation of 2 molecules of ATP.

2.4.3.2 Oxidation of cytosol NADH

It is mediated by substrate shuttles because NADH cannot penetrate the mitochondrial membrane. The shuttles used are:

1. ***Glycerophosphate shuttle:*** The mitochondrial enzyme is linked to the respiratory chain via FAD rather NAD, only 2 molecules of ATPase are produced.
2. ***Malate shuttle:*** 3 ATP molecules will be produced per 2H+ transferred into mitochondria. The hydrogen atoms are transferred to NADH in mitochondria.

2.4.3.3 Oxidation of pyruvic acid

The pyruvic acid is oxidatively decarboxylated to acetyl co-enzyme A (active/acetate) before entering the citric acid cycle. The conversion of pyruvic acid to acetyl-CoA is an irreversible reaction. It takes place in mitochondria. This occurs in the mitochondrial matrix and forms a link between glycolysis and the citric acid cycle.

The reaction is catalyzed by the multienzyme complex known as pyruvic acid dehydrogenase complex which is an aggregate of three kinds of enzymes, namely,

1. Pyruvic acid decarboxylase – Enzyme 1
2. Lipoate reductase transacetylase –Enzyme 2
3. Dihydrolipoyl dehydrogenase –Enzyme 3

The reaction is also assisted by TPP (Thymine Pyrophosphate), lipoic acid, FAD, CoA, and NAD^+ which act as coenzymes. There are four steps in the conversion of pyruvic acid to acetyl CoA.

Step 1: In the first step pyruvic acid reacts with enzyme bound thiamine pyrophosphate or thiamine diphosphate in the presence of magnesium ions (Mg^{++}) and pyruvate dehydrogenase to form "active pyruvate".

Step 2: The active pyruvate undergoes decarboxylation to produce hydroxyethyl thiamine diphosphate "active acetaldehyde" in the presence of pyruvate dehydrogenase.

Step 3: Hydroxyethyl thiamine diphosphate reacts with oxidized lipomide in the presence of enzyme, dihydrolipoyl transacetylase to produce acetyl lipoamide.

Step 4: Acetyl lipoamide reacts with coenzyme A to form acetyl-CoA and reduced lipoamide in the presence of dihydrolipoyl transacetylase. Acetyl-CoA contains one high energy bond.

Reduced lipoamide is then reoxidized by a flavoprotein FAD^+ in the presence of dihydrolipoyl dehydrogenase. As a result, FAD^+ gets converted to $FADH^+$ which in turn transfer the hydrogen to NAD.

The oxidation of pyruvic acid to acetyl-CoA can be summarized as follows:

$$\text{Pyruvic acid} + NAD^+ + CoA \longrightarrow \text{Acetyl-CoA} + NADH + H^+ + CO_2$$

Acetyl CoA is the end product of this reaction. It is an important intermediary metabolite which is formed not only from pyruvic acid but also from certain amino acids and fatty acids.

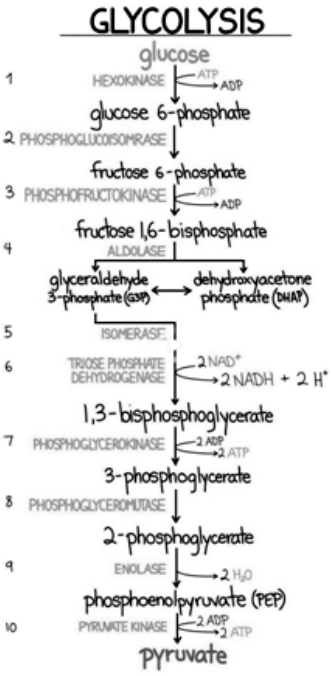


Figure 2.4 Glycolysis

2.4.4 Citric Acid Cycle

Citric acid cycle is a central pathway for the release of energy from acetyl CoA which is produced from the catabolism of carbohydrates, fatty acids and some amino acids.

The citric acid cycle is a series of reactions in mitochondria that bring about the catabolism of acetyl residues which are in the form of acetyl-CoA, an ester of coenzyme A.

The name citric acid cycle came from citric acid which is formed in the first step of this cycle. This cycle is also known as Krebs' cycle and tricarboxylic acid cycle or TCA cycle.

2.4.4.1 Reactions of the citric acid cycle

The citric acid cycle involves two important processes, namely electron transport and oxidative phosphorylation. The reduced enzymes of this cycle are oxidized and the available energy is used to synthesize ATP.

The various steps of citric acid cycle are as follows:

i. ***Condensation:*** The cycle starts with the joining of a 4 carbon unit, oxaloacetic acid and a two carbon unit, acetyl-CoA in the presence of a condensing enzyme, citrate synthetase to yield a six carbon unit, citric acid and coenzyme A(CoA).

$$\text{Acetyl-CoA + Oxaloacetate + } H_2O \longrightarrow \text{Citric acid + CoA}$$

ii. ***Dehydration:*** Under the action of enzyme aconitase, citric acid undergoes dehydration to form cis-aconitic acid.

$$\text{Citric acid} \xleftarrow{\text{Aconitase}} \text{Cis-aconitic acid + H2O}$$

iii. ***Hydration-I:*** Cis-aconitic acid undergoes hydration to form isocitric acid under the influence of aconitase.

$$\text{Cis – aconitic acid + } H_2O \xleftarrow{\text{Aconitase}} \text{Isocitric acid}$$

iv. ***Dehydrogenation I:*** Isocitric acid undergoes dehydrogenation to form oxalosuccinic acid. The pair of hydrogen atoms removed is accepted by NAD to form $NADH+H^+$ which enters the electron transport chain and 3 molecules of ATP are generated.

$$\text{Isocitric acid + NAD} \xleftarrow{\text{Isocitrate dehydrogenase}} \text{Oxalosuccinic acid + NADH + } H^+$$

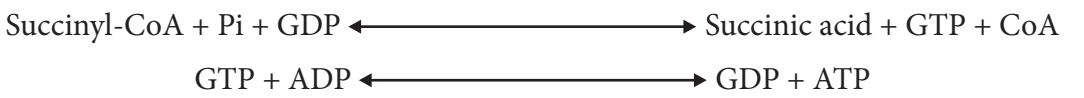
v. ***Decarboxylation I:*** The oxalosuccinic acid is oxidatively decarboxylated to α-ketoglutaric acid.One molecule of carbon dioxide is removed in the step and because of this loss of one carbon atom, the α- ketoglutaric acid molecule has five carbon atoms.

vi. ***Second Oxidative Decarboxylation (dehydrogenation II and decarboxylation II):*** α-Ketoglutaric acid undergoes oxidative decarboxylation and joins with coenzyme A to form succinyl CoA,a4 carbon atom derivate of coenzyme A.

This reaction is analogous to oxidative decarboxylation of pyruvic acid to acetyl CoA.This is an irreversible reaction and is catalyzed by α-ketoglutarate dehydrogenase complex which requires cofactors thiamine diphosphate, lipoate, NAD^+, FAD and CoA and results in the formation of succinyl-CoA, a thioester containing a high-energy bond.

vii. Formation of Succinic acid: Succinyl-CoA is converted to succinic acid by the enzyme succinate thiokinase (succinyl-CoA synthetase). The reaction requires GDP (Guanosine diphosphate) or IDP (inosine diphosphate) which undergoes phosphorylation in the presence of inorganic phosphate to produce GTP or ITP. GTP or ITP possesses a high energy phosphate bond, the energy is released.

In the citric acid cycle, this is the only example where energy is generated at the substrate level. By means of nucleoside diphosphate kinase, ATP may be formed from either GTP or ITP.



viii. ***Dehydrogenation III:*** Succinic acid undergoes dehydrogenation which is catalysed by succinate dehydrogenate, which is bound to the inner surface of the inner mitochondrial membrane. The hydrogen atoms are accepted directly by FAD.

ix. ***Hydration II:*** Fumaric acid is hydrated to malic acid and the reaction is catalyzed by the enzyme fumarase (fumarate hydrolase).

$$\text{Fumaric acid} + H_2O \longleftrightarrow \text{Malic acid}$$

x. ***Dehydration IV:*** In the final step malic acid is transformed into oxaloacetic acid by malate dehydrogenase. NAD+ accepts the hydrogen atoms to form NADH+ H^+ and by the passage of hydrogen atoms into the electron transport system 3 molecules of ATP are generated.

$$\text{Malic acid} + NAD^+ \longleftrightarrow \text{Oxaloacetic acid} + NADH + H^+$$

2.4.4.2 Energy yield in citric acid cycle

One molecule of glucose gives rise to two molecules of pyruvic acid by glycolysis, intermediates of citric acid cycle also result as two molecules.

2.4.4.3 Energetics of glucose metabolism

As a result of a glycolysis one molecule of glucose produces 2 molecules of pyruvic acid and in this process 6 molecules of ATP are synthesized.

In the conversion of the 2 molecules of pyruvic acid to 2 molecules of acetyl CoA, 6 ATP molecules are formed. The acetyl CoA enter the Krebs cycle and each acetyl CoA produces 122 molecules hence (2 × 12) 24 molecules of ATP are synthesized from 2 acetyl CoA molecules. Ultimately a total of 36 molecules of ATP are produced from the oxidation of a single molecule of glucose.

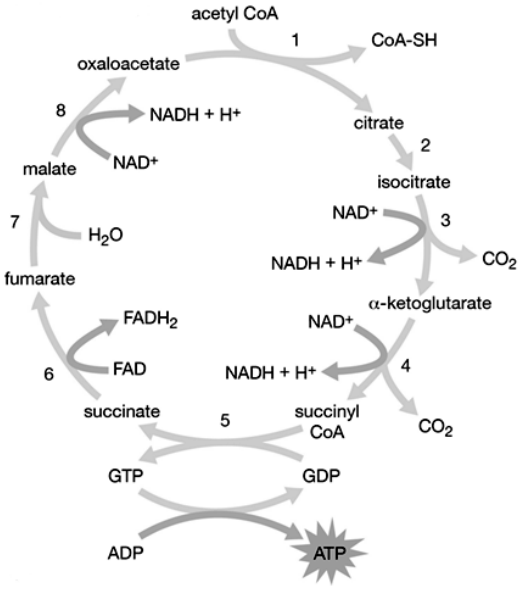


Figure 2.5 Citric Acid Cycle

2.4.5 Hexose Monophosphate (HMP) Shunt (Pentose Phosphate Pathway)

Hexose Monophosphate Shunt is an alternative pathway to glycolysis. It is an aerobic process. In this pathway glucose is used as the raw material.

HMP shunt is also known as alternative pathway or pentose phosphate pathway. This operates in liver, lactating mammary glands, adrenal cortex, adipose tissues, testis, thyroid, RBC and certain other tissues and is an alternative route for the oxidation of glucose.

The products formed in the HMP shunt are pentose sugar, CO, NADPH.The end products of HMP shunt can enter glycolysis and hence the name.

HMP shunt performs two major functions:

1. The generation of NADPH for reductive syntheses such as fatty acids and steroid biosynthesis
2. The provision of ribose for nucleotide and nucleic acid biosynthesis.

The HMP shunt reactions occur in the cytosol. The sequences of this shunt may be grouped into two phase,

1. The oxidative phase - conversion of hexose to pentose occurs
2. The non-oxidative phase - conversion of pentose to hexose occurs

1. **The oxidative phase:** During this phase NADPH are generated. The sequences of reactions are as follows

Step 1: Phosphorylation - The first step is the phosphorylation of glucose to glucose 6-phosphate in the presence of ATP and an enzyme hexokinase.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose 6-phosphate + ADP}$$

Step 2: Dehydrogenation - Glucose 6-phosphate is oxidized into 6-phosphogluconolactone in the presence of NADP and the enzyme glucose 6-phosphate dehydrogenase. NADP accepts the evolved hydrogen atoms and Mg^{2+} act as cofactors.

$$\text{Glucose6-phosphate} \xrightarrow[\text{Glucose 6-phosphate dehydrogenase}]{\text{NADP}^{+} \quad \text{Mg}^{+2} \quad \text{NADPH + H}^{+}} \text{6-phosphogluconolactone}$$

Step 3: Hydrolysis - 6-phosphogluconolactone is unstable and undergoes hydrolysis to produce 6-phosphogluconic acid. The enzyme that catalyzes the reaction is gluconolactone hydrolase in the presence of water and Mg^{+2} and Mn^{+2} ions.

$$\text{6-phosphogluconolactone} \xrightarrow[\text{Gluconolactone hydrolase}]{H_2O \quad Mg^{+2}, Mn^{+2}} \text{6-phosphogluconic acid}$$

Step4: Dehydrogenation - 6-phosphogluconic acid isoxidized by NADP into 3-keto-6-phosphogluconic acid in the presence of an enzyme 6-phosphogluconate dehydrogenase and cofactors Mg^{+2} and Mn^{+2} ions. NADP acts as hydrogen acceptor.

$$\text{6-phosphogluconic acid} \xrightarrow[\text{6-phosphogluconate dehydrogenase}]{NADP^+ \quad Mg^{+2}, Mn^{+2} \quad NADPH + H^+} \text{3-keto-6 phosphogluconic acid}$$

Step 5: Decarboxylation – Dehydrogenation is followed by decarboxylation to produce ketopentose, ribulose 5-phosphate. 3-keto-6-phosphogluconic acid acts as intermediate. Thus a hexose (glucose) is converted into a pentose (Ribulose 5-phosphate).

$$\text{3-keto-6-phosphogluconic acid} \longrightarrow \text{Ribulose 5-phosphate} + CO_2$$

Ribulose 5-phosphate is acted upon by two different enzymes. Ribulose 5-phosphate epimerase converts a portion of ribulose 5-phosphate to xylulose 5-phosphate while ribose 5-phosphate isomerase converts the rest of ribulose 5-phosphate into ribose 5 phosphate.

The above five steps constitute the first phase i.e. oxidative phase of the HMP shunt.

2. The non-oxidative phase: During this phase ribose precursors are generated. In the second phase of the HMP shunt the 5 carbon sugars, by a series of reversible reactions are converted into the glycolytic intermediates namely fructose 6-phosphate and glyceraldehyde 3-phosphate. The various sequences of reactions are as follows:

   Ribulose 5-phosphate generated during oxidative phase is acted upon by 2 different enzymes.

   Ribose 5-phosphateketoisomerase converts Ribulose 5-phosphate into an aldopentose, Ribose 5-phosphate and

   Ribulose 5-phosphate 3-epimerase converts ribulose 5-phosphate into xylulose 5-phosphate.

Ribose 5-phosphate keto Enediolform

Ribulose5-phosphate ⟷ isomerase ⟷

Ribose 5-phosphate

Ribulose 5-phosphate 3-epimerase

Ribulose 5-phosphate ⟷ xylulose 5-phophate

Step 6: Xylulose 5-phosphate and ribose 5-phosphate react to form sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate in the presence of the enzyme transketolase. Thus, the two pentose phosphate molecules react to form a heptose phosphate and a triose phosphate.

Ribose 5-phosphate + xylulose 5-phosphate ↔ Sedoheptulose7-phosphate + glyceraldehyde 3-phosphate

Transketolase thiamin – P_2 Mg^{+2}

Step 7: Sedoheptulose7-phosphate reacts with glyceraldehyde 3-phosphate to form erythrose 4-phosphate and fructose 6 phosphate and this reaction is catalyzed by transaldolase.

Sedoheptulose 7-phosphate + Glyceraldehyde 3-phosphate ↔ Fructose 6-phosphate + Erythrose 4-phosphate

Step 8: Xylulose 5-phophate may also react with erythrose 4-phosphate to produce fructose 6-phosphate and glyceraldehyde 3-phosphate in the presence of enzyme transketolase. The reaction also require thiamin-diphosphate and Mg+2 ions.

Xylulose 5-phosphate + Erythrose 4-phosphate ↔ Fructose 6-phosphate + Glyceraldehyde 3-phosphate

Transketolase thiamin – P_2 Mg^{+2}

Glyceraldehyde 3-phosphate and fructose 6-phosphate may enter the glycolytic cycle. These two products of the HMP shunt link up with the EMP pathway (Glycolysis). Fructose 6-phosphate is converted to glucose 6-phosphate in the presence of phosphoglucoisomerase and glyceraldehyde 3-phosphate is also converted to glucose 6-phosphate by the enzymes of the glycolytic pathway working in a reverse direction.

Figure 2.6 Hexose Monophosphate Shunt

2.4.6 Gluconeogenesis or neoglucogenesis

The synthesis of glucose from non-carbohydrate precursors is known as gluconeogenesis. The mechanism involved in gluconeogenesis is reversal of citric acid cycle and glycolysis. Gluconeogenesis mainly occurs in liver and kidney. The most important substrates for gluconeogenesis are the glucogenic aminoacids, lactic acid, propionic acid and glycerol.

Gluconeogenesis is an important source for supplying glucose to various tissues when glucose is otherwise not available and is regulated by certain key enzymes. These enzymes allow reversal of glycolysis. However, Gluconeogenesis is not an exact reversal of glycolysis.

2.4.6.1 Reactions of Gluconeogenesis

1. Pyruvate is carboxylated to oxaloacetate at the expense of an ATP which is catalyzed by pyruvate carboxylase. Then oxaloacetate is decarboxylated and phosphorylated to yield phosphoenolpyruvate at the expense of one molecule of GTP which is catalyzed by phosphoenolpyruvate carboxykinase.
2. Fructose 6-phosphate is formed from fructose 1, 6-diphosphate by hydrolysis and the enzyme fructose 1, 6-diphosphatase catalyzes this hydrolysis.
3. Glucose is formed by hydrolysis of glucose 6-phosphate catalyzed by glucose 6- phosphatase.

Here six high energy bond, are used to synthesize glucose from pyruvic acid whereas only two ATP are generated in glycolysis in the conversion of glucose to pyruvic acid. In gluconeogenesis four high energy phosphate bonds per glucose are synthesized from pyruvic acid.

2.4.6.2 Gluconeogenesis of amino acids

Amino acids which could be converted to glucose are called glucogenic amino acids. Most of the glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination. The amino acids are thus metabolically routed through oxaloacetic acid and phosphoenol pyruvic acid resulting in the formation of glucose.

2.4.6.3 Gluconeogenesis of propionic acid

In ruminants, propionic acid - a saturated fatty acid which is the end product of carbohydrate fermentation in the rumen is converted to succinyl CoA where by citric acid cycle enters the process of gluconeogenesis.

2.4.6.4 Gluconeogenesis of lactic acid

The liver and skeletal muscles exhibit a special metabolic co-operation as far as carbohydrates are concerned by way of a cycle of conversions known as cori cycle or lactic acid cycle. This is the cycle where liver glycogen may be converted into muscle glycogen and vice versa and the major raw material of this cycle is lactic acid produced by the active skeletal muscles.

Hence glycogen stored up in the muscle is converted into lactic acid by glycogenolysis followed by anaerobic glycolysis and thus lactic acid gets accumulated in the muscles.The lactic acid diffuses out of the muscle and enters the liver through the blood. In the liver lactic acid is oxidized to pyruvic acid which undergoes the process of gluconeogenesis resulting in the resynthesis of glucose.

The glucose is then converted into liver glycogen by a process of glycogenesis. The glycogen may be once again converted to glucose and may be recycled to the muscle through the blood. The process of glycogenesis completes the cycle by converting glucose once again to muscle glycogen. This cycle of conversion which repeats during muscle contraction is called the cori cycle.

2.4.6.5 Gluconeogenesis of glycerol

At the time of starvation glycerol can also undergo gluconeogenesis. When the triglycerides are hydrolyzed in the adipose tissue, glycerol is released. Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerolkinase necessary to phosphorylate it. Instead, the glycerol passes to the liver, where it is phosphorylated to glycerol 3-phosphate by the enzyme glycerolkinase.

The glycerol 3 phosphate is then oxidized to dihydroxyacetone phosphate catalyzed by the enzyme glycerol phosphate dehydrogenase. This dihydroxy acetone phosphate enters the gluconeogenic pathway and gets converted to glucose.

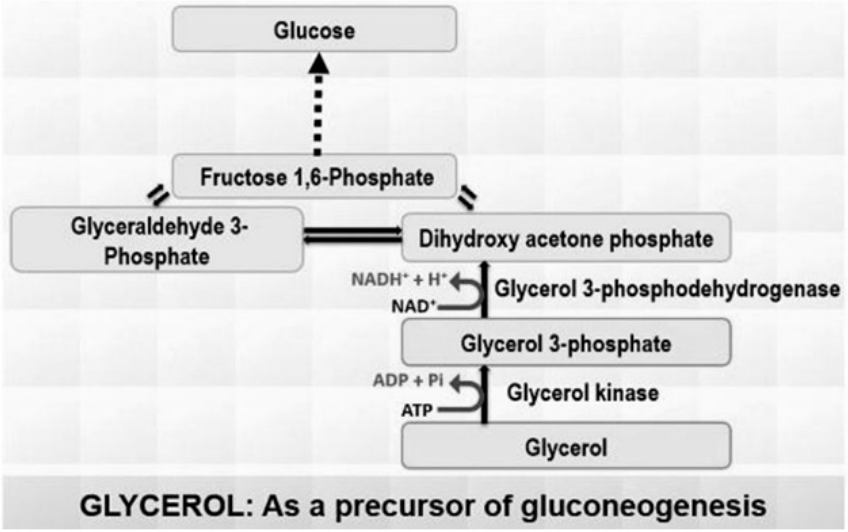


Figure 2.7 Gluconeogenesis

Overview:

Total net ATP produced under aerobic conditions:

a)	Glycolysis	8
b)	Pyruvic acid oxidation	6
c)	Citric acid cycle	24
	Total	**38**

Total net ATP produced under Anaerobic conditions = 2

If NADH produced during glycolysis is transported into mitochondria via glycerophosphate shuttle, only two ATP are produced hence total production of ATP would be 36 only instead of 38. If malate shuttle is used 38 ATP are produced.

CHAPTER

3 METABOLISM OF PROTEINS AND AMINO ACIDS

3.1 Protein Metabolism

The proteins present in the food are digested in the stomach and intestine and are hydrolysed into amino acids. The protein digestive enzymes are

1. Endopeptidases – pepsin, trypsin, chymotrypsin
2. Exopeptidases – carboxipeptidases, aminopeptidases, dipeptidases

The dietary proteins consist of 20 amino acids which exhibit L-configuration. The amino acids are classified into two types i.e. essential and non-essential amino acids. After digestion in the alimentary canal, the amino acids are absorbed through the intestinal epithelium into the blood. The sources of amino acids are dietary proteins, intra-cellular synthesis and tissue protein breakdown which constitute the "amino acid pool".

The amino acids undergo two main metabolic pathways:

1. Catabolic pathways
   i. Deamination
   ii. Transamination
   iii. Decarboxylation
2. Anabolic pathway
   i. Protein synthesis

3.2 Catabolic Pathways

3.2.1 Deamination

In the process of deamination an amino acid is converted into an α-keto acid and ammonia.

It occurs in the following ways:

i. **Amino acid oxidases –** The amino acid oxidases are flavoproteins and oxidize amino acids to the corresponding keto acids after generating a molecule of ammonia. Oxygen takes part in this oxidation process.

$$\text{Amino acid} + \tfrac{1}{2}\,O_2 \longrightarrow \alpha\text{-Keto acid} + NH_3$$

ii. **Amino acid dehydrogenases –** Amino acid dehydrogenases are NAD or NADP linked enzymes. This enzyme catalyses the removal of hydrogen atoms from an α-amino acid. The hydrogen atoms are accepted by NAD or NADP. The amino acid is hydrolysed to produce α-keto acid and ammonia.

$$\alpha\text{-Amino acid} \xrightarrow{NAD^+} \alpha\text{-Imino acid} + NADH + H+$$

$$\alpha\text{-Imino acid} + H_2O \xrightarrow{\text{Amino acid dehydrogenase}} \alpha\text{-Keto acid} + NH_3$$

3.2.2 Non-oxidative deaminatio

Serine and threonine dehydratases catalyse a non-oxidative deamination reaction resulting from a primary dehydration of the substrate and yield pyruvate or α-ketobutyrate.

$$\text{Serine} \longrightarrow \alpha\text{-imino acid} \xrightarrow{H_2O} \text{Pyruvate} + NH_3$$

$$\text{Threonine} \xrightarrow{H_2O} \alpha\text{-imino acid} \xrightarrow{H_2O} \alpha\text{-ketobutyrate} + NH_3$$

Non-oxidative deamination reactions also occur in microorganisms. Some of the reactions are as follows:

$$\text{Aspartate} \xrightarrow{\text{Aspartase}} \text{Fumarate} + NH3$$

$$\text{Histidine} \xrightarrow{\text{Histidinase}} \text{Uroeonic acid} + NH3$$

3.2.3 Transamination

In transamination reactions α-amino group is transferred to α-carbon atom of an α-keto acid. Transamination is catalysed by transaminases or aminotransferases. Pyridoxal phosphate acts as a cofactor.

$$\text{Amino acid 1 + Keto acid -1} \xleftrightarrow{\text{Transaminases}} \text{Keto acid 2 + Amino acid 2}$$

The transaminases are specific in their reactions. These are widely distributed in the tissues, however, their concentration in heart and liver is relatively high. Some specific examples of transaminases are as follows:

1. **GOT (Glutamic-oxaloacetate transaminase):** GOT activity is very high in heart tissue. It catalyses the reaction between glutamic acid and oxaloacetic acid to produce aspartic acid and α-ketoglutaric acid.

   $$\text{Glutamic acid + Oxaloacetic acid} \xleftrightarrow{\text{GOT}} \text{Aspartic acid + α-ketoglutaric acid}$$

   In a state of cardiac infarction, GOT is released in blood and its level in serum (SGOT) is abnormally raised.

2. GPT (Glutamic pyruvate transaminase): GPT concentration is very high in liver. Glutamic acid and pyruvic acid react in the presence of GPT to produce α-ketoglutaric acid and alanine.

   $$\text{Glutamic acid + Pyruvic acid} \xleftrightarrow{\text{GPT}} \text{α-ketoglutaric acid + Alanine}$$

   If liver cells are damaged, SGPT activity is abnormally raised.

3.2.4 Decarboxylation

Amino acids undergo decarboxylation to produce CO2 and an amine in the presence of amino acid decarboxylases. These enzymes require pyridoxal phosphate for their activity. The amines produced are called biogenic amines and have important pharmacological effects.

$$\text{Amino acids} \xrightarrow[\text{Pyridoxal phosphate}]{\text{Decarboxylase}} \text{Amine + } CO_2$$

Some metabolically significant amines formed as a result of decarboxylation are as follows:

1. **γ-Aminobutyric Acid (GABA):** GABA is formed in brain by the decarboxylation of glutamic acid. It stimulates neural activity and inhibits synaptic transmission in the central nervous system.

$$\text{Glutamic acid} \xrightarrow{\text{Glutamic acid decarboxylase}} \gamma\text{-Aminobutyric acid} + CO_2$$

2. **Tyramine:** Tyramine is obtained as a result of decarboxylation of tyrosine. It increases blood pressure.

$$\text{Tyrosine} \xrightarrow{\text{Tyrosine decarboxylase}} \text{Tyramine} + CO_2$$

3. **Histamine:** L-histidine is decarboxylated to produce histamine.

$$\text{Histidine} \xrightarrow{\text{Histine decarboxylase}} \text{Histamine} + CO_2$$

It's concentration is high in animal tissues such as liver, kidney, duodenum, large intestine and lungs. Histamine is a powerful vasodilator.

4. **Serotonin (5-hydroxytryptamine):** Serotonin is produced by the decarboxylation of 5-hydroxytryptophan.

$$\text{5-hydroxytryptophan} \xrightarrow{\text{5-hydroxytryptophan decarboxylase}} \text{5-hydroxytryptamine} + CO_2$$

Serotonin acts as a potent vasoconstrictor substance. It is mainly found in brain, blood platelets and intestinal tissues.

5. Dopamine (3,4-dihydroxyphenyl ethylamine): Dopamine is produced by the decarboxylation of 3,4-dihydroxyphenyl ethylamine (Dopa).

$$\text{Dopa} \xrightarrow{\text{Dopa decarboxylase}} \text{Dopamine} + CO2$$

Dopamine is present in kidney and adrenal tissues as well as sympathetic ganglia and nerves. It acts as an inhibitory neurotransmitter.

3.3 Anabolic Pathway

3.3.1 Protein Synthesis:

Protein synthesis is a process of synthesizing proteins in a chain of amino acids known as polypeptides. It takes place in the ribosomes found in the cytosol or those attached to the rough endoplasmic reticulum. It carries the information from DNA in the nucleus to a ribosome in the cytoplasm and then helps assemble the protein. It is called the central dogma of biology.

$$DNA \rightarrow RNA \rightarrow Protein$$

The protein synthesis involves two major steps:

- Transcription
- Translation

i. Transcription:

Transcription is the first part of the central dogma of molecular biology.

DNA → RNA

It is the transfer of genetic instructions in DNA to mRNA. During transcription, a strand of mRNA is made to complement a strand of DNA.

Steps of transcription

Transcriptiontakes place in three steps, called initiation, elongation, and termination.

1. Initiation is the beginning of transcription. It occurs when the enzymeRNA polymerase binds to a region of a genecalled the promoter. This signals the DNA to unwind so the enzymecan "read" the bases in one of the DNA strands. Theenzymeis ready to make a strand of mRNA with a complementary sequence of bases. The promoteris not part of the resulting mRNA.
2. Elongation is the addition of nucleotides to the mRNA strand.
3. Termination is the ending of transcription. As RNA polymerase transcribes the terminator, it detaches from DNA. The mRNA strand is complete after this step.

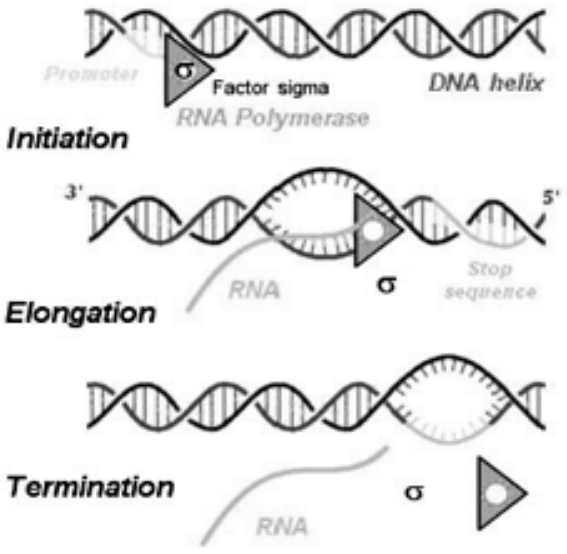


Figure 3.1 Protein Synthesis

Processing mRNA

In eukaryotes, the new mRNA is not yet ready for translation. At this stage, it is called pre-mRNA, and it must go through more processing before it leaves the nucleus as mature mRNA. The processing may include splicing, editing, and polyadenylation. These processes modify the mRNA in various ways. Such modifications allow a single gene to be used to make more than one protein.

- **Splicing** removes introns from mRNA. **Introns** are regions that do not code for the protein. The remaining mRNA consists only of regions called **exons** that do code for the protein. The ribonucleoproteins in the diagram are small proteins in the nucleus that contain RNA and are needed for the splicing process.
- **Editing** changes some of the nucleotides in mRNA. For example, a human protein called APOB, which helps transport lipids in the blood, has two different forms because of editing. One form is smaller than the other because editing adds an earlier stop signal in mRNA.
- **5′ Capping** adds a methylated cap to the "head" of the mRNA. This cap protects the mRNA from breaking down, and helps the ribosomes know where to bind to the mRNA
- **Polyadenylation** adds a "tail" to the mRNA. The tail consists of a string of As (adenine bases). It signals the end of mRNA. It is also involved in exporting mRNA from the nucleus, and it protects mRNA from enzymes that might break it down.

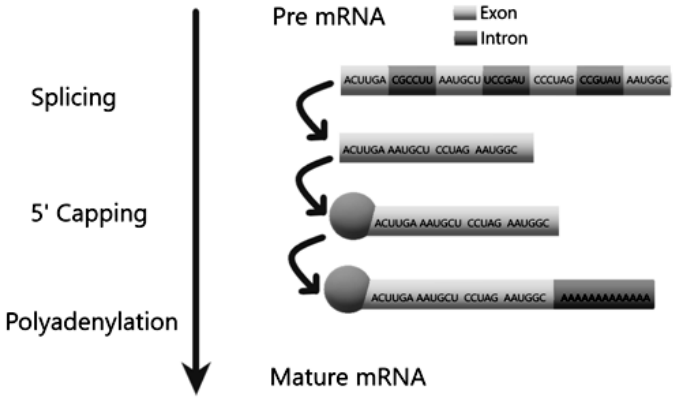


Figure 3.2 Pre mRNA processing

ii. Translation

Translation is the second part of the central dogma of molecular biology.

RNA → Protein

It is the process in which the genetic code in mRNA is read to make a protein. After mRNA leaves the nucleus, it moves to a ribosome, which consists of rRNA and proteins. The ribosome reads the sequence of codons in mRNA, and molecules of tRNA bring amino acids to the ribosome in the correct sequence.

Translation occurs in three stages: Initiation, Elongation and Termination.

a. Initiation

After transcription in the nucleus, the mRNA exits through a nuclear pore and enters the cytoplasm. At the region on the mRNA containing the methylated cap and the start codon, the small and large subunits of the ribosome bind to the mRNA. These are then joined by a tRNA which contains the anticodons matching the start codon on the mRNA. This group of molecues (mRNA, ribosome, tRNA) is called an initiation complex.

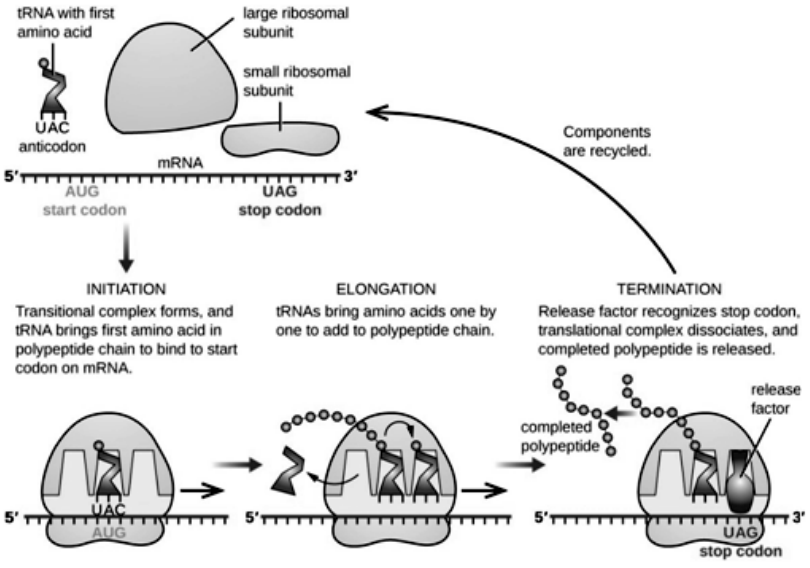


Figure 3.3 Translation takes place in three stages: Initiation, Elongation and Termination

b. Elongation

tRNA keep bringing amino acids to the growing polypeptide according to complementary base pairing between the codons on the mRNA and the anticodons

on the tRNA. As a tRNA moves into the ribosome, its amino acid is transferred to the growing polypeptide. Once this transfer is complete, the tRNA leaves the ribosome, the ribosome moves one codon length down the mRNA, and a new tRNA enters with its corresponding amino acid. This process repeats and the polypeptide grows.

c. Termination

At the end of the mRNA coding is a stop codon which will end the elongation stage. The stop codon doesn't call for a tRNA, but instead for a type of protein called a release factor, which will cause the entire complex (mRNA, ribosome, tRNA, and polypeptide) to break apart, releasing all of the components.

3.4 Amino acid Metabolism

The dietary proteins consist of 20 amino acids which exhibit L-configuration.

3.4.1 Essential amino acids (Indispensable amino acids)

Indispensable amino acids are those which cannot be synthesized in the body but must be supplied through food. These amino acids are:

- Arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine.

3.4.2 Non-essential amino acids (Dispensable amino acids)

Dispensable amino acids are those which can be synthesized at the desired rates in the body. These amino acids are:

- Alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine.

After digestion in the alimentary canal, the amino acids are absorbed through the intestinal epithelium into the blood.

3.5 Ornithine Cycle

Ammonia produced by deamination is highly toxic. It is converted into urea in liver. The formation of urea is known as urea cycle or ornithine cycle or Krebs-Henseleit cycle. The various steps of this cycle are as follows:

Step 1: Synthesis of Carbamyl phosphate - One molecule of NH3 reacts with CO2 to produce carbamyl phosphate. ATP donates phosphate group. Carbamyl phosphate synthetase catalyses the reaction in the presence of Mg^{+2} and N-acetyl glutamic acid.

$$CO2 + NH3 + 2ATP \xrightarrow[Mg+2]{\text{Carbamyl phosphate synthetase}} \text{Carbamyl phosphate} + 2ADP + Pi$$

Step 2: Synthesis of Citrulline – Carbamyl phosphate reacts with ornithine in the presence of ornithine carbamyl transferase to produce citrulline.

$$\text{Ornithine} + \text{Carbamyl phosphate} \xrightarrow{\text{Ornithine carbamyl transferase}} \text{Citrulline} + Pi$$

Step 3: Synthesis of Arginosuccinic acid – Citrulline reacts with aspartic acid (which injects one more NH3) to produce arginosuccinic acid. The reaction is catalysed by arginosuccinate synthetase in the presence of Mg^{+2}. ATP provides energy.

$$\text{Citrulline} + \text{Aspartic acid} + ATP \xrightarrow{\text{Arginosuccinate synthetase}} \text{Arginosuccinic acid} + AMP + Pi$$

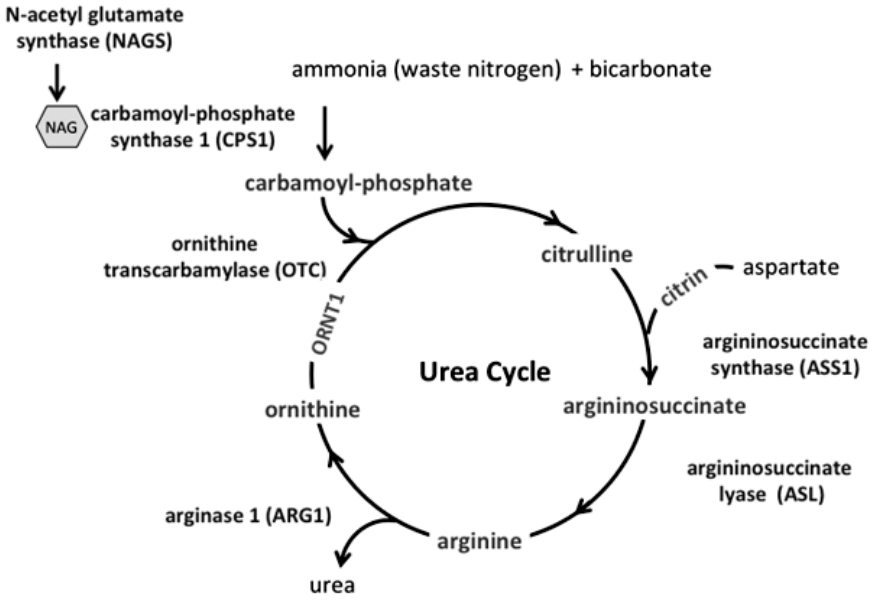


Figure 3.4 Ornithine Cycle

Step 4: Cleavage of Arginosuccinic acid: Arginosuccinase catalyses the cleavage of arginosuccinic acid into arginine and fumaric acid. The fumaric acid enters the Krebs cycle.

$$\text{Arginosuccinic acid} \xrightarrow{\text{Arginosuccinase}} \text{Arginine + Fumaric acid}$$

Step 5: Hydrolysis of Arginine: Arginine undergoes hydrolysis releasing urea and regenerating ornithine. This reaction is catalysed by the enzyme arginase.

$$\text{Arginine} + H_2O \xrightarrow{\text{Arginase}} \text{Urea + ornithine}$$

Urea is the main excretory products of ureotelic animals. It is less toxic than that of NH_3.

3.6 Catabolism of Phenylalanine and Tyrosine

Phenylalanine and tyrosine, the two aromatic amino acids have a common degradative pathway. The degradation is physiologically very important as it produces various substances of importance, namely thyroxine, adrenaline, melanin etc. The various steps of the catabolism are:

1. Phenylalanine undergoes hydroxylation to form tyrosine. This reaction is irreversible and is catalyzed by Phenylalanine hydroxylase which incorporates one oxygen atom from molecular oxygen into phenylalanine to yield tyrosine. The other oxygen atom is reduced to water. NADPH acts as a reducing agent which functions with another reductant namely tetrahydrobiopterin (a coenzyme which is a folic acid derivative).
2. Tyrosine formed from phenylalanine or taken from the diet enters into any one of the pathways mentioned below:

1. Acetoacetate and Fumarate Pathway:

This is the major pathway of degradation and most of the tyrosine and phenylalanine undergo degradation through this pathway. The various reactions in the pathway are:

i. Tyrosine is transaminated to parahydroxyphenyl pyruvic acid.

ii. Parahydroxyphenyl pyruvic acid is oxidized to homogentisic acid. Ascorbic acid is essential for this reaction which acts as a cofactor. The oxidation step is very complex which involves hydroxylation of the phenylring and decarboxylation, oxidation and migration of the side chain.

iii. The aromatic ring of homogentisic acid is then cleaved by oxidative reaction catalyzed by homogentisic acid oxidase resulting in 4-maleylacetoacetic acid. This reaction also needs ascorbic acid.

iv. 4-Maleylacetoacetic acid is isomerized to 4-fumaryl acetoacetic acid.

v. 4-Fumarylacetoacetic acid on hydrolysis by the enzyme fumarylacetoacetate hydrolase form fumaric acid (oxidized via citric acid cycle) and acetoacetate (a ketone body) hence phenylalanine and tyrosine are both glucogenic and ketogenic.

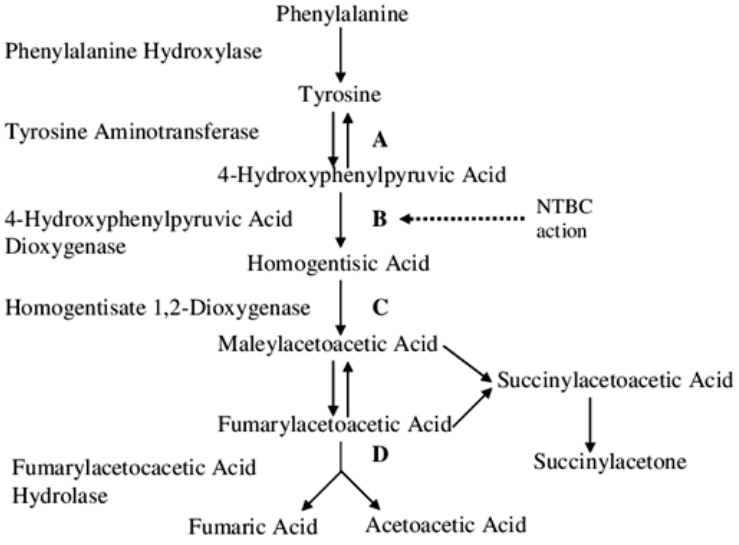


Figure 3.5 Acetoacetate and Fumarate Pathway

2. Epinephrine (adrenaline) Pathway:

i. Tyrosine is first oxidized to form 3,4-dihydroxy phenylalanine (DOPA) in the presence of tyrosine hydroxylase with tetrahydropteridine as cofactor.

ii. DOPA is then decarboxylated by a decarboxylating enzyme to dopamine (hydroxy tyramine). Pyridoxal phosphate acts as a cofactor in this reaction.

iii. Dopamine is further hydroxylated to nor-epinephrine and this reaction, catalyzed by dopamine hydroxylase needs ascorbic acid and molecular oxygen.

iv. Nor-epinephrine is converted to epinephrine by transmethylation and the active methionine (S-adenosyl methionine) is the source of methyl group.

3. Pathway to Melanin:

i. Tyrosine is oxidized to dihydroxy phenylalanine (DOPA) by tyrosinase in the presence of ascorbic acid which acts as a cofactor. This reaction occurs in specialized cells called melanocytes located in skin and eyes.

ii. DOPA is further oxidized to dopaquinone.

iii. Dopaquinone is further converted into 5,6-dihydroxy indole-2-carboxylic acid.

iv. 5,6-dihydroxy indole-2-carboxylic acid is oxidized to dihydroxy indole which then polymerises to melanin.

4. Pathway to Thyroxine:

This pathway leading to the synthesis of the hormone thyroxine occurs in the thyroid gland. The various steps of conversion are:

i. Tyrosine is iodinated at the 3rd position to form monoiodotyrosine.

ii. This is followed by the second iodination in the 5th position to form 3,5-di-iodotyrosine.

iii. Two molecules of di-iodotyrosine undergoes oxidative coupling to form one molecule of tetra-iodotyrosine, the hormone thyroxine. In this process, alanine is also eliminated.

iv. Coupling of one molecule of mono-iodotyrosine and one molecule of di-iodotyrosine results in the production of tri-iodothyronine.

Other Pathways:

i. A minor pathway in which tyrosine gets decarboxylated to tyramine which occurs in the kidney.

ii. Another minor pathway in which phenolic hydroxyl group of tyrosine conjugates with sulphate to form tyrosine-O-sulphate. This ester is a constituent of the peptide liberated in the transformation of fibrinogen to fibrin.

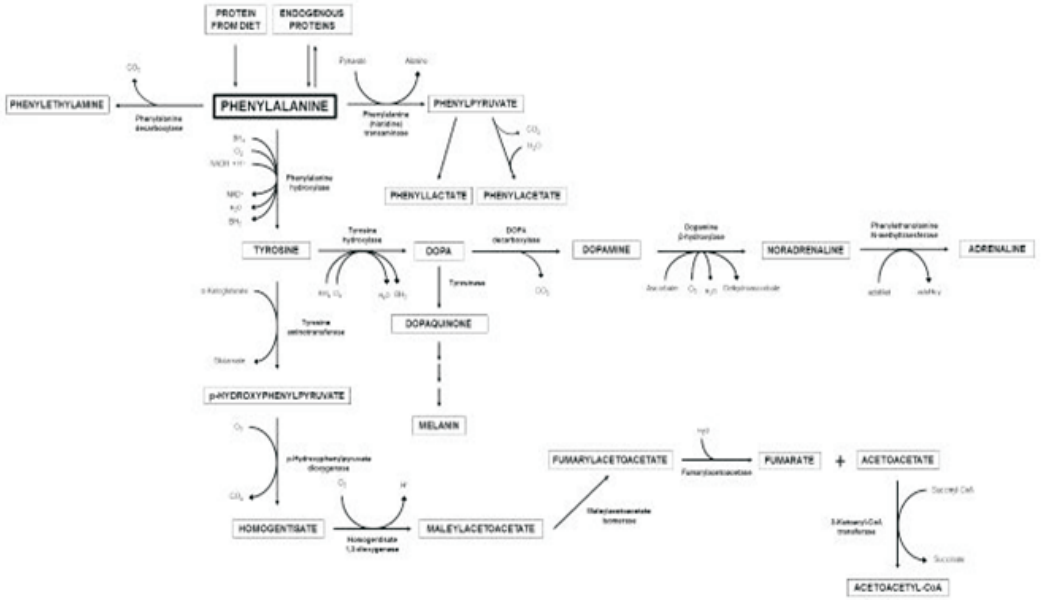


Figure 3.6 Epinephrine Pathway

3.7 Metabolism of Phenylalanine

3.7.1 Catabolism of Tryptophan:

The catabolism of tryptophan involves several pathways and it is both ketogenic and glucogenic. The various pathways are as follows:

1. Kynurenine-anthranilic acid pathway

This is the major pathway in the catabolism of tryptophan and the intermediates of this pathway are associated with some minor pathways which also result in the production of substances of physiological interest.

The various reactions of the pathway are:

i. The first step in the catabolism is catalyzed by the enzyme 2,3-dioxygenase also called tryptophan pyrrolase. This enzyme contains copper and heme groups which oxidizes tryptophan in the presence of molecular oxygen to N-formyl-L-kynurenine. In some human beings the enzyme is genetically defective, giving rise to mental retardation.

ii. Kynurenine formylase catalyzes the hydrolytic removal of the formyl group of N-formyl-kynurenine producing kynurenine.

iii. Kynurenine may take two different pathways.

   a. It may be hydroxylated to 3-hydroxy kynurenine by the enzyme kynurenine hydroxylase with molecular oxygen in the presence of NADPH.

   b. It may undergo spontaneous ring formation to form kynurenic acid. This is one of the side steps and not occurring in the main pathway.

iv. 3-hydroxy kynurenine of the major pathway may again undergo any one of the following steps.

   a. 3-hydroxy kynurenine is cleaved to alanine and 3-hydroxy anthranilic acid which is catalyzed by the enzyme kynureninase that needs Vitamin B6 (pyridoxal phosphate) as coenzyme. Alanine which is got in this step enters the citric acid cycle to be converted to glucose hence tryptophan is glucogenic.

   b. In deficiency of Vitamin B6 in mammals, large amount of kynurenine derivatives reach the extra hepatic tissues (for Eg.Kidney) where they are converted to xanthurenic acid which is found in the urine of mammals deficient in Vitamin B6.

   c. In some insects 3-hydroxy kynurenine is utilized as precursor of the pigment ommochrome.

v. 3-hydroxy anthranilic acid of the major pathway is then oxidized to 2-acroleyl-3-amino fumaric acid by the specific oxidase namely 3-hydroxy anthranilic acid oxidase.

vi. 2-acroleyl-3-amino fumaric acid is very unstable and it may take 2 different routes of catabolism.

   a. In the first route 2-acroleyl-3-amino fumaric acid is decarboxylated to form 2-amino muconic acid 6-semialdehyde.

   b. In the second route 2-acroleyl-3-amino fumaric acid is dehydrated to quinolinic acid which on decarboxylation produces nicotinic acid. This reaction is only a side step which further forms nicotinamides (NAD+ and NADP+).

vii. In the major pathway, 2-aminomuconic acid-6-semialdehyde on deamination and oxidation produces oxalocrotonic acid.

viii. Oxalocrotonic acid on reduction forms α-ketoadipic acid.

ix. α-ketoadipic acid is ultimately converted into acetoacetyl CoA and thus tryptophan is ketogenic.

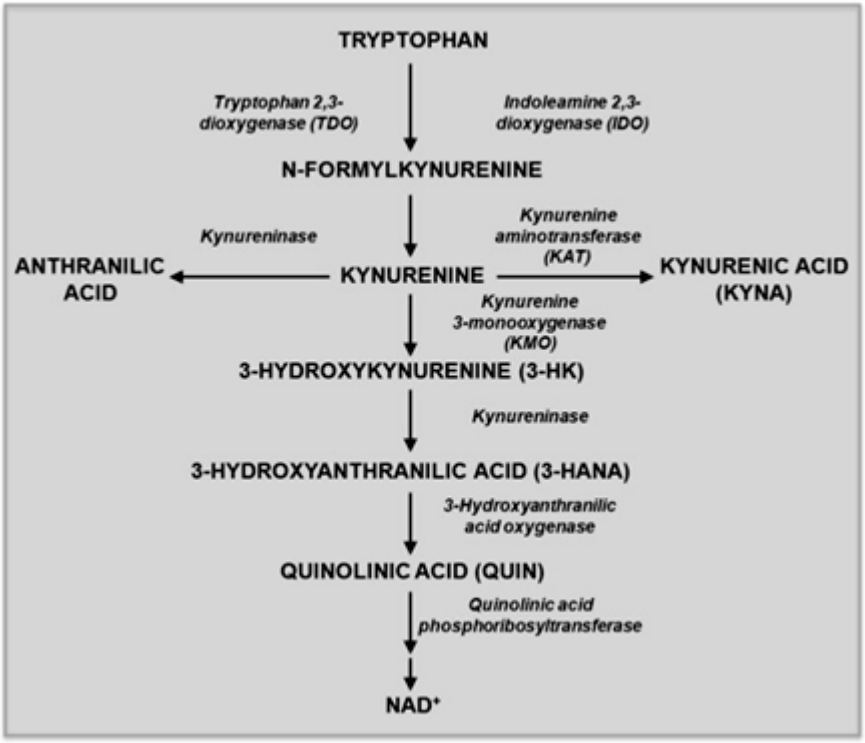


Figure 3.7 Kynurenine-anthranilic acid pathway

2. Serotonin Pathway

i. In the second pathway, namely serotonin pathway, tryptophan on dehydroxylation in the liver forms 5-hydroxy tryptophan by a hydroxylase enzyme.

ii. Next, 5-hydroxy tryptophan on decarboxylation produces 5-hydroxy tryptamine which is otherwise known as serotonin. Serotonin is a neurohormone and a vasoconstrictor in vertebrates. It also stimulates the contraction of smooth muscles. It is stored in blood platelets, gastro-intestinal tract and central nervous system. When platelets disintegrate during blood clotting, serotonin is liberated. Serotonin enters into two different minor pathways.

a. Serotonin is oxidatively deaminated to 5-hydroxy indole acetic acid (5-HIAA) by monoamine oxidase. About 5 to 10 mg of 5-HIAA is normally

excreted in the urine every 24 hours but when there is tumour of the argentaffin cells of the small intestine (malignant tumour) large amount of 5-HIAA are excreted in the urine and the amount of serotonin in the blood is also high.

b. Serotonin on acetylation with acetyl CoA and further methylation produces N-acetyl-5-methoxy tryptamine which is a hormone, otherwise known as melatonin. This is the hormone of the pineal gland and peripheral nerve of mammals. This hormone in frog has a lightening effect on the colour of the skin.

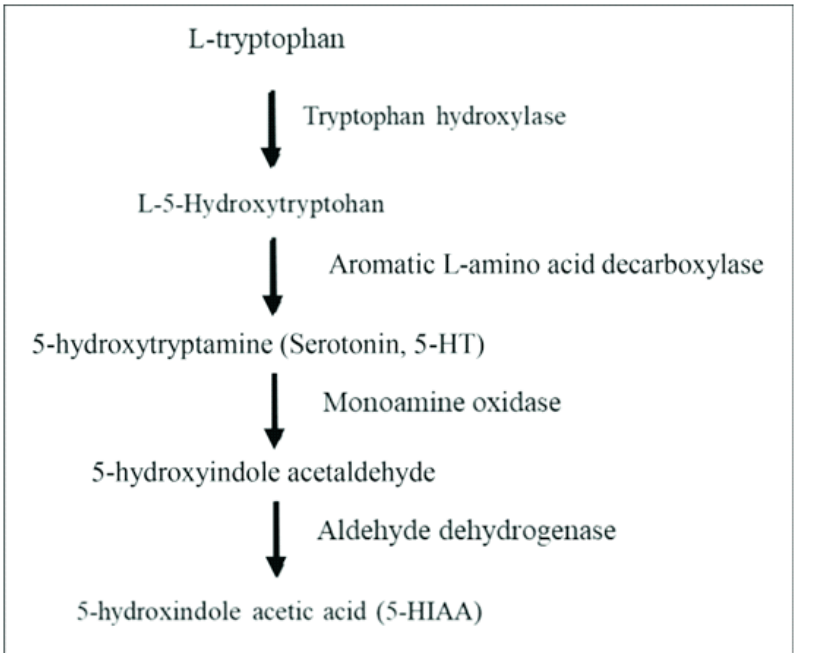


Figure 3.8 Serotonin Pathway

Hartnup Syndrome: It is an inborn error in the metabolism of tryptophan. It leads to increased excretion of tryptophan derivatives like indoleacetic acid and tryptophan itself, skin rash, mental deterioration.

Other pathways

1. Formation of Indole Acetic Acid: Under certain conditions, such as are found in the colon of human beings, tryptophan is converted to indole acetic acid and the various steps of conversion are:

a. Tryptophan is oxidatively deaminated to form indole acetic acid. In plants indole acetic acid functions as a growth hormone.

b. In humanbeings indole acetic acid is broken down by the intestinal bacteria to produce the evil smelling compounds skatole and indole which are responsible for the characteristic foul smell of the faeces and the related substance indoxyl which appears both in the faeces and urine.

2. Formation of Tryptamine: Some amount of tryptophan is converted by tryptophan carboxylase into tryptamine which is the precursor of the plant growth hormone indole acetic acid.

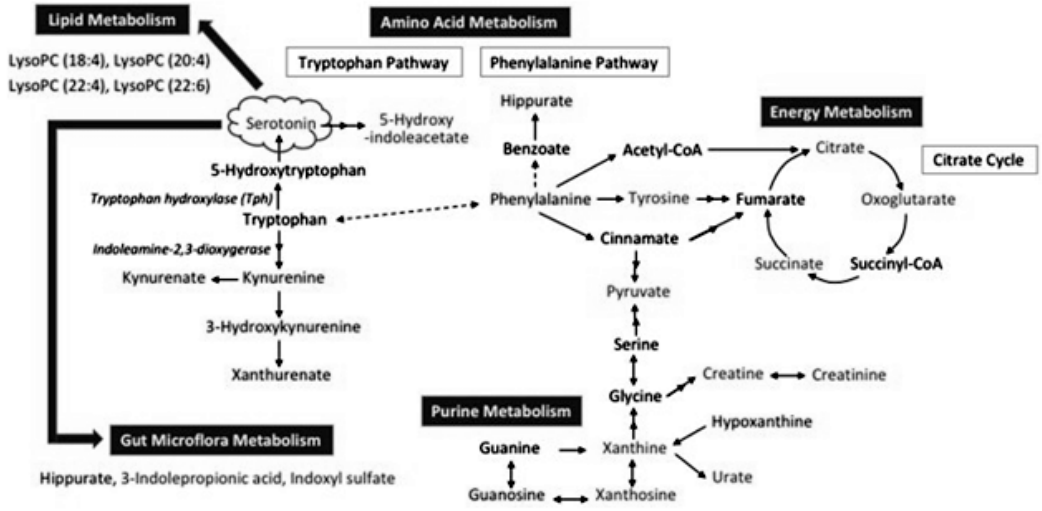


Figure 3.9 Amino Acid Metabolism

CHAPTER

4 LIPID METABOLISM

4.1 Introduction

Lipid metabolism refers to all chemical reactions concerned with lipids taking place in the cells. It includes anabolism and catabolism. Anabolism is the synthesis of lipids and catabolism is the breakdown of lipids. Lipids play a major role in the nutrition of man and other animals. The energy value of fat is extremely high when compared to carbohydrate or protein. One gram of fat releases 9.3 calories of energy; at the same time one gram of carbohydrate releases only 4.2 calories.

4.2 Catabolic Pathway

Fatty acid catabolism is the mechanism by which the body accesses energy stored as triglycerides. There are three steps in fatty acid catabolism. First the body must mobilize the lipid stores by breaking down triglycerides into free fatty acids and glycerols. Catabolism of fat (lipolysis) involves two separate pathways, glycerol pathway and fatty acids pathway.

4.2.1 Oxidation of glycerol

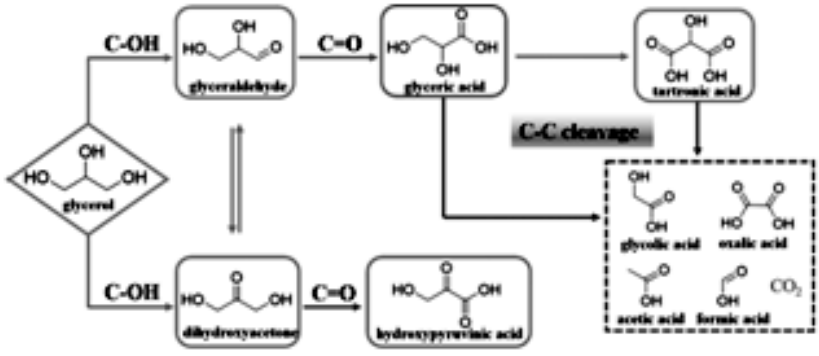


Figure 4.1 Oxidation of Glycerol

1. Glycerol with ATP converted into glycerol-3-phosphate by glycerol kinase and release one inorganic phosphate yields ADP.

2. Then, it is oxidized by NAD^+ into dihydroxyacetone-phosphate using glycerol-phosphate dehydrogenase. Theoxidized products will enter glycolysis pathway and produce energy.

4.2.2 Oxidation of fatty acids

Fatty acid catabolism through beta oxidation (the broken down process of Acyl-CoA molecules into Acetyl-CoA) occurred in mitochondria and/or in peroxisomes. Betaoxidation will produce:

1. Two-carbon acetic acid fragments, which are converted to acetyl-CoA and enter the Krebs cycle.
2. Reduced coenzymes, will enter the electron transportchain.
3. An acetyl-CoA used in the Krebs Cycle will make one ATP, 3 $NADH^+$, H^+ and 1 $FADH_2$. If a fatty acid has 18 carbon units, then 9 acetyl CoA units would be made.

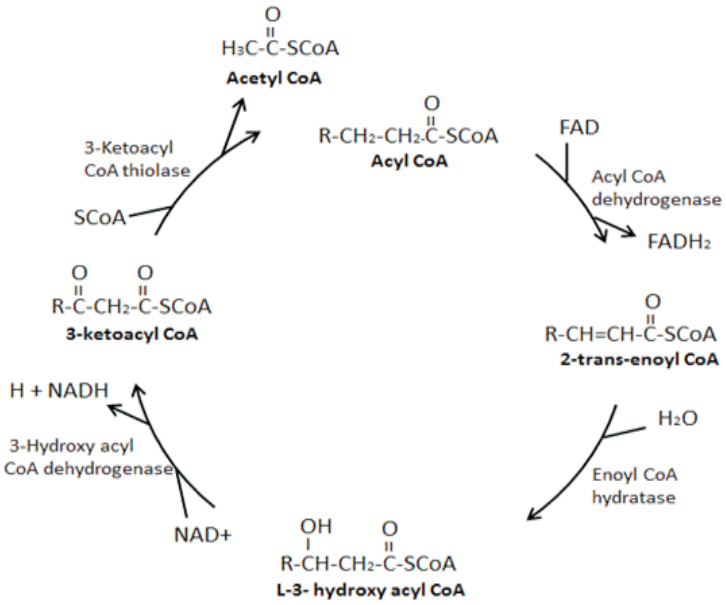


Figure 4.2 Oxidation of Fatty Acids

4.3 β - Oxidation

β – oxidation was first proposed by Knoop in 1905. In β – oxidation, the fatty acid is oxidised at the β carbon atom (second carbon atom from the carboxyl group) and is converted into fatty acid having two carbon atoms less and acetyl CoA.

This process is repeated where two carbon atoms are removed in each oxidation until a four carbon atom residue is left as butyric acid. The butyric acid is also oxidised in the β-position to form aceto-acetic acid. Both of these compounds undergo final oxidation into CO2 and water.

β – oxidation occurs in mitochondria. The acetyl CoA formed in β-oxidations of fatty acids is oxidised to CO2 and H2O with the release of energy through Krebs cycle. β-oxidation has the following steps:

1. Activation
2. Dehydrogenation I
3. Hydration
4. Dehydrogenation II
5. Thiolytic cleavage

1. Activation

The fatty acids, as such, are inert chemically. So in the first step, they are activated. The enzyme thiokinase (acyl-CoA synthetase) in the presence of ATP and CoA converts free fatty acid.

2. Dehydrogenation I

The acyl CoA is then oxidised by the removal of two hydrogen atoms, one form α-carbon and the other from the β-carbon. This reduction is brought about by the enzyme acyl CoA dehydrgenase and coenzyme flavin adenine dinucleotide (FAD). This reduction leads to the formation of an unsaturated double bond –CH=CH- and the substance is called α,β-unsaturated acyl-CoA.

3. Hydration

The α,β - unsaturated acyl-CoA undergoes a process of hydration with the addition of water under the influence of enol hydrase. The resulting compound is called beta-hydroxy acyl-CoA.

4. Dehydrogenation II

The beta-hydroxy acyl –CoA is again dehydrogenated by the removal of 2 hydrogen atoms from the beta carbon. This reaction leads to the formation of the beta-keto-acyl-CoA. It is catalyzed by beta-hydroxy acyl dehydrogenase and the coenzyme NAD functions as the hydrogen acceptor.

5. Thiolysis

The beta-keto-acyl-CoA is unstable and is cleaved into acetyl CoA and an activated fatty acid (acyl CoA), which is shorter by 2 carbon atoms than the original fatty acid. The cleavage is brought about by the addition of a new CoA to the beta carbon of the fatty acid. This reaction is catalyzed by beta-keto-acyl thiolase.

4.3.1 Energetics of Fatty acid Oxidation

The net production of energy from a single molecule of palmitic acid (a fatty acid) is 130 molecules of ATP.

4.3.2 Energetics of β Oxidation

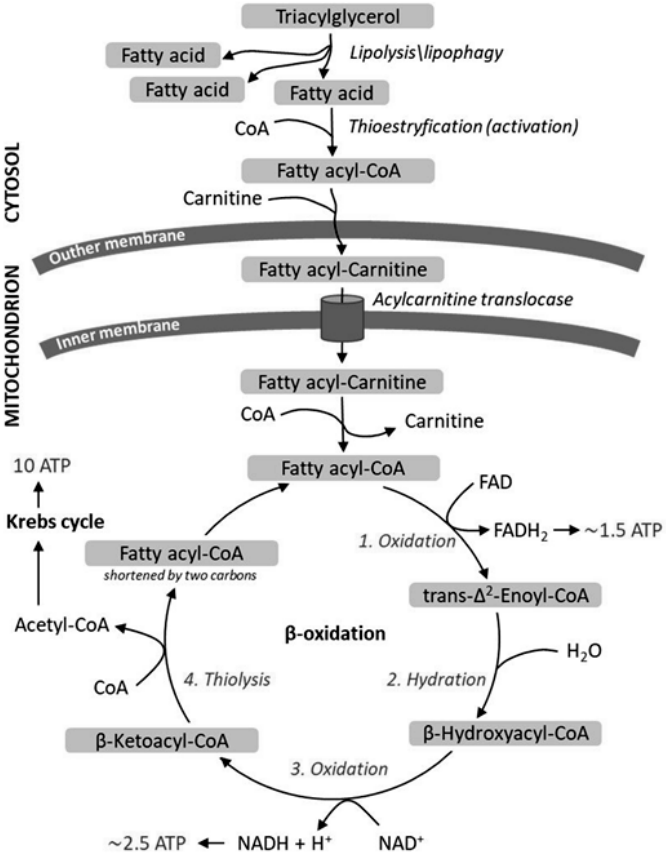


Figure 4.3 β Oxidation

The energetics of beta oxidation can be calculated by taking a typical fatty acid, palmitic acid – $C_{15}H_{31}COOH$.

Number of beta oxidation cycles - 7

Number of acetyl CoA produced - 8

Number of $FADH_2$ produced - 7

Number of $NADH_2$ produced - 7

Number of ATP produced by one acetyl CoA in Krebs cycle - 12

Number of ATP produced by 8 acetyl CoA – $8 \times 12 =$ 96

Number of ATP form each $FADH_2$ - 3

Number of ATP form 7 $FADH_2$ – $7 \times 3 =$ 21

Number of ATP form each $NADH_2$ – 2

Number of ATP form 7 $NADH_2$ – $7 \times 2 = 14$

131

ATP consumed for fatty acid activation 1

Net gain 130

So each palmitic acid can produce 130 ATP molecules.

4.4 Ketogenesis

- The process of formation of theketone bodies is known as ketogenesis.
- Acetoacetate, 3-hydroxybutryic acid and acetone are collectively referred to as ketone bodies (also called acetone bodies or ketones).
- Ketogenesis occurs only in the mitochondria of liver and the ketone bodies which are water soluble, lipid fuels are released continuously.
- Ketogenesis occurs when fatty acids undergo excessive oxidation in the liver, producing large amount of acetyl CoA. The entry of acetyl CoA into Kreb's cycle depends on the availability of oxaloacetate.
- When the amount of oxaloacetate is less, the acetyl CoA is diverted to form ketone bodies. In normal conditions, when carbohydrates are plenty and glucose is readily available to the tissues, the amount of ketone bodies in the blood is very low (1 mg/100 ml of blood) and the average excretion in the

urine in 24 hours is less than 125 mg. But, if break down of fat predominates, acetyl CoA is diverted to form ketone bodies.

- Certain chemical substances such as ammonia and phlorhizin are found to increase the formation of ketone bodies and such substances are known as ketogenic substances.
- On the other hand substances such as carbohydrates, oxaloacetic acid, thiamine pyrophosphate and α-keto acids such as pyruvic acid and α-keto glutaric acid are known as anti-ketogenic substances, as they are found to lower the formation of ketone bodies.

4.5 Ketosis

- The overall condition of increased concentration of ketone bodies in tissues and fluids is termed ketosis.
- Excretion of abnormally high amount of ketone bodies in urine is known as ketonuria and appearance of high levels of ketone bodies in the blood is known as ketonemia.
- Ketosis may occur due to many different physiological as well as pathological factors such as prolonged starvation, availability of less amount of carbohydrates or high amount of fat in the diet, severe exercise in the post absorptive state, increased metabolic demand (as in pregnancy and lactation) glycogen storage diseases, continued fever, mild pancreatic dysfunction, deficiency of insulin, diabetes mellitus and toxemia of pregnancy.
- In all the above cases there is a diminished utilization of carbohydrates and increased mobilization of fats.

4.5.1 Formation of Ketone Bodies

There are 3 steps in the formation of ketone bodies.

1. In the first step two molecules of acetyl CoA condense to form acetoacetyl CoA with the loss of one molecule of CoA and this reaction is catalyzed by 3-keto thiolase.
2. Next, acetoacetyl CoA reacts with one more molecule of acetyl CoA and H2O to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) in the presence of hydroxyl methylglutaryl synthase. In this process one molecule of CoA is also split off.

3. 3-hydroxy-3-methylglutaryl CoA is then cleaved to acetyl CoA and acetoacetate in the presence of hydroxyl methylglutaryl CoA lyase. Acetoacetate that is formed, spontaneously decarboxylates to form acetone. The odour of acetone may be detected in the breadth of a person who has a high level of acetoacetate in the blood. In the liver acetoacetate may also be reduced to 3-hydroxybutyric acid in the presence of NADH and this reaction is catalyzed by the enzyme 3-hydroxybutyric acid dehydrogenase.
4. Acetoacetate and 3-hydroxybutyric acid are normal fuels of respiration and quantitatively important as sources of energy. Tissues such as heart muscle and renal cortex use acetoacetate in preference to glucose whereas in well nourished individuals, the brain tissue use glucose as the major fuel. However, the brain adapts to the utilization of acetoacetate during starvation and diabetes.

4.6 Ketolysis

- The oxidation of ketone bodies to CO2 and water is known as ketolysis. Acetoacetate and 3-hydroxybutyric acid were carried to extrahepatic tissues such as kidney and muscle where they are converted to acetoacetyl CoA.
- The acetoacetyl CoA is then split by thiolase to acetyl CoA which is then oxidized by way of citric acid cycle to CO_2 and water with the liberation of energy.
- Acetoacetate and 3-hydroxybutyric acid are readily oxidized by extra-hepatic tissues. Whereas acetone is oxidized with difficulty and it is also utilized very slowly.
- Ketone bodies are not toxic if they are properly metabolized by the extra hepatic tissues.
- In this property of removing sodium ions along with them in the process of excretion results in acidosis which is accompanied by excretion of large amounts of water that is necessary to carry the ketone bodies.
- As a result the person becomes dehydrated and passes into a coma stage. In case of severe acidosis, death may result.

4.7 Anabolic Pathway

Lipid anabolism (lipogenesis) is synthesis oflipids on liver cells from amino acids which areconverted to acetyl-CoA and from glucose intoglyceraldehyde 3-phosphate. Both of acetyl-CoAand glyceraldehyde 3-phosphate converted intotriglycerides.

4.8 Biosynthesis of Fatty Acids

- Synthesis of fatty acids occurs in the cytoplasm and endoplasmic reticulum of the cell and is chemically similar to the beta-oxidation process, but with a couple of key differences.
- The first of these occur in preparing substrates for the reactions that grow the fatty acid. Transport of acetyl-CoA from the mitochondria occurs when it begins to build up.
- Two molecules can play roles in moving it to the cytoplasm – citrate and acetylcarnitine. Joining of oxaloacetate with acetyl-CoA in the mitochondrion creates citrate which moves across the membrane, followed by action of citrate lyase in the cytoplasm of the cell to release acetyl-CoA and oxaloacetate.
- Additionally, when free acetyl-CoA accumulates in the mitochondrion, it may combine with carnitine and be transported out to the cytoplasm.
- Starting with two acetyl-CoA, one is converted to malonyl-CoA by carboxylation catalyzed by the enzyme acetyl-CoA carboxylase (ACC), the only regulatory enzyme of fatty acid synthesis.

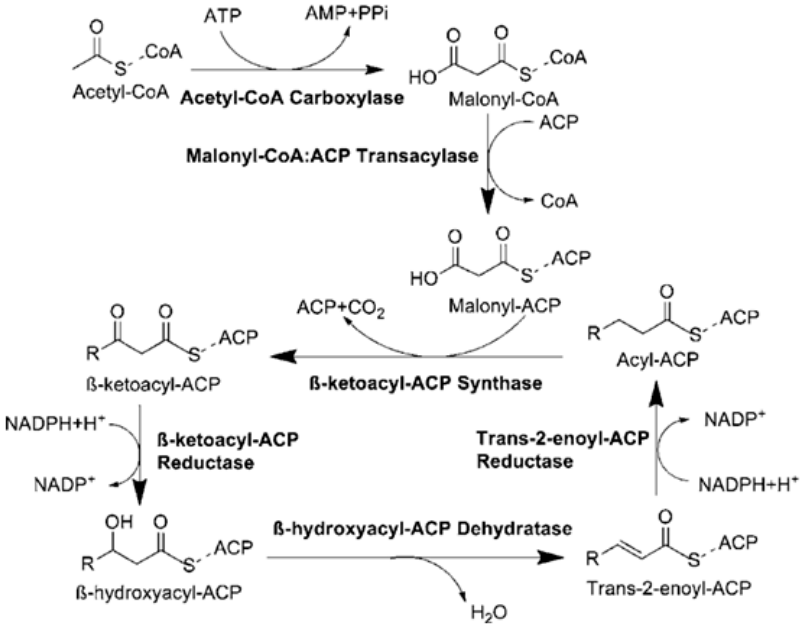


Figure 4.4 Biosynthesis of Fatty Acids

- Both molecules have their CoA portions replaced by a carrier protein known as ACP (acyl-carrier protein) to form acetyl-ACP and malonyl-ACP. Joining of a fatty acyl-ACP (in this case, acetyl-ACP) with malonyl-ACP splits out the carboxyl that was added and creates the intermediate.
- From this point forward, the chemical reactions resemble those of beta oxidation reversed. First, the ketone is reduced to a hydroxyl using NADPH.
- In contrast to the hydroxylated intermediate of beta oxidation, the beta intermediate here is in the D-configuration. Next, water is removed from carbons 2 and 3 of the hydroxyl intermediate to produce a trans doubled bonded molecule. Last, the double bond is hydrogenated to yield a saturated intermediate. T
- This process cycles with the addition of another malonyl-ACP to the growing chain until ultimately an intermediate with 16 carbons is produced (palmitoyl-CoA). At this point, the cytoplasmic synthesis ceases.

4.8.1 Enzymes of Fatty Acid Synthesis

- Acetyl-CoA carboxylase - catalyzes synthesis of malonyl-CoA, is the only regulated enzyme in fatty acid synthesis. Its regulation involves both allosteric control and covalent modification. The enzyme is known to be phosphorylated by both AMP Kinase and Protein Kinase A.
- Dephosphorylation is stimulated by phosphatases activated by insulin binding. Dephosphorylation activates the enzyme and favors its assembly into a long polymer, while phosphorylation reverses the process. Citrate acts as an allosteric activator and may also favor polymerization. Palmitoyl-CoA allosterically inactivates it.
- In animals, six different catalytic activities necessary for the remaining catalytic actions to fully make palmitoyl-CoA are contained in a single complex called Fatty Acid Synthase. These include transacylases for swapping CoA with ACP on acetyl-CoA and malonyl-CoA; a synthase to catalyze addition of the twocarbon unit from the three carbon malonyl-ACP in the first step of the elongation process; a reductase to reduce the ketone; a dehydrase to catalyze removal of water, and a reductase to reduce the trans double bond. In bacteria, these activities are found on separate enzymes and are not part of a complex.

4.8.2 Elongation of Fatty Acids

- Elongation to make fatty acids longer than 16 carbons occurs in the endoplasmic reticulum and is catalyzed by enzymes described as elongases.
- Mitochondria also can elongate fatty acids, but their starting materials are generally shorter than 16 carbons long. The mechanisms in both environments are similar to those in the cytoplasm (a malonyl group is used to add two carbons, for example), but CoA is attached to the intermediates, not ACP. Further, whereas cytoplasmic synthesis employs the fatty acid synthase complex, the enzymes in these organelles are separable and not part of a complex.

4.8.3 Desaturation of Fatty Acids

- Fatty acids are synthesized in the saturated form and desaturation occurs later. Enzymes called desaturases catalyze the formation of cis double bonds in mature fatty acids. These enzymes are found in the endoplasmic reticulum.
- Animals are limited in the desaturated fatty acids they can make, due to an inability to catalyze reactions beyond carbons 9 and 10. Thus, humans can make oleic acid, but cannot synthesis linoleic acid or linolenic acid. Consequently, these two must be provided in the diet and are referred to as essential fatty acids.

4.9 Synthesis of Triglycerides

- Glycerol accepts fatty acids from acyl-CoAs to synthesize glycerol lipids. Glycerol phosphate comes from glycolysis—specifically from the reduction of dihydroxyacetone phosphate using NADH as a cofactor.
- Then the glycerol phosphate accepts two fatty acids from fatty acyl-CoA. The fatty acyl-CoA is formed by the expenditure of two high-energy phosphate bonds from ATP.
- Fatty acyl-CoA is the donor of the fatty acyl group to the two non-phosphorylated positions of glycerol phosphate to make a phosphatidic acid.
- The third fatty acid can be added after the removal of the phosphate of the phosphatidic acid. This scheme results in a triacylglycerol, although other phosphatidic acids can be used as precursors to various membrane lipids.

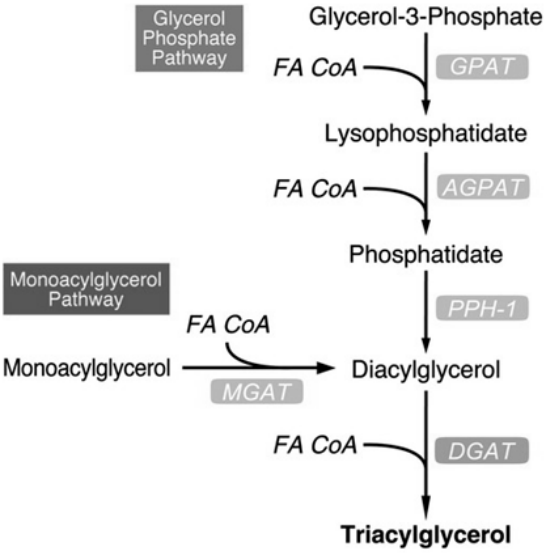


Figure 4.5 Synthesis of Triglycerides

4.10 Disorders of Fat Metabolism

4.10.1 Hypercholestrolemia

Hypercholesterolemia is an autosomal dominant disease is caused by the deficiency of the LDL receptor on the surface of cells in the liver and other organs. As a result, cholesterol is not moved into the cells. Under normal conditions, when enough cholesterol is present in the cell, feedback mechanisms signal enzymes to cease cholesterol synthesis. In familial hypercholesterolemia, these enzymes are relieved of feedback inhibition, thus inducing the production of still more cholesterol. The disease is characterized by early coronary vascular disease, strokes, and fatty deposits on the tendons. Blood cholesterol levels are very high from birth, and LDL cholesterol is also elevated. Treatment is by a low-cholesterol diet and drugs that inhibit cholesterol synthesis or increase its excretion in the gastrointestinal tract.

4.10.2 Hyperlipoproteinemia

Hyperlipoproteinemia is a common disorder. It results from an inability to break down lipids or fats in your body, specifically cholesterol and triglycerides. There are several types of hyperlipoproteinemia. The type depends on the concentration of lipids and which are affected. High levels of cholesterol or triglycerides are serious because they're associated with heart problems.Hyperlipoproteinemia can be a primary or secondary condition.

- Primary hyperlipoproteinemia is often genetic. It's a result of a defect or mutation in lipoproteins. These changes result in problems with accumulation of lipids in your body.
- Secondary hyperlipoproteinemia is the result of other health conditions that lead to high levels of lipids in your body.

These include:

- diabetes
- hypothyroidism
- pancreatitis
- use of certain drugs, such as contraceptives and steroids
- certain lifestyle choices

4.10.3 Atherosclerosis

Atherosclerosis is a hardening and narrowing of your arteries caused by cholesterol plaques lining the artery over time. It can put blood flow at risk as your arteries become blocked.It's the usual cause of heart attacks, strokes, and peripheral vascular disease together are known ascardiovascular disease.

Arteries are blood vessels that carry blood from your heart throughout your body. They're lined by a thin layer of cells called the endothelium. It keeps the inside of your arteries in shape and smooth, which keeps blood flowing.

Atherosclerosis begins with damage to the endothelium. Common causes include:

- High cholesterol
- High blood pressure
- Inflammation, like from arthritis or lupus
- Obesity or diabetes
- Smoking

CHAPTER

5 Metabolism of Nucleic Acids

5.1 Introduction

- Nucleotides consist of a nitrogenous base, a pentose and a phosphate.
- The pentose sugar is D-ribose in ribonucleotides of RNA while in deoxyribonucleotides of DNA, the sugar is 2- deoxy D-ribose.
- Nucleotides participate in almost all the biochemical processes, either directly or indirectly.
- They are the structural components of nuclei acid, coenzymes and are involved in the regulation of several metabolic reactions.

5.2 Biosynthesis of Purine Ribonucleotides

Many compounds contribute to the purine ring of the nucleotides.

1. N_1 of purine is derived from amino group of aspartate
2. C_2 and C_8 arise from formate of N10- formyl THF
3. N_3 and N_9 are obtained from amide group of glutamine
4. C_4, C_5 and N- are contributed by glycine.
5. C_6 directly comes from CO_2.

It should be remembered that purine bases are not synthesized as such, but they are formed as ribonucleotides. The purines are built upon a pre-existing ribose 5- phosphate. Liver is the major site for purine nucleotide synthesis erythrocytes, polymorphonuclear leukocytes and brain cannot produce purines.

The pathway for the synthesis of inosine monophosphate, the parent purine nucleotide. The reaction are briefly described in the next column.

1. Ribose 5-phosphate, produced in the hexose monophosphate shunt of carbohydrate metabolism is the starting material for purine nucleotide synthesis. It reacts with ATP to form phosphoribosyl pyrophosphate.

2. Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate and produce 5-phosphoribosylamine. The enzyme PRPP glutamyl amidotransferase is controlled by feedback inhibition of nucleotides. This reaction is the committed step in purine nucleotide biosynthesis.

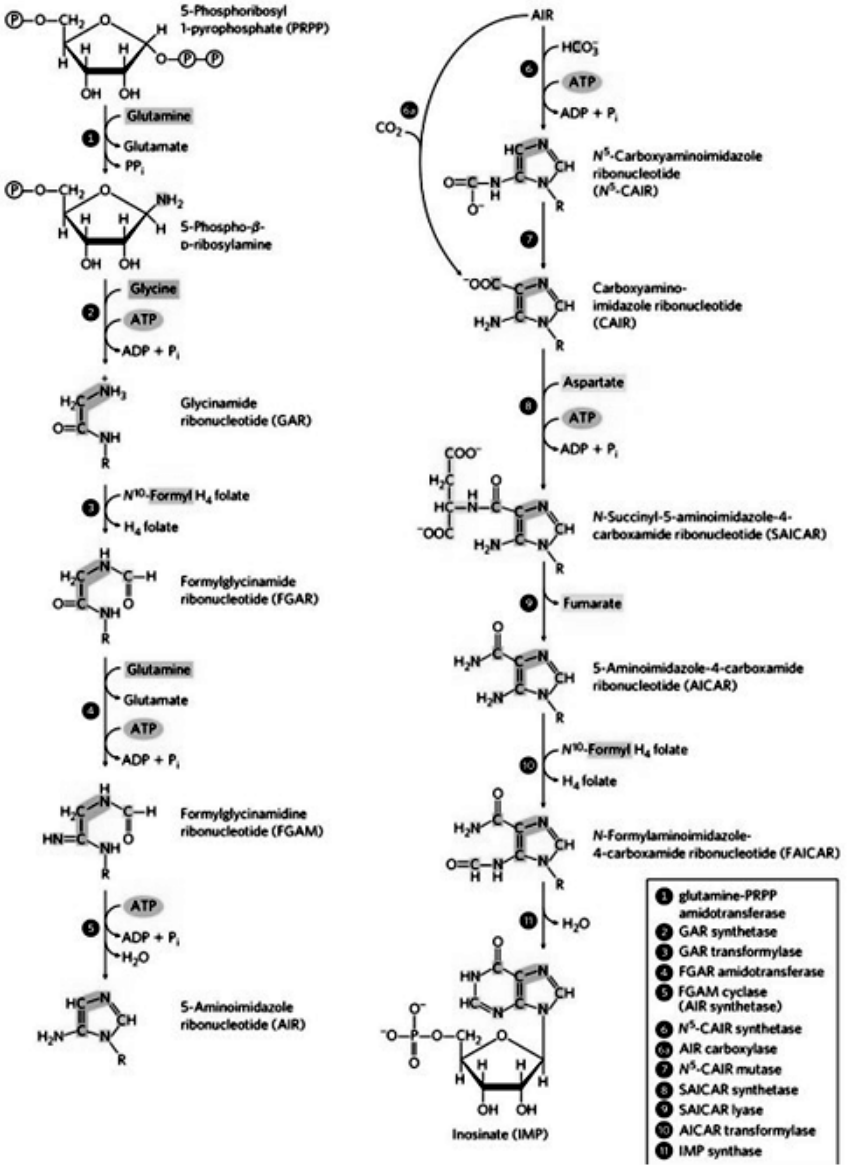


Figure 5.1 Biosynthesis of Purine Bionucleotides

3. Phosphoribosylamine reacts with glycine in the presence of ATP to form glycinamide ribosyl 5-phosphate or glycinamide ribotide.
4. N^{10} formyl tetrahydrofolate donates the formyl group and the product formed is formyl glycinamide ribosyl 5-phosphate.
5. Glutamine transfers the second amido amino group to produce formylglycinamidine ribosyl 5-phosphate.
6. The imidazole ring of the purine is closed in an ATP dependent reaction to yield 5 amino imidazole ribosyl 5-phosphate.
7. Incorporation of co2 occurs to yield aminoimidazole carboxylate ribosyl 5-phosphate. This reaction does not require the vitamin biotin and or ATP which is the case with most of the carboxylation reactions.
8. Aspirate condenses with the product in reaction 7 to form aminoimidaze 4-succinyl carboxamide ribosyl 5-phosphate
9. Adenosuccinate lyase cleaves off fumarate and only the amino group of asparatae is retained to yield aminoimidazole 4- carboxamide ribosyl 5-phosphate,
10. N^{10} formyl tetrahydrofolate donates a one carbon moiety to produce formamino imidazole 4 carboxamide ribosyl 5-phosphate with this reaction all the carbon and nitrogen atoms of purine rings are contributed by the respective sources.

5.2.1 Inhibitors of Purine Synthesis

Folic acid (THF) is essential for the synthesis of purine nucleotide (reaction 4 and 10). Sulfonamides are the structural analogs of para-aminnobenzoic acid (PABA). These sulfa drugs can be used to inhibit the synthesis of folic acid by microorganisms. This indirectly reduces the synthesis of purines and therefore, the nucleic acids (DNA and RNA). Sulfonamides have no influence on humans, since folic acid is not synthesized and is supplied through diet.

The structural analogs of folic acid (e.g. methotrexate) are widely used to control cancer. They inhibit the synthesis of purine nucleotides (reaction 4 and 10) and thus, nucleic acid. Both these reactions are concerned with the transfer of one carbon moiety (formyl group). These inhibitors also effect the proliferation of normally growing cells. This causes many side effects including anemia, baldness, scaly skin etc.

5.2.2 Synthesis of AMP and GMP from IMP

Inosine monophosphate is the immediate precursor for the formation of AMP and GMP. Aspartate condenses with IMP in the presence of GTP to produce adenylsuccinate which, on cleavage forms AMP.

For the synthesis of GMP, IMP undergoes NAD dependent dehydrogenation to form xanthosine monophosphate (XMP). Glutamine then transfers amide nitrogen to XMP to produce GMP.

6-Mercaptopurine is an inhibitor of the synthesis of AMP and GMP. It acts in the enzymes adenylsuccinase (of AMP pathway) and IMP dehydrogenase (of GMP pathway).

5.2.3 Formation of Purine Nucleoside Diphosphates and Triphosphates

The nucleoside monophosphate (AMP and GMP) have to be converted to the corresponding di and triphosphates to participate in most of the metabolic reactions. This is achieved by the transfer of phosphate group from ATP, catalysed by nucleoside monophosphate (NMP) kinases and nucleoside diphosphate (NDP) kinases.

5.3 Salvage Pathway For Purines

The free purines (adenine, guanine and hypoxanthine) are formed in the normal turnover of nucleic acid (particularly RNA) and also obtained from the dietary sources. The purines can be directly converted to the corresponding nucleotides and this process is known as salvage pathway.

Adenine phosphoribosyl transferase catalyses the formation of AMP from adenine. Hypoxanthine- guanine phosphoribosyl transferase (HGPRT) converts guanine and hypoxanthine respectively to GMP and IMP. Phosphoribosyl pyrophosphate (PRPP) is the donor of ribose 5-phosphate in the salvage pathway.

The salvage pathway is particularly important in certain tissues such as erythrocytes and brain where de novo (a new) synthesis of purine nucleotides is not operative.

A defect in enzyme HGPRT causes Lesch Nyhan syndrome.

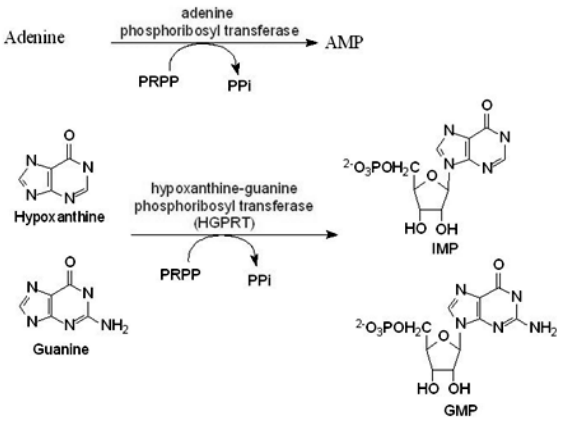


Figure 5.2 Purine Salvage Pathway

5.4 Regulation of Purine Nucleotide Biosynthesis

The purine nucleotide synthesis is well coordinated to meet the cellular demands. The intracellular concentration of PGPR regulates purine synthesis to a large extent. This, in turn is dependent on the availability of ribose 5-phosphate and the enzyme PROP synthetase.

PRPP glutamyl amidotransferase is controlled by a feedback mechanism by purine nucleotides. That is, if AMP and GMP are available in adequate amounts to meet the cellular requirements their synthesis is turned off at the amidotransferase reaction.

Another important stage of regulation is in the conversion of IMP to AMP and GMP. AMP inhibits adenylsuccinate synthetase while GMP inhibits IMP dehydrogenase. Thus, AMP and GMP control their respective synthesis from IMP by a feedback mechanism.

5.4.1 Conversion of Ribonucleotides to Deoxyribonucleotides

The synthesis of purine and pyrimidine deoxyribonucleotides occurs from ribo nucleotides by a reduction at the C2 of ribose moiety. This reaction is catalysed by a multisubunit enzyme ribonucleotides reductase.

5.4.2 Supply of reducing equivalents

The enzyme ribonucleotides reductase itself provides the hydrogen atoms needed for reduction from its sulfhydryl groups. The reducing equivalents, in turn are supplied by thioreedoxin a monomeric protein with two cysteine residues.

NADPH-dependent thioredoxin reductase converts the oxidized thioredoxin to reduced form which can be recycled again and again. Thioredoxin thus serves as a protein cofactor in an enzymatic reaction.

5.4.3 Regulation of deoxyribonucleotides synthesis

Deoxyribbonucleotides are mostly requires for the synthesis of DNA. The activity of the enzyme ribonucleotides reductase maintains the adequate supply of deoxyribonucleotides.

Ribonucleotides reductase is a complex enzyme with multiple sies (active sites and allosteric sites) that control the formation of deoxyribonucleotides.

5.5 Degradation of Purine Metabolism

The end product of purine metabolism in humans is uric acid. The sequence of reactions in purine nucleotide degradation is given in

1. The nucleotides monophosphates (AMP, IMPand GMP)are converted to their respective nucleoside forms (adenosine, inosine and guanosine) by the action of nucleotidase .
2. The amino group, either from AMP or adenosine, can be removed to produce IMP or inosinerespectively.
3. Inosine and guanosine are, respectively, converted to hypoxanthine and guanine (purine bases) by purine nucleoside phosphorylase. Adenosine is not degraded by this enzyme, hence it has to be converted to inosine.
4. Guanosine undergoes deamination by guanase to form xanthine.
5. Xanthine oxidase is an important enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid. This enzyme contains FAD, molybdenum and iron, and is exclusively found in liver and small intestine. Xanthine oxidase liberates H_2O_2 which is harmful to the tissues. Catalase cleaves H_2O_2 to H_2O and O_2.

Uric acid is the final excretory product of purine metabolism in humans. Uric acid can serve as an important antioxidant by getting itself converted to allantoin it is believed that the antioxidant role of ascorbic acid in primates is replaced by uric acid, since these animals have lost the ability to synthesize ascorbic acid.

Most animals, however, oxidize uric acid by the enzyme uricase to allantoin, where the purine ring is cleaved. Allantoin is then converted to allantoic acid ad excreted in

some fishes. Further degradation of allantoic acid may occur to produce urea and, later to ammonia (in marine invertebrates).

5.6 Disorders of Purine Metabolism

5.6.1 Hyperuricemia and gout

- Uric acid is the end product of purine metabolism in humans. The normal concentration of uric acid in the serum of adults is in the range of 3-7 mg/dl. In women, it is slightly lower (by about 1 mg) than in men. The daily excretion of uric acid is about 500-700 mg.
- Hyperuricemia refers to an elevation in the serum uric acid concentration. This is sometimes associated with increased uric acid excretion (uricosuria).
- Gout is a metabolic disease associated with overproduction of uric acid. At the physiological pH, uric acid is found in a more soluble form as sodium urate. In severe hyperuricemia, crystals of sodium urate get deposited in the soft tissues, particularly in the joints. Such deposits are commonly known as tophi. This causes inflammation in the joints resulting in a painful gouty arthritis. Sodium urate and/or uric acid may also precipitate in kidneys and ureters that results in renal damage and stone formation.
- Historically, gout was found to be often associated with high living, over-eating and alcohol consumption. In the previous centuries, alcohol was contaminated with lead during its manufacture and storage. Lead poisoning leads to kidney damage and decreased uric acid excretion causing gout.
- The prevalence of gout is about 3 per 1,000 persons, mostly affecting males. Post menopausal women, however, are as susceptible as men for this disease. Gout is of two types primary and secondary.

1. Primary gout

It is an inborn error of metabolism due to overproduction of uric acid. This is mostly related to increased synthesis of purine nucleotides. The following are the important metabolic defects associated with primary gout.

- PRPP synthetase: In normal circumstances, PRPP synthesis is under feedback control by purine nucleotides (ADP and GDP). However, variant forms of PRPP synthetase –which are not subjected to feedback regulation –have been detected. This leads to the increased production of purines.

- PRPP glutamylamidotransferase: The lack of feedback control of this enzyme by purine nucleotides also leads to their elevated synthesis.
- HGPRT deficiency: This is an enzyme of purine salvage pathway, and its defect causes Lesch-Nyhan syndrome. This disorder is associated with increased synthesis of purine nucleotides by a two-fold mechanism. Firstly, decreased utilization of purines by salvage pathway, resulting in the accumulation and diversion of PRPP for purine nucleotides. Secondly, the defect in salvage pathway leads to decreased levels of IMP and GMP causing impairment in the tightly controlled feedback regulation of their production.
- Glucose 6-phosphate deficiency: In type 1 glycogen storage disease, glucose 6-phosphate cannot be converted to glucose 6-phosphatae. This leads to the increased utilization of glucose 6-phosphate by hexose monophosphate shunt (HMP shunt), resulting in elevated levels of ribose 5-phosphate and PRPP and, ultimately, purine overproduction. Von Gierke's disease is also associated with increased activity of glycolysis. Due to this, lactic acid accumulates in the body which interferes with the uric acid excretion through renal tubules.
- Elevation of glutathiosine reductase: Increased glutathiosine reductase generates more NADP+ which is utilized by HMP shunt. This causes increased ribose 5- phosphate and PRPP synthesis.

  Among the five enzymes described, the first three are directly involved in purine synthesis. The remaining two directly regulate purine production. This is a good example to show how an abnormality in one metabolic pathway influences the other.

2. Secondary gout

Secondary hyperuricemia is due to various diseasecausing increased synthesis or decreased excretion of uric acid. Increased degradation of nucleic acid is observed in various cancers (leukemias, polycythemia, lymphomas, etc).

The disorders associated with impairment in renal function cause accumulation of uric acid which may lead to gout.

5.6.2 Uric acid pool in gout

By administration of uric acid isotope (N15), the miscible uric acid pool can be calculated. It is around 1,200 mg in normal subjects. Uric acid pool is tremendously increased to 3,000 mg. or even more, in patients suffering from gout.

Treatment of gout

The drug of choice for the treatment of primary gout is allopurinol. This is a structural analog of hypoxanthine that competitively inhibits the enzyme xanthine oxidase. Further, allopurinol is oxidized to alloxanthine by xanthine oxidase. Alloxanthine, in turn, is a more effective inhibitor of xanthine oxidase. This type of inhibition is referred to as suicide inhibition.

Inhibition of xanthine oxidase by allopurinol leads to the accumulation of hypoxanthine and xanthine. These two compounds are more soluble than uric acid , hence easily excreted.

Besides the drug therapy, restriction in dietary intake of purines and alcohol is advised. Consumption of plenty of water will also be useful.

The anti-inflammatory drug colchicine is used for the treatment of gouty arthritis. Other anti-inflammatory drugs such as phenylbutazone, indomethacin, oxyphenbutazone, corticosteroids-are also useful.

5.6.3 Pseudogout

The clinical manifestations of pseudogout are similar to gout. But this disorder is caused by the deposition of calcium pyrophosphate crystals in joints. Further, serum uric acid concentration is normal in pseudogout.

5.6.4 Lesch –Nyhan syndrome

This disorder is due to the deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme of purine salvage pathway .it was first described in 1964 by Michael Lesch and William L.Nyhan .

Lesh- Nyhan syndrome is a sex-linked metabolic disorder since the structural gene for HGPRT is located on the X-chromosome. It affects only the males and is characterized by excessive uric acid production , and neurological abnormalities such as mental retardation, aggressive behaviour, learning disability etc. the patients of this disorder have an irresistible urge to bite their fingers and lips, often causing self-multilation.

The overproduction of uric acid in Lesch-Nyhan syndrome is explained. HGPRT deficiency results in the accumulation of PRPP and decrease in GMP and IMP , ultimately leading to increased synthesis and degradation of purines (more details given under primary gout).

The biochemical basis for the neurological symptoms observed in Lesch – Nyhan syndrome is not clearly understood. This may be related to the dependence of brain on the salvage pathway for de novo synthesis of purine nucleotides. Uric acid is not toxic to the brain, since patients with severe hyperuricemia do not exhibit any neurological symptoms. Further, allopurinol treatment that helps to decrease uric acid production, has no affect on the neurological manifestations in these patients.

5.6.5 Immunodeficiency diseases associated with purine metabolism

Two different immunodeficiency disorders associated with the degradation of purine nucleosides are identified. The enzyme defects are adenosine deaminase and purine nucleoside phosphorylase, involved in uric acid synthesis.

The deficiency of adenosine deaminase (ADA)causes severe combined immunodeficiency (SCID)involving T-cell and usually B-cell dysfunction. It is explained that ADA deficiency results in the accumulation of dATP which is an inhibitor of ribonucleotide reductase and, therefore, DNA synthesis and cell replication.

The deficiency of purine nucleotide phosphorylase is associated with impairment of T-cell function but has no effect on B-cell function uric acid synthesis is decreased and the tissue levels of purine nucleosides and nucleotides are higher. It is believed that dGTP inhibits the development of normal T-cell.

5.6.6 Hypouricemia

Decreased uric acid levels in the serum (2mg/dl) represent hypouricemia. This is mostly associated with a rare genetic defect in the enzyme xanthine oxidase. It leads to the increased excretion of xanthine and hypoxanthine. Xanthinuria frequently causes the formation of xanthine stones in the urinary tract.

5.7 Biosynthesis of Pyrimidine Ribonucleotides

5.7.1 Introduction

The synthesis of pyrimidines is a much simpler process compared to that of purines. Aspirate, glutamine and CO_2 contribute to atoms in the formation of pyrimidines ring. Pyrimidines ring is first synthesized and then attached to ribose 5-phosphate. This is in contrast to purine nucleotide synthesis wherein purine ring is built upon a pre-existing ribose 5-phosphate. The pathway of pyrimidines synthesis are:

- Glutamine transfers its amido nitrogen to CO_2 to produce carbamoyl phosphate. This reaction is ATP-dependent and is catalysed by cytosomal enzyme carbamoyl phosphate synthetase 2(CPS 2).

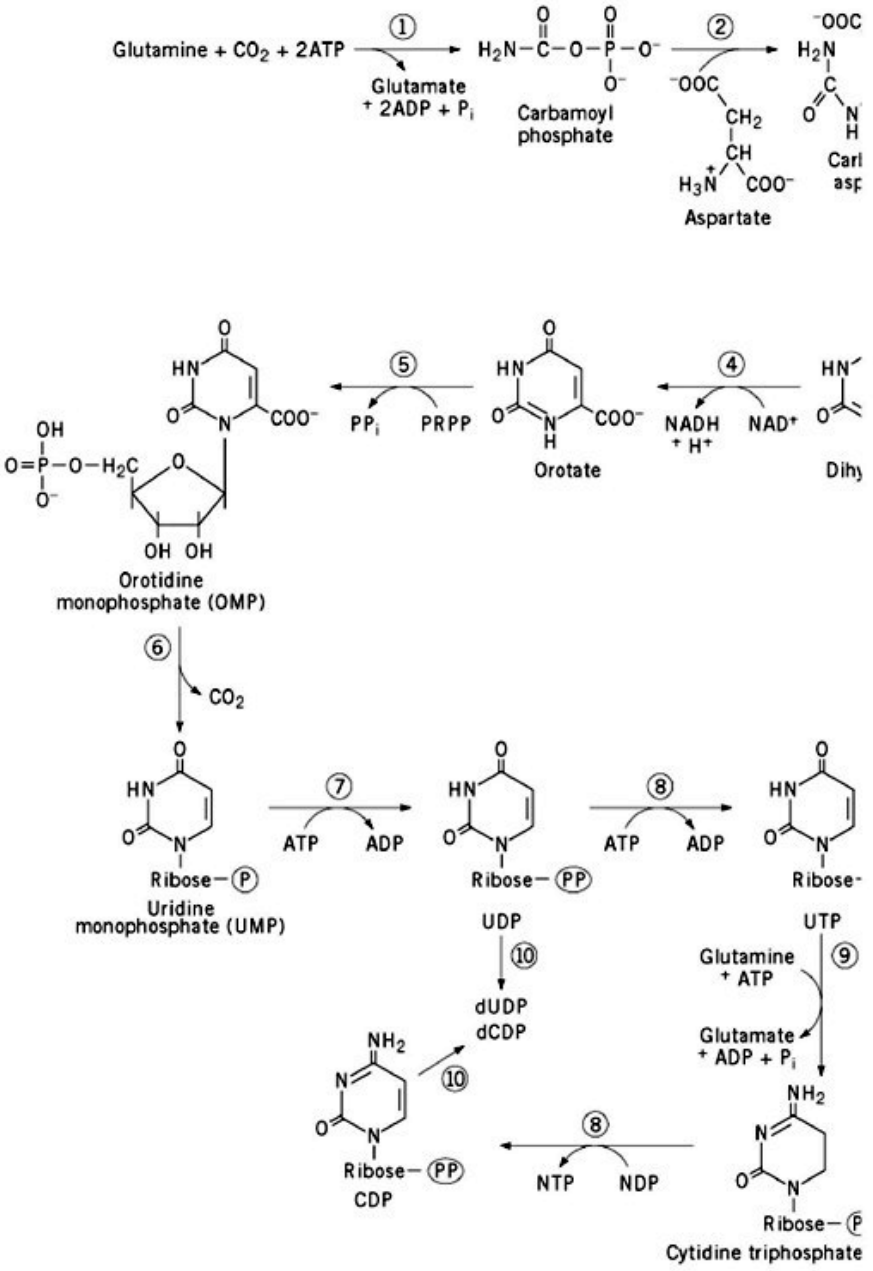


Figure 5.3 Biosynthesis of Pyrimidine Ribonucleotides

- CPS 2 is activated by ATP and PRPP and inhibited by UTP. Carbamoyl phosphate synthetase 1 (CPS 1) is a mitochondrial enzyme which synthesizes

carbamoyl phosphate from ammonia and CO_2 and, in turn urea. Prokaryotes have only one carbamoyl phosphate synthetase which is responsible for the biosynthesis of arginine and pyrimidines.

- Carbamoyl phosphate condenses with aspirate to form carbamoyl aspartate. This reaction is catalysed by aspartate transcarbamoylase. Dihydroorotase catalyses the pyrimidines ring with a loss of H_2O.
- The three enzymes –CPS 2, aspartate transcarbamoylase and dihydroorotase are the are the domins of the same protein. This is a good example of a multifunctional enzyme.
- The next step in pyrimidines synthesis is an NAD^+ dependent dehydrogenation, leading to the formation of orotate.
- Ribose 5-phosphate is now added to orotate to produce orotidine monophosphate(OMP). This reaction is catalysed by orotate phosphoribosyltransferase, an enzyme comparable with HGPRT in its function. OMP undergoes decarboxylation to uridine mono-phosphate (UMP).
- Orotate phosphoribosyltransferaseand OMP decarboxylase are domains of a single protein. A defect in this bifunctional enzyme causes orotic aciduria.
- By an ATP dependent kinase reaction, UMP is converted to UDP which serves as a precursor for the synthesis of dUMP, dTMP, UTP and CTP.
- Ribonucleotide reductase converts UDP to dUDP by a thioredoxin dependent reaction. Thymidylate synthetase catalyses the transfer of a methyl group from N5, N10 methylene tetrahydrofolate to produce deoxythymidines monophosphate (dTMP).
- UDP undergoes an ATP dependent kinase reaction to produce UDP. Cytidine triphosphate (CTP) is synthesized from UTP by amination CTP synthetase is the enzyme and glutamine provides the nitrogen.

5.7.2 Regulation of pyrimidine synthesis

In bacteria, aspartate transcarbamoylase (ATCase) catalyses a committed step in pyrimidine biosynthesis. ATCase is a good example of an enzyme controlled by feedback mechanism by the end product CTP. In certain bacteria, UTP also inhibits ATCase. ATP, however, stimulates ATCase activity.

Cabamoyl phosphate synthetase2 (CPS2) is the regulatory enzyme of pyrimidine synthesis in animals. It is activated by PRPP and ATP and inhibited by UDP and UTP. OMP decarboxylase, inhibited by UMP and CMP, also controls pyrimidine formation.

5.7.3 Degradation of Pyrimidine Nucleotides

The pyrimidine nucleotides undergo similar reactions (dephosphorylation, deamination and cleavage of gycosidic bond) like that of purine nucleotides to liberate the nitrogenous bases- cytosine, uracil and thymine. The bases are then degraded to highly soluble products β-alanine and β-aminoisobutyrate. These are the amino acids which undergo transamination and other reaction to finally produce acetyl CoA and succinyl CoA.

5.7.4 Salvage Pathway

The pyrimidines (like purines) ca also serve as precursors in the salvage pathway to be converted to the respective nucleotides. This reaction is catalysed by pyrimidine phosphoribosyltransferase which utilize PRPP as the source of ribose 5-phosphate.

5.7.5 Disorder of Pyrimidine Metabolism

Orotic aciduria: This is a rare metabolic disorder characterized by the excretion of orotic acid in urine, severe anemia and retarded growth. It is due to the deficiency of the enzymes orotate phosphoribosyl transferase and OMP decarboxylase of pyrimidine synthesis. Both these enzyme activities are present on a single protein as domains (bifunctional enzyme).

Feeding diet rich in uridine and\or cytidine is an effective treatment for orotic aciduria. These compounds provide (through phosphorylation) pyrimidine nucleotides required for DNA and RNA synthesis. Besides this, UTP inhibits carbomyl phosphate synthetase II and blocks synthesis of orotic acid.

Reye's syndrome: It is considered as secondary orotic aciduria. It is believed that a defect in ornithine transcarbomoylase (of urea cycle) causes the accumulation of carbamoyl phosphate. This is then diverted for the increased synthesis and excretion of orotic acid.

CHAPTER

6

HORMONES

Hormones are the chemical messengers run through the blood stream to various organs in our body. They are very essential for various activities in our body. Exocrine glands have ducts to carry their secretions and endocrine glands have no ducts to carry their secretions. Endocrine glands are the ductless glands. Plants have no specialized glands for the secretion of plant hormones.

6.1 Hormones

The chemical substance or messenger is produced from one part of body by endocrine gland entered into the circulation. This was carried to distal target or cell to modify their structure and function is called hormone. Hormones may be steroids, proteins, peptides or amino acid derivatives. Steroid hormones (testosterones) are secreted by testes, adrenal cortex, placenta and ovaries. Proteinaceous hormones are secreted by pituatory gland and pancreas. Peptide hormones are secreted by pituatory gland, thyroid gland and parathyroid gland. Aminoacid derivatives are secreted by the thyroid gland and adrenal medulla. Some important activities of hormones in our body are

- Thyroid stimulating hormone controls the secretion of the hormones released by the thyroid.
- Adrenaline mobilizes glucose, increases heart rate and blood flow to skeletal muscles.
- Insulin controls blood glucose by lowering blood glucose levels.
- Glucagon increases blood glucose.
- Testosterone helps for the development of male sex organs and for change in voice.
- Oestrogen helps for the development of female sex organs and regulates the menstrual cycle.
- The main hormone secreting glands are intenstinal mucosa, pancreas, adrenals, thyroids, parathyroid, pituitary, ovaries and testes.

- Cranial endocrine glands are lying in the head. Example: Pineal and pituitary glands.
- Pharyngeal endocrine glands are lying on the neighborhood of the pharynx. Example: Thyroid and parathyroid glands.
- Abdominal glands are lying in the abdomen. Example: Pancreas, intenstinal glands and adrenals.

6.2 Properties

Hormones have low molecular weight and it can pass easily through capillaries.

- They are produced in trace amounts.
- They are soluble in water.
- They are carried by the blood to the target tissue.
- They act as catalysts.
- A single hormone may have multiple effects on a single target tissue or on several different target tissues.
- They can act in very low concentrations.
- They are destroyed or inactivated as soon as their functions are over.
- They are non-antigenic.
- They function as organic catalysts and act as coenzymes.
- The hormones show a high degree of target-specificity.
- Hormones act in very low concentration.
- The endocrine system is under the control of nerves.
- Hormonal activities are not heredity based.
- Hormones retard the reaction rate.
- Hormonal reactions are irreversible.

6.3 Biological Functions of Hormones

- Hormones control the growth of a body.
- Hormones maintain constant internal environment of organisms. Example:Insulin maintains a constant sugar level in the blood.

- Hormones promote Sexual maturation. Hormones are responsible for the sexual maturation in animals. Example: Lactogenic hormone brings about the secretion of milk, Testosterone in male produces male characters, Progesterone maintains the embryo in the uterus.
- Metabolic reactions are regulated by hormones.
- Emergency reactions are brought about by hormones. Example: Adrenaline is responsible for emergency reaction in the body.
- The development of an organism is controlled by hormones.

  Example: Thyroxin converts a tadpole into a frog. Ecdyson converts a pupa into a moth.

6.4 Plant Hormones

Plant Hormones are produced within the plants. They control the growth and development of plants. These hormones are involved in Cell enlargement, Cellular division, Phototropism, seed germination. Some hormones inhibit the growth. The organic compounds produced by higher plants regulate growth or other physiological functions. Plant hormones have no ducts. Plant hormones occur in very low concentration and they are transmitted through various parts of the plant.

Plant hormones available in plants are

1. Auxins
2. Gibberllins
3. Cytokinins
4. Ethylene
5. Traumatic acid
6. Abscisic acid
7. Marphactins.

6.4.1 Auxins

- Auxins are phytohormones that promote the plant growth. They organic compounds which promote growth along the longitudinal axis. Auxins occur in plants are Natural Auxin. It can also be synthesized artificially. Auxins are abundant in the growing tips of roots and leaves.
- Auxins induce cell differentiation and stimulate intake of water.
- Auxins control the premature fall of leaves.
- Auxins include elongation of plant cells, roots, buds and stem.

- Auxins promote cell division of parenchyma cells.
- In stem cuttings, rooting is favored by Auxins.
- Auxins modify flowering process.
- Auxins are used for the development of seedless fruits without pollination and fertilization.
- Auxins stimulate the process of respiration.
- Auxins act as herbicides.

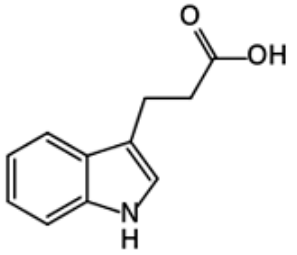


Figure 4.1 Structure of Auxin (Indole 3-acetic acid)

6.4.2 Gibberllins

- Gibberllins are plant hormones that promote stem elongation between nodes on the stem.
- The organs of flowering plants contain Giberllins.
- Gibberllins helps for the recovery from Genetic Dwarfism.
- Gibberllins induce bolting (shoot elongation) and flowering.
- Gibberllins helps for the reversal of light induced inhibition of stem growth.
- Gibberllins helps some plants like tobacco to germinate even in dark.
- Gibberllins regulate various developmental processes of plants.
- Gibberllins promote cell elongation that helps for the plant growth and they are very useful in agriculture.

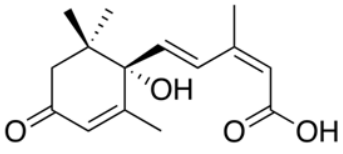


Figure 4.2 Structure of Gibberllin

- Gibberllins are used to increase the crop production. Gibberllins help for the germination of seeds.

6.4.3 Cytokinins

- Cytokinins are plant hormones promote cell division of plants.
- Cytokinins enhance the formation of chloroplast.
- Roots are the richest sources of vitamins.Fruits and endosperm contain large amount of cytokinins.
- Cytokinins promote Cell elongation.
- Cytokinins are effective in breaking seed dormancy in tobacco, lettuce.
- Cytokinins can be employed successfully to induce flowering in short day plants.
- Cytokinins helps for the shoot growth in plants and promote nutrient metabolism.

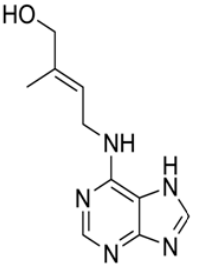


Figure 4.3 Structure of Cytokinin

6.4.4 Ethylene

- Ethylene plant hormone acts as both promoter and inhibitor. Ethylene is a gas produced by fungi and by the leaves and flowers. It is the most important hormone used much in agriculture
- Ethylene hormone promotes the elongation of petioles and internodes.
- It accelerates the colouring of harvested lemons.
- It fasten the fruit ripening.
- It induces root growth and root hair formation.
- It accelerates the ripening of fruits.

- It induces rooting.
- In pineapple, it induces flowering.

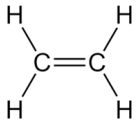


Figure 4.4 Structure of Ethylene

6.4.5 Jasmonates

- Jasmonates are isolated from Jasmine oil. In plants, it exist as jasmonic acid.

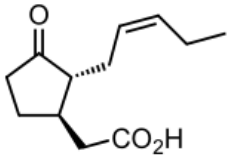


Figure 4.5 Structure of Jasmonic acid

6.4.6 Salicylic acid

- Salicylic acid hormone provide resistance to bio attacks

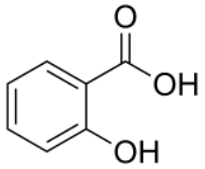


Figure 4.6 Structure of Salicylic acid

6.4.7 Traumatic acid

- Traumatic acid is a wound hormone. It promotes the healing of wounds and injured cells secret this hormone.This hormone is effective in inducing cell division.

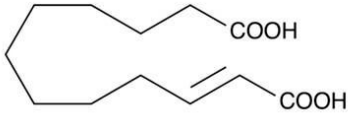


Figure 4.7 Structure of Traumatic acid

6.4.8 Abscisic Acid

- Abscisic Acid is a plant hormone.It is a weak organic acid exists in cis and trans forms.
- It helps for the developmental process including seed and bud dormancy.
- It inhibits growth and seed germination in lettuce.
- It helps the plants to tolerate heat and cold.

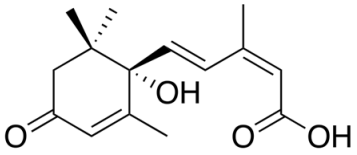
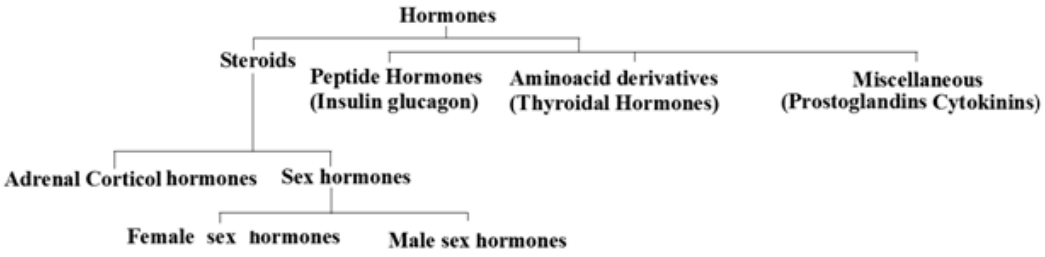


Figure 4.8 Structure of Abscisicc acid

6.4.9 Morphactins

- Morphactins are inhibiting hormones.They are the derivatives of fluorine compounds.
- It promotes the formation of branches.
- Morphactins are effective in inducing lateral bud development.

6.5 Animal Hormones



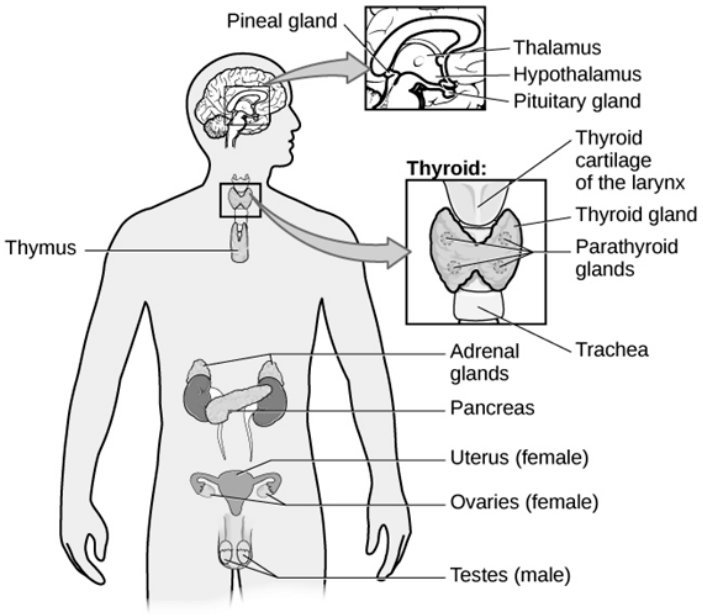


Figure 4.9 Human Physiology

6.5.1 Growth Hormone or Somatotrophic Hormone

- Growth hormone is also called as human growth hormone
- It is secreted by the adenohypophysis anterior lobe of the pituitary gland and stimulates the growth of tissues..
- It is protein in nature and is formed of a straight polypeptide chain having about 188 amino acids. It promotes the children growth.
- It stimulates the multiplication of cells.
- It increases the body growth and are essential for tissue growth.
- It increases the secretion of milk during lactation.
- STH has a remarkable effect on metabolism.It increases the release of fatty acids from the adipose tissue.
- STH decreases the utilization of carbohydrate for energy.
- GH increases intestinal absorption of calcium as well as its excretion. In addition to calcium, Na, K, Mg, PO4 and chloride are also retained.
- Children with growth hormone deficiency can be treated with the injections,

- Gigantism is a disorder caused by the over activity of pituitary glands in the child. This leads to over secretion of growth hormone. Acromegaly is caused by the hyperactivity of pituitary gland in the adult. It is due to the over secretion of growth hormone. Acromegaly is characterized by the over growth of the two jaws, the molar bones and the supraorbital ridges. Dwarfism is caused by the hypo activity of the pituitary in the child.

6.5.2 AdrenoCorticotrophic Hormone

Adreno Corticotropic hormone is secreted by the anterior lobe of the pituitary gland. It is a polypeptide hormone. It is made up to 39 amino acids. In morning, the hormone level is high and it will get decrease during the day time. It stimulates the activity of the adrenal cortex, inducing the secretion of glucocorticoids.

Oversecretion of this hormone leads to Cushing's disease Deficiency causes rheumatoid fever, Addison's disease, etc.

6.5.3 Thyrotrophin or Thyroid Stimulating Hormone

Thyroid Stimulating Hormone is secreted by the anterior lobe of pituitary gland. Hypothalamus in brain produces this hormone. It is a protein hormone. It stimulates thyroid gland there by increasing the thyroxine secretion.

6.5.4 Follicle Stimulating Hormone

FSH is secreted by the anterior lobe of the pituitary. It is a protein hormone. This hormone is important in sexual development. FSH in women changes in the menstrual cycle. In females it increases the number and size of Graffian follicles. FSH control the production of sperm. In males it stimulates the testis for spermatogenesis. Along with luteinizing hormone it control the sexual functions.

6.5.5 Luteinising Hormone

- LH is a gonadotrophic hormone secreted by the anterior lobe of pituitary.It is a glycoprotein with a molecular weight of about 3000. It makes the Graffian follicles grow and mature.It causes the Graffian follicles to secrete another sex hormone called oestrogen.
- In co-operation with FSH, it causes the rupture of the follicle and ovulation.LH causes the appearance, growth and persistence of corpus luteum in the ovary. In the male, LH stimulates the interstitial cells of testis and consequently the production of androgen.

6.5.6 Lactogenic Hormone or Prolactin or Luteotrophic Hormone (LTH)

- LTH is secreted by the anterior lobe of the pituitary gland.It is a protein with several disulphide bridges. It has a molecular weight of about 25000.It helps in initiating milk secretion in the breast.
- It stimulates the proliferation of the glandular elements of mammary glands during pregnancy and thus helps to complete the development of breasts.It helps the corpus luteum in the secretion of progesterone in co-operation with LH.

6.5.7 Thyroxin

Thyroxin is a thyroid hormone. It is a protein hormone. It is a derivative of tyrosine and it contains iodine. It increases basal metabolic rate (BMR). Hence it stimulates the production of more energy.

- It plays an important role in digestion, heart and muscle function, brain development.
- It improves growth.
- It stimulates protein synthesis.
- In increases the absorption of monosaccharaides.
- Deficiency of this hormone in children causes cretinism.
- In an adult deficiency causes **myxoedema**(myxa = mucus oedema = swelling). It is characterized by swelling of certain parts of skin, low BMR, low body temperature, under sensitivity of cold, anaemia, etc.

Over activity of thyroid gland or hyperthyroidism leads to a disease called exophthalmic goitre. It is characterized by considerable enlargement and protrusion of the gland below the chin; increased pulse rate and nervousness, bulging of the eyes, etc.

6.5.8 Insulin

Insulin is a peptide hormone. secreted by the islets of Langerhans. Chemically, is a polypeptide. Insulin helps to lower the blood sugar level. Deficiency of insulin causes a disease called diabetes mellitus. Insulin has two polypeptide chains namely A chain and B chain. A chain is acidic and it contains 21 amino acids. B chain is basic and it contains 30 amino acids. This two chains are joined by two disulphide (S-S) bonds.

- Insulin helps to break down fats or protein.
- Diabetes occurs because of this hormonal secretion will have the following symptoms. Hyper glycaemia is a pronounced increase in blood sugar level. Glycosuria shows the appearance of sugar in the urine. Polyuria is a condition in which Large volumes of urine about 10 litres per day will be released. Ketonuria: Ketonemia occurs due to increased appearance of ketone bodies in the blood.. Diabetes can be cured by the injection of protamine zinc solution.

CHAPTER

7

CELL RESPIRATION AND BIOLOGICAL OXIDATIONS

7.1 Introduction

Oxygen plays a major role in respiration. The exchange of gases between the air and organism is called respiration. We need energy to carry out all reactions and this energy is acquired through food.

7.2 Cellular Respiration

The process of respiration starts from cytoplasm and the process will complete in mitochondria. The exchange of gases between the body fluid and environment takes place by diffusion is called as breathing or external respiration in which oxygen is taken in and carbon dioxide is given out. Oxidative energy-yielding reactions of the cell is known as cell or internal respiration or biological oxidation. The main product of cellular respiration is ATP. By the process of cellular respiration, nutrients are converted into chemical energy

Biochemical reactions involve catabolic reactions, combustion and redox reactions. Biological oxidation refers to all enzyme catalyzed chemical reactions which utilize oxygen or lose hydrogen or electron (e). The complex organic food molecules are oxidized in the cells to release energy. During oxidation process, Carbohydrates and proteins are oxidized to form CO_2 and H_2O. Fats are oxidized to form acetate which was converted to CO_2 and H_2O by the process of oxidation and combustion.

7.3 Biological Oxidation

Biological oxidation is the process which takes place in living organisms in which the oxidation and reduction reaction takes place simultaneously.

- Biological oxidation is an important energy producing reaction in cell.
- Oxidation in living organism is a complex process.
- By the process of biological oxidation, living organism will get energy.
- Oxidation in living organism is a controlled process.

7.4 Theories of Biological Oxidation

There are two theories of Biological oxidation.

Oxygen Activation Theory

Oxidation reactions are catalyzed by oxidase enzymes which activate molecular oxygen.

$$SH_2 + \tfrac{1}{2}\, O_2 \xrightarrow{\text{oxidase}} S + H_2O$$

Hydrogen Activation Theory

Oxidation reactions are catalyzed by dehydrogenase enzymes which activate hydrogen atoms of the substrate. The hydrogen atoms are removed by a hydrogen acceptor 'A'.

$$SH_2 + A \xrightarrow{\text{Dehydrogenase}} S + AH_2$$

$$AH_2 + O_2 \longrightarrow A + H_2O_2$$

The above reactions will be catalysed by the enzymes known as oxidoreductases. Biological oxidations mostly take place by the removal of hydrogen in pairs. This process is known as dehydrogenation. The enzyme which catalyzes the dehydrogenation is known as dehydrogenase. The important hydrogen acceptors and carriers in biological oxidation are Nicotinamide nucleotides, Flavin Nucleotide and Cytochromes. NAD is the major electron acceptor in biological oxidation.

7.5 Cytochromes

Cytochromes are iron containing hemoproteins and they play a major role in electron transport. They transfer electrons from flavoproteins to molecular oxygen. They are mostly present in the mitochondria of aerobic cells. Cytochromes have a porphyrin ring with iron atom. It is a small water soluble protein.

7.6 Mitochondria

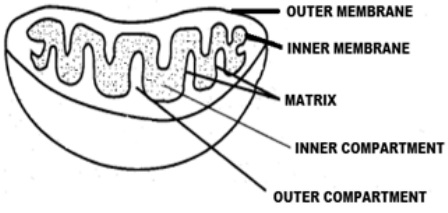


Figure 7.1 Mitochondria

- Mutochondria is found in the eukaryotic organism. They are the major sites of biological oxidations.
- Mitochondria float within the cytoplasm of the cell and they generate energy required for the chemical reactions. They are the "power house" of the cell because they are the sites of production of high energy compounds like ATP.
- All the enzymes and coenzymes required for biological oxidation are present in mitochondria.
- Mitochondria helps for the thermogenesis process. They support for the production of heat in living organism.
- The mitochondria have two membranes an outer membrane and an inner membrane.
- The inner mitochondrial membrane form folds called cristae. The inner compartment is filled with fluid matrix. The inner membrane has two layers a protein layer and a lipid layer. The protein layer is associated with electron transport chain of enzymes and the lipid layer with the enzymes of oxidative phosphorylation. The fluid matrix is associated with the enzymes of citric acid cycle and fatty acid oxidation.
- Mitochondria synthesis certain hormones and biochemicals.
- Mitochondria is very essential for the cholesterol and neurotransmitter metabolism.

7.7 Intermediatory Metabolism

Intermediary metabolism is the total intacellular processes in which the nutritive component is converted to cellular components. It is the total of all enzymatic reactions, occurring in the cell. A metabolic pathway is a series of chemical reactions that takes place within the cell. The metabolic pathways can be divided into 3 major types

1. ***Catabolic pathway:*** In this pathway, energy is released by breaking the molecules into simpler forms. It is responsible for degradation of energy rich nutrient molecules to release energy. Example: Krebs Cycle, Glycolysis.
2. ***Anabolic Pathway:*** It is responsible for synthesis of cellular components.

   Example: Synthesis of proteins from aminoacids.
3. ***Amphibolic Pathway:*** It includes both catabolic and anabolic pathways.

   Example: Glycogenosis, Lipogenosis.

Metabolites are the reactants, products and intermediates of enzymatic reactions. Biological oxidations occur in both catabolic and in amphibolic pathways.

7.8 Oxidative Decarboxylation

- Oxidative decarboxylation occurs in the mitochondrial matrix in which involves both oxidation and loss of carboxyl group as CO2 will takes place in the cell.
- In this process, the pyruvic acid is converted into acetyl coenzyme A and the removal of carbon dioxide will also takes place. The process takes place with the help of enzymes Pyruvic acid decarboxylase, Dihydroxylipoyl transacetylase, Dihydroxylipoyl dehydrogenase and the Co-factors such as Co-enzyme A, NAD, Lipoic acid and Thiamine pyrophosphate
- ***Step – 1:*** Thiamine pyrophosphate combines with pyruvic acid to form hydroxyethyl thiamine pyrophosphate . The carboxyl group is removed as CO2 with the help of enzyme Pyruvic dehydrogenase and forms acetaldehyde.

Pyruvic acid + Thiamine pyrophosphate $\xrightarrow[\text{dehydrogenase}]{}$ CO_2 + Pyruvic Acetaldehyde

- ***Step – 2:*** It is then oxidized to form acetyl lipoic acid.

Active acetaldehyde + lipoic acid $\longrightarrow$ 6-S-acetyl lipoic acid

- ***Step – 3:*** The acetyl group is then transferred to coenzyme A to form acetyl coenzyme A.

6-S-acetyl lipoic acid + CoA $\longrightarrow$ Acetyl CoA + Dihydrolipoic acid

Electron Transport System

- Energy is transferred from electron to ATP molecules by the process of cellular respiration. In the electron transport chain, 3 main steps will occur which involves the generation of proton across the mitochondrial membrane, reduction of molecular oxygen to water and ATP synthesis.
- Electron transport system is present in the cristae of mitochondria. Cristae contains coenzymes which acts as carrier and transfer molecules. The electron flow takes place through various components of the respiratory chain are arranged in the inner mitochondrial membrane in sequence an in the following order:

- Electrons from reduced NAD and reduced FAD are passed on to a common acceptor coenzyme Q. From CoQ electrons are passed on to cytochrome b, then to cytochrome c. From cytochrome c, the electrons are transferred to cytochrome a.
- The electrons are accepted by oxygen. Oxygen combines with hydrogen to form water.
- The electron transport chain is a enzymatic reactions which involves the electron acceptance and donors. Each electrons will be will passed to an acceptor of higher redox potential. The electrons are donated to another acceptor till the electrons are passed to oxygen. Energy is released in each step since the higher-energy donor and acceptor is converted into lower-energy products.
- The protein complexes which are involved for the electron transport from NADH to O_2 are NADH-Q reductase complex, Cytochrome C reductase complex, Cytochrome C, Cytochrome C oxidase complex.
- For the conversion of $FADH_2$ to O_2, the four protein complexes that catalyse the redox reaction are succinate dehydrogenase, Cytochrome C reductase complex, Cytochrome C, Cytochrome c oxidase complex.
- The electron transfer potential can be determined by placing an electrode in a solution containing 1M NADH and 1M NAD^+. The reference electrode is connected to 1M saturated H_2 gas. Then the electrodes are connected to voltmeter. Then the potential is measured.

Respiratory Chain	**Redox potential in Volt**
NADH	–0.32
↓	
FAD	–0.03
↓	
CoQ	–0.1
↓	
Cyt. b	0.04
↓	
Cyt.c	0.25

↓ Cyt. a ↓ O_2	0.29 0.8

Three greater jumps in the redox-potential values are observed in the conversion of NAD to FAD, Coenzyme Q to Cytochrome B, Cytochrome A to O_2.

Oxidative Phospholylation

- Oxidative phosphorylation is a final step of cellular respiration.
- The electrons are transported through redox reactions through the mitochondrial membrane. During biological oxidation, a large amount of energy is released.
- Energy is used to convert ADP into ATP.
- The conversion of oxidation-reduction energy into high energy phosphate bond is known as oxidative phosphorylation.
- Transfer of electrons takes place and the protons are pumped out of mitochondrial matrix.
- Hence a membrane potential is generated. When the protons are pumped in, ATP is synthesized. 4 ADP molecules are phosphorylated to 3 ATP molecules.
- The first molecule of ATP is formed in the reaction between NAD and FAD, the second between Cyt b and Cyt c and the third between Cyt a and O_2.

$$3\ NADH + H^+ + 3\ ADP + 3\ Pi + ½\ O_2 \longrightarrow NAD^+ + 4\ H_2O + 3\ ATP$$

The electron transfer takes place as follows

$$NADH + H^+ + ½\ O_2 \rightarrow NAD+ + H_2O;\ \Delta G = -51700\ cal/mole$$

In this reaction NADH is oxidized to NAD^+ and pair of electron is transferred to O_2 to form water. The process releases a free energy of 52700 cal/mole.

$$3\ ADP + 3\ Pi \rightarrow 3\ ATP + 3\ H_2O;\ \Delta G = +21900\ cal/mole$$

ADP is phosphorylated to ATP. The process requires a free energy of 21900 calories for the formation of one mole of ATP.

CHAPTER

8 BIOCHEMICAL TECHNIQUES

8.1 Introduction

Biochemical Techniques helps to identify the substance present in living organisms. Biochemical techniques are very important in the field of biology. Biochemical techniques helps to understand the biochemical process.

8.2 Microscopy

Microscope is a technique used to see objects that are not seen with the naked eye. Microscopy involves diffraction, reflection and refraction of electromagnetic radiation. Biochemical analysis is frequently accompanied by light and electron microscopic examination of tissue, cell or organelle preparations to evaluate the integrity of samples and to correlate structure with function.

8.2.1 Optical Microscope

Optical or light microscope involves passing light through a lens to magnify the image of an object. A condenser lens is used to produce a parallel beam of light. Thhe image is enlarged by objective and eyepiece lenses. The resolving power (resolution) of a microscope is given by Abbe's formula

$$R = 0.5 \lambda / n. \sin \alpha \ \mu m \text{ (or nm)}$$

Where λ = the wavelength of radiation in (or nm)

n = the refractive index (RI) of the medium between the specimen and the first lens

α = ½ angle of the aperture

As per Abbe's formula, small value for R gives a better resolution. Smaller values of R is obtained by making λ smaller (short wave) than longer wavelength. Also by using special oil immersion lenses, the value of n can be increased which lowers the value of R.

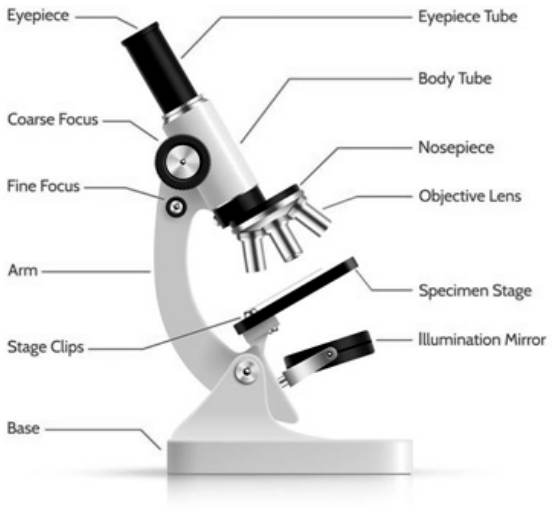


Figure 8.1 Optical Microscope

Making α as close to 90° as possible, R values can be smaller. The image clarity is reduced outside the focal plane. This technique can be used for strongly refracting objects.

The important features of optical microscopy are

- Sample preparation normally takes a few minutes / hours.
- Live or dead specimens is used for analysis.
- Running cost is very low.
- Morphology is detected in colour or black and white.
- Poor depth of focus
- Resolution 170 nm at best magnification x 2000 at best

8.2.2 Electron Microscope

For getting a high resolution, the electron beam is used in electron microscoy. This technique is carried out under vacuum since the electrons are easily scattered. Electrons are charged particles and they have wave-like properties, so they respond to magnetic fields. Powerful magnets are used to focus electrons. The wavelength of an electron is given by the formula:

$$\lambda = 1.23 / \sqrt{E} \text{ nm}$$

Where, E is the voltage through which an electron is accelerated.

Higher the voltage, the shorter the wavelength. Abbe's formula applies equally to both optical and electron microscopes, the best resolution are achieved at high voltages. The main types of electron microscopy are transmission electron microscopes (TEMs), scanning electron microscopes (SEMs) and Scanning Probe Microscopy (SPM).

Transmission Electron Microscopy:

In TEMs electrons are transmitted through the specimen. The image produced is due to the interaction of electrons with the sample. The image is then magnified using photographic film, fluorescent screen or a sensor. This technique is widely used in environmental research, medicinal and electronic applications.

Scanning Electron Microscopy:

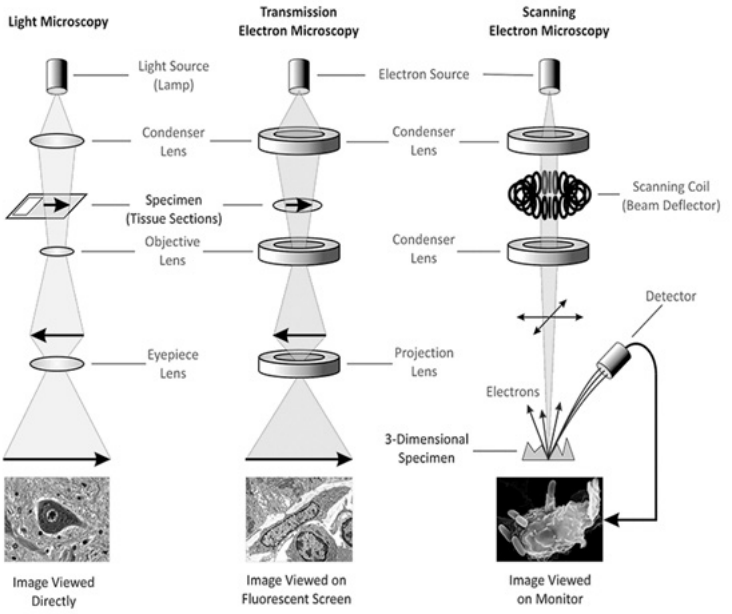


Figure 9.2 Electron Microscope

In SEMs the surface is scanned with a beam of electrons. Interaction between electron and the atoms take place. As a result, surface morphology of the sample can be determined. The important features of scanning electron microscope are

- Sample preparation often takes several days.
- Dead, dried specimens are used.
- Heavy running costs.
- Surface Morphology is detected in black and white only.
- Good depth of focus.
- Resolution 0.5 nm at best. Magnification x 5,00,000 at best

From electron gun, electron beam is emitted thermionically. Tungsten is used in thermionic electron guns. The beam passes through the scanning coils. The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons. Images are created. Electronic amplifiers are used to amplify the signals.

8.3 Centrifuge

Centrifuge involves the process of centrifugation. The centrifugal force is involved to separate the particles in a solutioin. Centrifuge instrument is used to spin substances at a high speed. According to their size, shape, density, medium viscosity and rotor speed, the particles present in the liquid sample will be separated. On increasing the effective gravitational force of the solution, precipitate will settle down quickly to the bottom of the tube. The remaining liquid that lies above the precipitate is called a supernatant. Larger the size and the density of the particles, the faster they separate from the mixture. The rate of centrifugation is expresses by the angular velocity usually expressed as revolutions per minute (RPM), or acceleration expressed as g. The conversion factor between RPM and g depends on the radius of the centrifuge rotor. The sedimentation rate of each particle is directly proportional to the applied centrifugal force.

Centrifugation techniques in research involves Microcentrifuge, Low speed centrifuge, High-speed centrifuge, Ultra centrifugation, Differential centrifugation, Density gradient and Isopycnic centrifugation.

Ultracentrifugation involves preparative centrifugation and analytical centrifugation.Preparative centrifugation technique requires large quantity of sample and it involves separation, isolation and purification of sub-cellular organelles, plasma membranes, ribosomes, chromatin, nucleic acids and viruses. This technique helps in the study of their morphology, composition and biological activity.

Analytical centrifugation technique requires only small amount of sample and is applied mainly to the study of purified macromolecules. By this technique, the purity,

chang in the molecular mass of complexes, relative molecular weight and shape of the material can be determined.

Depending on the operating speed, the centrifuge is classified as hand centrifuge, Desktop centrifuge, Continuous flow centrifuge, Gas centrifuge, Hematocrit centrifuge, low speed centrifuge, high speed centrifuge,Ultra centrifuge, Microcentrifuge, Refrigerated centrifuge and Vacuum centrifuge.

8.3.1 Hand Centrifuge

It is manually operated to separate the solid part of a solution.

Figure 9.3 Hand Centrifuge

8.3.2 Desktop Centrifuge or Small Bench Centrifuge

Figure 9.4 Desktop Centrifuge

Desktop centrifuge consists of an electric motor to rotate the tubes. They are used in clinical and research laboratories to separate red blood cells, yeast cells or bulky precipitate of chemical reactions. Their maximum speed is usually 3000 rpm They do not usually have any temperature regulatory system. Large volumes of crude samples can be used to separate. The centrifuge tubes must be placed opposite to each other after balancing their weights accurately.

8.3.3 Continuous Flow Centrifuge

Continuous Flow Centrifuge is a rapid centrifuge technique. It is used to separate large volumes of sample. A high centrifugal force is applied to remove the solid components of sample.

8.3.4 High Speed Centrifuge

High speed centrifuge operate at a very high speed. This centrifuge operates at a speed of 15,000 to 30,000 rpm. Due to large number of rotations, more heat energy will be generated. Hence it is connected with arefrigeration equipment to remove the heat generated due to friction between the air and the spinning rotor. The temperature can easily be maintained in the range 0°C to 4°C. A maximum volume of about 150 mL can be loaded. This centrifuge is used to separate sensitive biological samples.It can be used to isolate sub-cellular organelles such as the nuclei, mitochondria and lysosomes and to collect microorganisms, cell debris, precipitates of chemical reactions and immune precipitates.

Figure 8.5 High Speed Centrifuge

8.3.5 Gas Centrifuge

Gas centrifuge is used for the separation of gases based on their isotopes. Gas molecules are separated on the basis of their masses.

8.3.6 Hematocrit Centrifuge

Hematocrit Centrifuge is used to separate the blood samples. They are used for the volume fraction of RBCs in the blood sample. By using this technique, blood loss, anaemia, polycythemia, leukemia can be determined. This centrifuge quickly attains a speed of 11000rpm.

8.3.7 Ultracentrifuge

Ultracentrifuge is used for the separation of very smaller molecules. This centrifuge operates at a higher speed. The molecules like ribosomes, proteins and viruses can be separated. This centrifuge operates at a speed of 150, 000 rpm. Ultracentrifuge is used for both preparative and analytical methods. Due to large number of rotations, refrigeration system is equipped with this centrifuge. It is used for the determination of properties of macromolecules like size, shape and density.

8.3.8 Microcentrifuge

Microcentrifuge technique is used to separate very smaller volumes. It is operated at a speed of 12000 to 13000rpm. Also temperature control is attached to operate the temperature sensitive samples. It is used for the molecular separation of cell organelles like nuclei, DNA.

8.3.9 Refrigerated Centrifuge

Refrigerated centrifuges are used for the separation of various biological molecules like yeast cells, chloroplasts and erythrocytes. It is operated with temperature control ranging –20°c to 30°C.

8.3.10 Vacuum Centrifuge

Vacuum Centrifuge can centrifuge large number of samples at a time. This centrifuge is used on chemical and biological laboratories. By lowering the vapor pressure of the sample, the boiling point of the sample decreases which causes the solvent to get evaporated. The particles will get separated.

8.3.11 Advantages

The centrifugation technique is used to

- To separate the miscible substance.
- To purify the components.
- To separate crystalline drugs.
- To test the emulsion and suspensions for creaming and sedimentation at an accelerated speed.

8.3.12 Application Of Centrifugation

- Separation of particles in air.
- Removing fat from milk.
- Production of bulk drugs.
- Purification of cells.
- Production of biological products.
- Evaluation of suspensions and emulsion.
- Determination of molecular weight of colloids.
- Separating chalk powder from water.
- Purification of water.

8.4 pH meter

P.L.Sorenson introduced the concept of pH in 1909. pH meter is used to measure the acidity or alkalinity of a sample. It is used for various applications.

8.4.1 Principle

pH meter is a potentiometer which measures the voltage between two electrodes placed in a solution.pH meter has a glass and reference electrode or a combination electrode. An electric potential is generated when a thin glass membrane separates two solutions of different H^+ ion concentrations. The two electrodes used in pH meter are a calomel electrode and a glass electrode. Reference electrode used for this instrument is calomel electrode and the glass electrode is the standard test electrode whose electrical potential depends on the pH of the test solution.

8.4.2 Electrodes Used

The calomel electrode contains mercury, mercury chloride and a saturated solution of potassium chloride and it does not allow H^+ ions which indicates that its potential

is independent of pH. The calomel electrode is dipped in saturated solution of KCl. The glass electrode contains silver, silver chloride and 0.1M HCl solution which is permeable only to H^+ ions. This electrode is dipped in 0.1M HCl solution.

An electrical potential develops across the glass electrode and calomel electrode, which results in a flow of current between the electrodes. The magnitude of this current depends on the concentration of H^+ / OH- ions in the test solution.

Combination electrodes consist of a glass and a reference electrode in a single unit. The electrode is of high cost andsmaller volumes of solution are enough to measure.

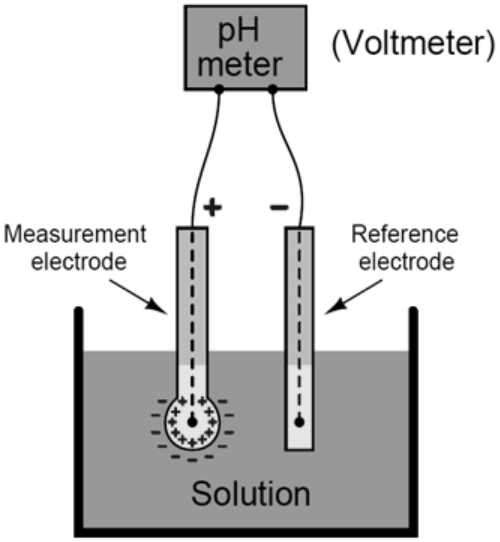


Figure 8.6 pH Meter

8.4.3 Application

- pH meter is used to measure pH of a given solution
- It is used in all industries.
- It is used in the clinical laboratory
- It is used in biochemical research.
- Used in agriculture for fertilizer testing
- Used for soil testing.
- Used to check water quality.

8.5 Electrophoresis

The movement of charged particles under the influence of an electric current to oppositely charged electrodes is called electrophoresis. The movement of the charged particles in an electric field depends upon time, electric currentand conductivity of the solvent and charge of the molecule to be separated. Electrophoresis of positively charged cation is called cataphoresis and the electrophoresis of negatively charged anion is called anaphoresis.

The electrophoretic movement is observed in clayparticles dispersed in water. Macromolecules can be separated. This technique is used in analysis of DNA, RNA and protein.

Electrophoretic mobility is defined as the distance travelled by the particles in one second under the potential gradient of one volt per centimeter. The different compounds in a mixture will have different electrophoretic mobility's and hence they can be separated. The two main types of electrophoretic methods are Moving boundary and Zone electrophoresis.

8.5.1 Zone Electrophoresis

In Zone electrophoresis, the migration of charged particles takes place with the supporting media. On the supporting medium, the components will get separate. For this technique, small volume of the smaple is enough. This technique is easy to maintain and it involves low cost. It is highly applicable to biochemical research.

This technique involves electrophoretic chamber, supportimg media, electrodes and diffusion barrier.On the basis of supporting media, zone electrophoresis is classified into

a) Paper Electrophoresis
b) Gel Electrophoresis
c) Thin Layer Electrophoresis
d) Cellulose acetate Electrophoresis

a) Paper Electrophoresis:

Under the influence of electric current, the charged particles migrate towards the positive and negative pole according to their charge. Paper Electrophoresis is a simple and low cost method. It is used for testing water samples, pharmaceutical industries and in clinical applications. The separation of particles depends upon size, charge, shape, electric field and pH.

Higher the charge of the sample, the mobility of the particles will be more. Larger particles have smaller electrophoretic mobility. Paper electrophoresis is very important for the study of normal and abnormal plasma proteins.The serum under investigation is mixed with bromophenol blue, a blue coloured stain and spotted at the centre of as trip of special filter paper, saturated with barbitone buffer of pH 8.6

When an electric current and voltage is passed through the paper, charged protein fractions bearing different charges migrate at different rates. The different fractions of plasma will migrate toward the anode at characteristically different rates. The paper is dried and stained with a solution containing bromophenol blue after a run of about 5 to 6 hours. Using paper electrophoresis, five different bands is observed in human serum in the order of decreasing mobility as albumin, alpha1-glubulin, alpha2-glubulin, beta-globulin and gamma-globulin. A band was identified in which Albumin, the fastest moving fraction of the proteins of plasma, forms the last band of the paper. Gamma globulin, which is the slowest moving protein, forms a band at the other end. The remaining fractions are seen in between these two bands.

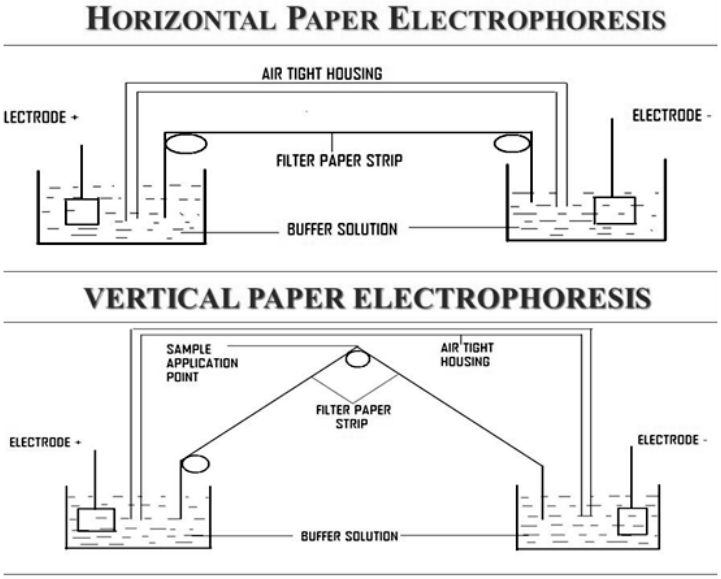


Figure 8.7 Paper Electrophoresis

b) Gel Electrophoresis

Based on the molecular size of the substances, separation is done by molecular sieving in gel electrophoresis. The supporting medium used in gel electrophoresis is electrically neutral. The gel sieves the macromolecules and allows only the smaller molecules to migrate.

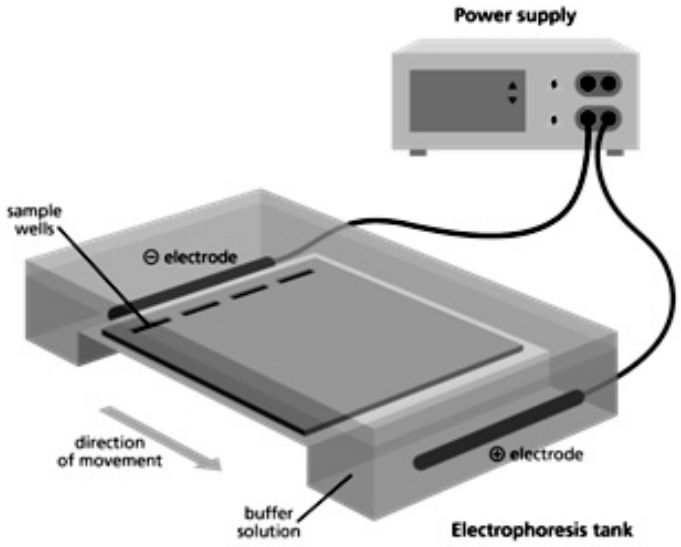


Figure 8.8 Paper Electrophoresis

Various types of gels are used as the supporting medium are starch gel, Agar gel, Polyacrylamide gel and Sephadex gel. The use of gels in electrophoresis is highly applicable for proteins and amino acids. Serum proteins can be separated into 15 bands.

Polacrylamide gel electrophoresis combined with sodium dodecyl sulphate is known as SDS-PAGE. It is the most widely used method for analyzing protein mixtures qualitatively. It is particularly useful for monitoring protein purification. It is also used to determine the relative molecular weight of proteins. PAGE is the most versatile electrophoretic system for the analysis and separation of proteins, small RNA molecules and very small fragments of DNA

c) Thin Layer Electrophoresis

In Thin layer Electrophoresis, thin layers of silica, alumina are used. Along with chromatography, this technique is useful in the study of proteins and nucleic acids. This technique requires less time and give good resolution. It is more advantageous than paper electrophoresis.

d) Cellulose Acetate Electrophoresis

This technique is more advantageous than paper electrophoresis.Biological acetate membrane is used for this type of electrophoresis. This membrane gives sharp bands. The cellulose acetate paper was dipped in the buffer solution. The sample is then applied

at one end. Then current was passed. Then the particles will migrate depending on their charge. This technique os used in the clinical and biological applications.

8.5.2 Moving Boundary Electrophoresis

Large volumes of sample is required for moving boundary electrophoresis. This technique is carried out in the absence of supporting medium.The apparatus consists of U- shaped cell containing buffer solution. On applying an electrical current, proteins migrate towards the anode. The migration of negatively charged proteins from the macromolecule solution to the pure buffer forms a boundary. A sharp change in the refractive index of the solution is identified. The changes in the refractive index are measured by Schlerin optics.

a) Capillary Electrophoresis

b) Isotachophoresis

c) Isoelectric Focusing

d) Immuno Electrophoresis

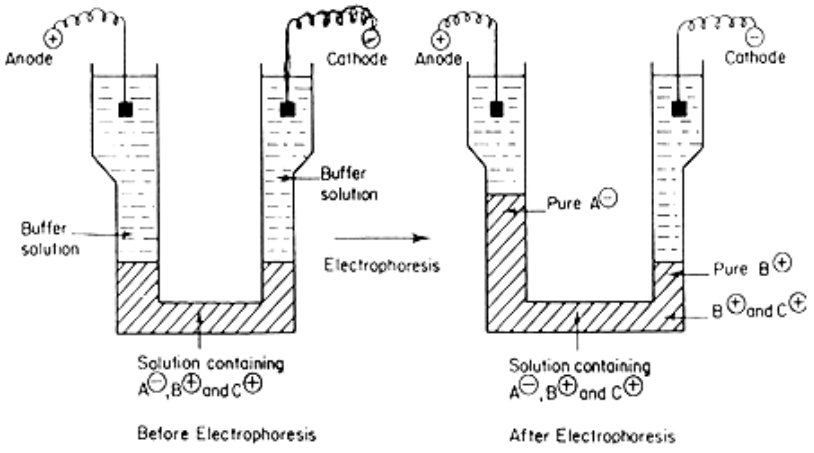


Figure 8.9 Moving Boundary Electrophoresis

Capillary electrophoresis is a separation method that takes place under the influence of electric field. Small highly charged solute will migrate faster. Thus the migration of solute depends on the charge and size of the particle.Proteins, Nucleic acids, organic and inorganic analysis can be done using this technique. UV, laser induced flourescence detectors can be used.

Capillary isoelectric focusing is used to separate peptides and proteins.

Capillary Isotachophoresis involves the migration of sample between electrolytes. Serum proteins can be separated into 40 bands. This technique is highly applicable for the purification of proteins.

Immuno Electrophoresis is used for the analysis of antigens and antibodies.

Spot test techniques are helpful to identify the separated components. Proteins are usually located by staining and enzymes by their specific activities. Amino acids can be detected by fluorescence under UV light. Radioactive substances can be located by autoradiography or by staining. Lipoproteins can be detected by staining with the fat-soluble dye such as sudan dye. Glycoprotein is detected by using modified Schiff's reagent.

In hepatic Cirrhosis, a decrease in albumin and an increase in globulin is identified. Albumin decrese is seen in protein malnutrition.In chronic infection (hepatitis) a relative decrease in albumin with a notable elevation in γ-globulin is observed.
The presence of an abnormal band (M protein) usually between ß and γ-globulin bands, closer to the γ band is identified in multiple myeloma.

In hypogammaglobulinemia a considerable drop in γ-globulin is readily observed. A slight increase in α2-globulin is also seen. In chronic liver diseases, a decrease in albumin band is observed. Occasionally an increase in γ and ß-globulin is also seen. α2-globulin level increase in nephritic syndrome.

8.5.3 Applications of Electrophoresis

- Used to separate insulin from plasma proteins.
- Helps to isolate a large number of proteins.
- Identify the purity of the isolated proteins.
- Molecular weight of proteins can be detected.
- Used for the separation of carbohydrates and vitamins.
- Helpful to determine the sequences of DNA.
- Used to find out the point of mutation in DNA or RNA.
- Helps to detect the precursor molecules of tRNA, rRNA and mRNA .
- Used to find out the number of subunits present in a protein.
- Used to determine the molecular weight of proteins and DNA.
- Haemoglobin separation can be done using this techniques.

8.6 Colorimeter

A colorimeter is a device which measures the transmittance and absorbance of a solution.

8.6.1 Principle

Colorimeter works on the principle of Beer-Lambert law which states that the concentration of a solute is directly proportional to the absorbance.

$$A = \epsilon cl$$

Where A is the absorbance, c is concentration, ϵ and l are constant.

Colorimeter involves a light source (Tungsten), Monochromator which selects the particular wavelength to pass, cuvette, filters and detector (Photocell).

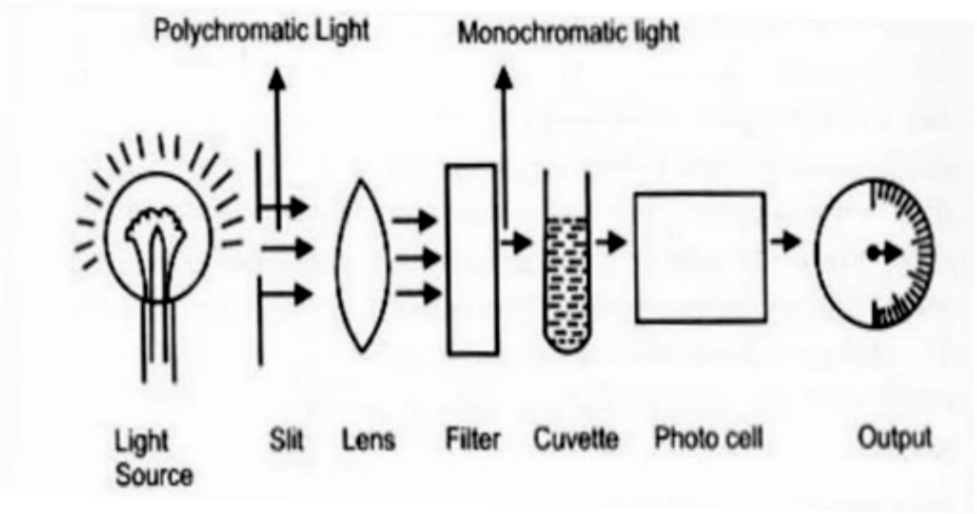


Figure 8.10 Colorimeter

A beam of light having particular wavelength is passed through a solution. A microprocessor then calculates the absorbance or percent transmittance. A sample of known concentration is first used to calibrate. Then the concentration of an unknown sample is determined.A graph is drawn between the concentration and absorbance, From the graph, the concentration of unknown sample is easily determined.

8.6.2 Applications

- Used to identify the food colours.
- Used in chemical Laboratories.

- Helps to monitor the growth of bacteria and yeast.
- Used to determine the concentration of plant nutrients.
- Used in paint industry.
- Used to determine the concentration of haemoglobin in blood.

CHAPTER

9 MINERALS

9.1 Introduction

Minerals play an important role and it is very essential for many activities in our body. For a healthy living, minerals play a major role. Minerals are responsible for growth. Also, they are required for hormones and enzymatic activities. Mineral balance must be maintained in our body or it will lead to many disease. Minerals does not decompose under the influence of heat or light. Cooking will not affect the composition of minerals. The two types of minerals required for our body are Macrominerals and microminerals.

9.2 Macrominerals

Macrominerals are the minerals which are required large amount in our body.

Example: Calcium, Sodium, Potassium, Phosphorous, Magnesium, Chloride and Sulphur.

9.3 Microminerals

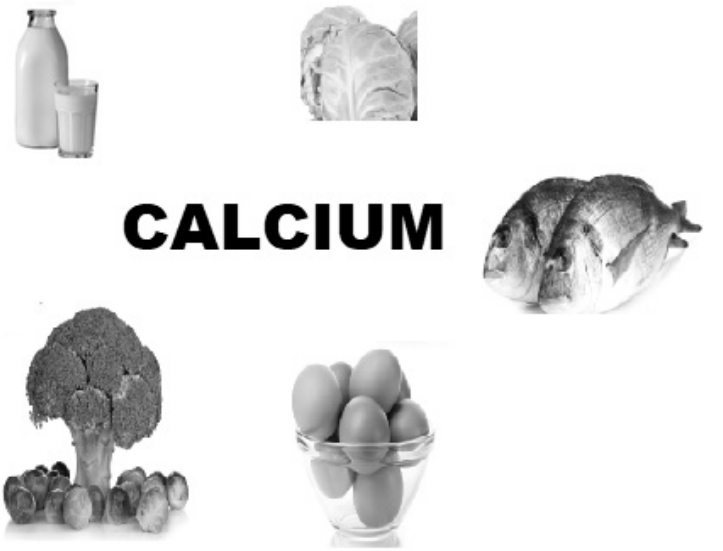
Microminerals are the minerals which are needed in trace amounts.

Example: Iron, Manganese, Copper, Iodine, Zinc, Cobalt, Fluoride and selenium.

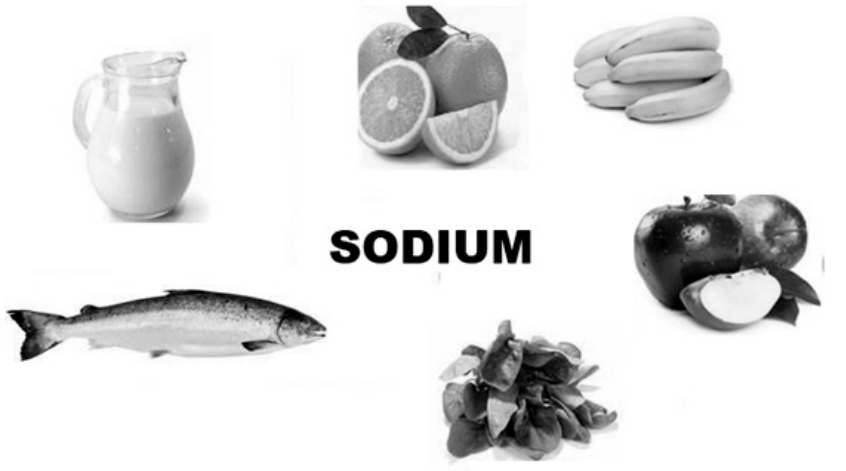
9.4 Role of Minerals In Human Body

9.4.1 Calcium

- The most abundant mineral in our body is calcium.
- Calcium along with vitamin D helps for good calcium absorption.
- Calcium is very essential to maintain healthy bones and teeth.
- Calcium is rich in milk, yoghurt, ghee, leafy green vegetables and cereals.
- Deficiency of calcium leads to rickets in children.
- Deficiency of calcium leads to osteoporosis for adults.
- Calcium helps for blood clotting.



9.4.2 Sodium

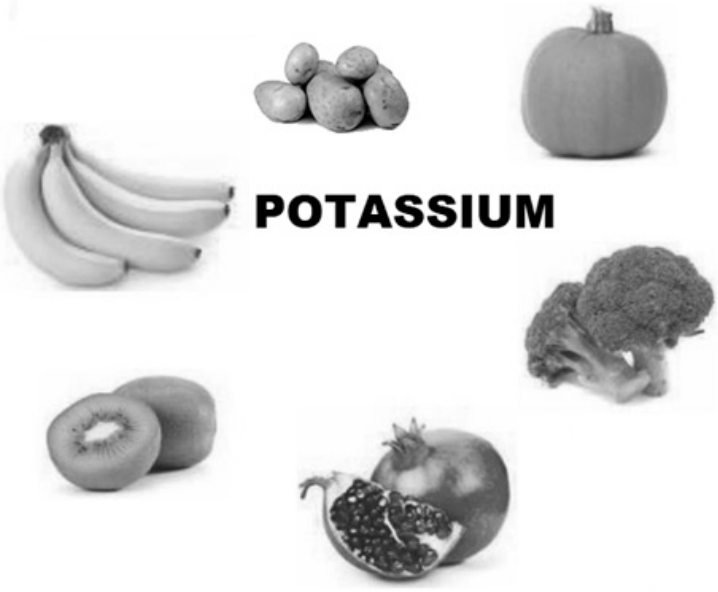


- Sodium is required in large amounts to keep our body healthy.
- Commonly we are taking sodium in the form of sodium chloride.
- Sodium helps to maintain water balance.
- Sodium also helps to control blood pressure of our body.
- Sodium transport nutrients and biomolecules in our body.

- Calcium imbalance will occur if we take excess of sodium.
- Deficiency of calcium leads to cardio vascular diseases.

9.4.3 Potassium

- Potassium mineral is required for organ function.
- Potassium is responsible for transmitting nerve impulses. It helps to keep the nervous system functioning properly.
- Potassium is third abundant mineral in our body.
- It helps in maintaining fluid balance of body.
- Potassium is high in banana, tomatoes, green vegetables and dairy products.
- Potassium is responsible for maintaining normal blood pressure and water balance of the body.
- Potassium helps to regulate digestion process.
- Deficiency of potassium leads to hypokalemia.



9.4.4 Phosphorous

- Phosphorous is rich in Mushrooms, Meat, Oats, Fish, Beans and Almonds.
- Phosphorous is essential for filtering waste secreted in the kidney.

- Phosphorous is required for cell repairing.
- ATP the energy bank of cell needs phosphorous.
- Phosphorous is essential for growth.

PHOSPHOROUS

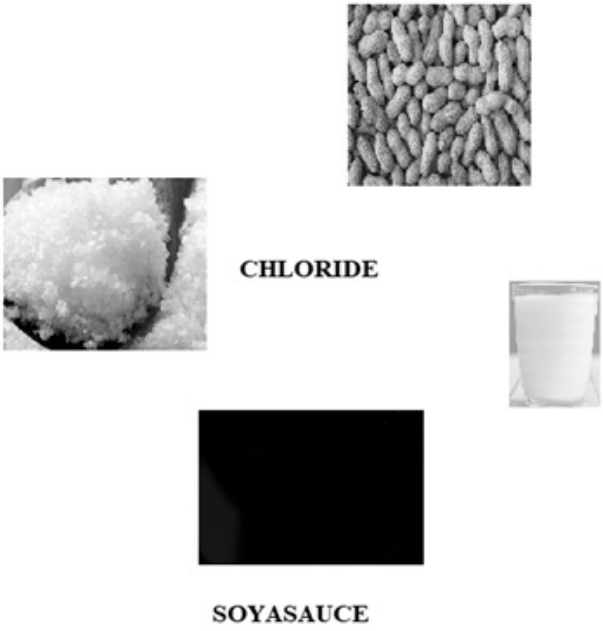
9.4.5 Magnesium

MAGNESIUM

- Magnesium is essential for healthy bones.
- Magnesium is essential for biochemical reactions in our body.

- Magnesium helps to regulate calcium and vitamin D in our body.
- Magnesium is essential for our heart to avoid cardio vascular problems. Magnesium intake lowers the risk of stroke.
- Magnesium plays a role in hypertension.
- Deficiency of Magnesium also leads to stress, anxiety and weakness.
- Magnesium deficiency causes hypomagnesemia.
- Excess of magnesium leads to gastrointestinal problems.
- Magnesium level fluctuates during menstrual cycle.

9.4.6 Chloride

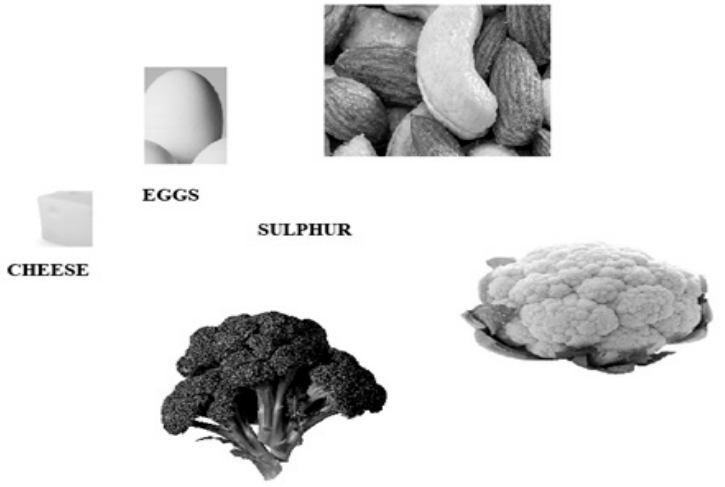


- Chloride helps to maintain pH and fluid level of our body.
- Chloride helps the red blood cells to exchange gases in our body.
- Cholride helps for the digestion of foods.
- Chloride helps to maintain blood pressure and blood volume.
- Chloride is rich in Table Salt, Soy Sauce, Milk and Peanuts.
- Excess of chloride leads to high blood pressure.

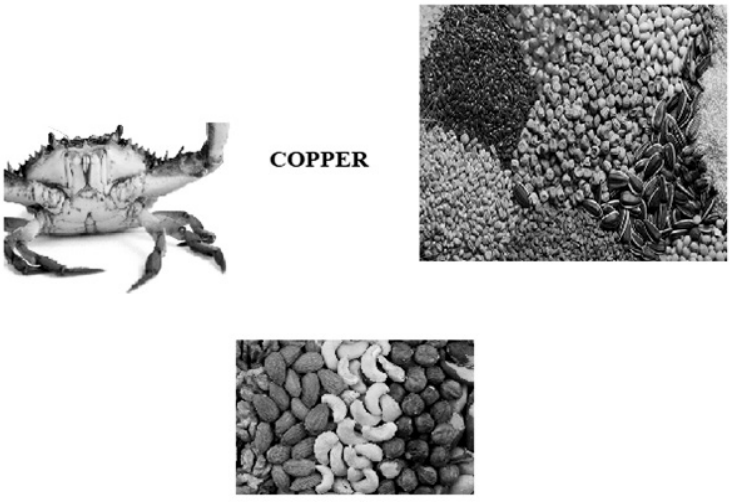
- Deficiency of chloride leads to vomiting, sweating and diarrhea.
- Immune system of our body needs chloride to perform it's functions.

9.4.7 Sulphur

- Suphur is an important mineral in protein synthesis.
- Sulphur is rich in Cheese, Eggs,Nuts, Onions, Cucumbers, Cauliflower and Broccoli.
- Sulphur has a special role to control cell damage.



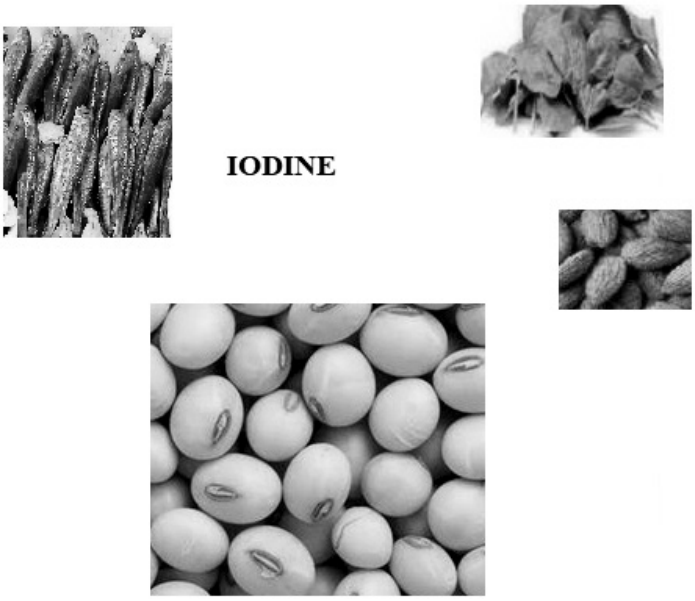
9.4.8 Copper



- Copper is rich in Oysters, Crab, Nuts, Wholegrains and Yeast extract.
- Copper is essential for the formation of red blood cells.

9.4.9 Iodine

- Iodine is an important mineral required for the normal functioning of the thyroid gland.
- Iodine is essential for normal growth and cell development.
- Iodine is necessary for thyroid hormones.
- Energy is generated from foods with the help of iodine.
- An iodine deficiency can lead to goiter.
- Swelling of thyroid gland occurs due to iodine deficiency.
- Radioactive iodine is used for treating thyroid cancer.
- Iodine promotes memory, concentration and thinking skills.
- Iodine is rich in salt, seaweeds and sea foods.

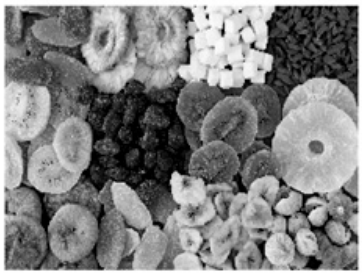


9.5 Microminerals

9.5.1 Iron

- Haemoglobin is an important constituent of blood and it contains iron.
- Iron is rich in meat and chicken, leafy green vegetables, dried fruits

- Iron helps for the transport of oxygen in blood.
- Deficiency of iron leads to anaemia.
- Excess of iron leads to liver and heart diseases and diabetes. In such cases, legumes, grains nuts can be taken to avoid iron absorption.
- APP

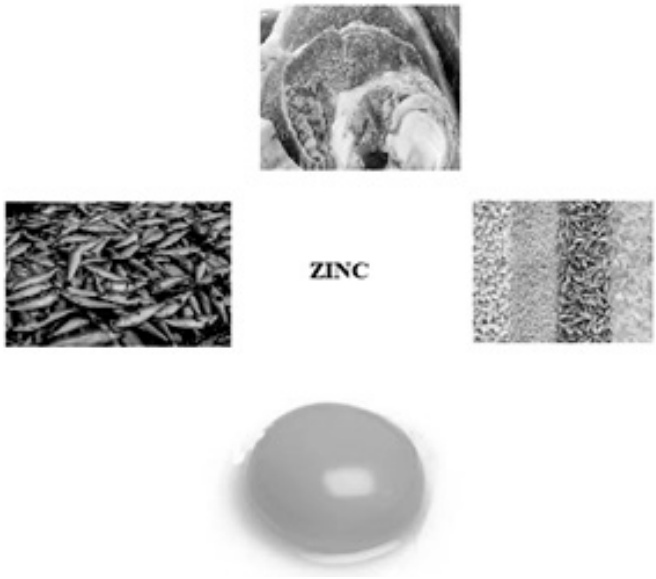


9.5.2 Boron

- Boron is important for strong bones.
- Boron is essential for muscle coordination.
- Boron helps to increase thinking skills.
- Boron is rich in coffee, apple, dried beans and milk.

9.5.3 Zinc

- Zinc is essential to maintain good health.
- Zinc is important for enzymatic reactions.
- Zinc helps to maintain growth and development.
- Zinc helps for supporting healthy skin and proper wound healing.
- Zinc supports sexual maturation and reproduction.
- Zinc is rich in egg yolk, fish, meat, seafood, seeds and grains.
- Zinc deficiency can result in loss of taste and smell.
- Zinc supports the immune system.
- Zinc controls the functioning of the sense organs in the nervous system.
- Zinc regulates division and reproduction.



9.5.4 Selenium

- Selenium helps to get healthy immune system.
- Selenium can be found in meat and grains.
- It helps to keep the blood sugar level.
- It helps to regulate cholesterol levels.

SELENIUM

9.5.5 Chromium

- Chromium is found in whole grains, cereals, mushrooms and meat.
- Chromium helps to break down fats and carbohydrates.
- Chromium is essential for metabolic processes.
- Deficiency of chromium leads to weight loss.
- It enhance protein, carbohydrate and lipid metabolism.

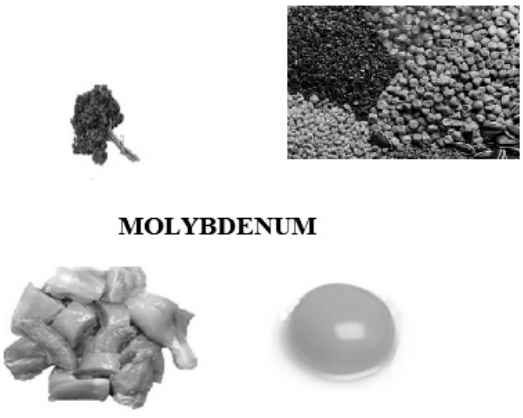
CHROMIUM

9.5.6 Manganese

- Manganese is essential for normal brain and nerve function.

- Manganese supports connective tissue, bones, blood clotting factors and sex hormones.
- It helps for fat and carbohydrate metabolism.
- Manganese is required for calcium absorption and blood sugar regulation.
- Manganese is rich in spinach, pineapple, nuts.
- Manganese deficiency leads to poor bone growth.

9.5.8 Molybdenum



- Molybdenum is rich in beans, liver, cereal grains, peas, legumes and dark green leafy vegetables.
- Molybdenum helps to break down proteins.
- Molybdenum is necessary to process proteins and genetic material.
- Molybdenum is vital for basic functions in our body.
- Molybdenum deficiency leads to poor growth.

CHAPTER

10 GENERAL BIOCHEMICAL PROCEDURES

10.1 Basics of Analysis

10.1.1 Qualitative Analysis

Qualitative analysis is the determination of chemical composition of a sample. By this method, the elements present in a sample can be identified.

10.1.2 Quantitative Analysis

Quantitative analysis is the determination of amount of substance present in a sample.

10.1.3 Solution

A homogeneous mixture of two or more substances which may be solids, liquids, gases, or a combination of solid, liquid and a gas.

10.1.4 Solvent

A substance in which another substance is dissolved, forming a solution.

10.1.5 Solute

The substance dissolved in a solvent in forming a solution.

10.1.6 Strength

The amount of solute in gram present in one litre of the solution.

10.1.7 Normality

Normality is the number of gram equivalents of the substance dissolved per litre of the solution. It is denoted by N.

For example, equivalent weight of NaOH is 40g.

For preparing 1N NaOH, 40g of NaOH is dissolved in 1L of water and for 0.1N NaOH, 4g of NaOH is dissolved in 1L of water.

For 1000mL of water, amount of NaOH required for 0.1N = 4g

For 100mL of water, amount of NaOH required for 0.1N = 0.4g

10.1.8 Molarity

Molarity is the number of moles of solute per litre of the solution. It is denoted by M.

10.1.9 Molality

Molality is the number of moles of the substance dissolved in 1000gms of the solvent.

10.1.10 Standard solution:

A solution whose concentration is known.

10.1.11 Percent Solution

A percentage solution is an amount or volume of chemical or compound per 100 mL of a solution. It is a relative expression of solute to solvent: X amount/100 ml = X%

For preparing 2% NaOH solution, we have to dissolve 2g of NaOH in 100 mL of water.

10.1.12 Buffer

A buffer is a solution that can resist pH change upon the addition of an acid or base. Using buffer solution, pH of a sample can be maintained. Blood acts as buffer in many biological reactions.

10.2 Qualitative Analysis of Biomolecules

10.2.1 Test for Carbohydrate

Molisch's Test

To about few ml of the substance , two drops of Molisch's reagent is added and shaken well. 2ml of conc.H_2SO_4 is added carefully along the sides of the test tube. A violet ring appears which indicates the presence of carbohydrate.

Fehling's Test

To few ml of the solution, 2ml of Fehling's solution(1ml of Fehling's A and 1ml of Fehling's B) is added. It is then boiled in a water bath for 5 mins. Reddish brown precipitate indicates the presence of monosaccharide.

Benedict's test

To 1ml of the solution, 2 drops of Benedict's solution is added. It is then boiled in a water bath for 5 mins. The Colour of solution change from blue to green , yellow, orange or red which indicates the presence of monosaccharide.

Tollen's test

To 1ml of the solution, 2ml of Tollen's reagent (1ml of Tollen's A and 1ml of Tollen's B) is added to the test tube It is then boiled in a water bath for 5 mins.

Iodine test

To about 1ml of the solution , 2 drops of 0.1N HCl and 2 drops of iodine solution are added.

Blue colouration indicates the presence of starch.

10.2.2 Test for monosaccharide

1. Barfoed's Test

To 1ml of the solution, 2ml of Barfoed's solution is added. It is then boiled in a water bath for 5mins. Indication of brick red precipitate shows the presence of monosaccharide.

2. Anthrone test

To 1ml of the solution, 2 drops of anthrone reagent is added along the sides of the test tube and shaken well. (If there is no colour change keep it in a water bath). Green coloration of solution shows the presence of monosaccharide.

3. Seliwanoff's test

To 1ml of the solution, 3 drops of Seliwanoff's reagent is added and boiled in a water bath for 5 mins.Cherry red colour indicates the presence of fructose.

4. Fougler's test

To one drop of a solution in a test tube, 3 drops of Foulger's reagent is added and boiled for 2minutes.Appearance of deep blue colour indicates the presence of fructose.

5. Bial's test

To 2ml of the solution in a test tube, 5 drops of Bial's reagent is added. It is then boiled in a water bath for 5 minutes. Appearance of green colour within 10 minutes shows the presence of pentose.

10.2.3 Test for Proteins

To 1ml of the solution, 5 drops of biuret reagent is added and mixed well. A violet or purple colour indicates the presence of proteins.

10.2.4 Test for Aminoacids

To 1ml of the solution , 1ml of 2% ninhydrin is added. It is then heated in a water bath for 5 mins. A purple colour will appear which shows the presence of aminoacids.

1. Xanthoproteic test

To about 1ml of the solution in a test tube, 0.5ml of conc.HNO3 is added, boiled, cooled. To this excess of 40% NaOH is added. Yellow coloured solution will formed which indicates the presence of aromatic amino acids Tyrosine and Tryptophan.

2. Pauly's test

To 1ml of the solution in a test tube, 1 drop of sulphanilic acid is added and cooled in ice. To this, add 1 drop of sodium nitrite solution, heat, cool and add 2 drops of 1% Na2CO3 solution. Solution turns to red colour which shows the presence of tyrosine and tryptophan.

3. Millon's test(Modified Millon's Test)

To 1ml of the solution in a test tube, 0.5ml of Millon's reagent is added and boiled in a water bath for 10 mins. Cool the mixture and add 5 drops of 1% sodium nitrite solution. Solution turns red which indicates the presence of tyrosine and tryptophan.

4. Ehrlich's Test

To 1ml of the solution, add 1 drop of Ehrlich's reagent is added. The appearance of deep red colour indicates the presence of tryptophan.

5. Hopkins-Cole Test

To 1ml of the solution added 2ml of glacial acetic acid, mixed well and then carefully added 2 drops of conc.H_2SO_4 along the sides of the test tube. A violet ring appears at the junction of liquids indicates the tryptophan.

6. Sodium Nitroprusside Test

To 1ml of the solution 5 drops of freshly prepared 2% solution of sodium nitroprusside and 5 drops of 10% NaOH is added. The solution turns red in colour which shows the presence of cysteine and cystine.

7. Sulphur Test

To 1ml of the solution in a test tube, add 2 drops of 40% NaOH and 1 drop lead acetate solution. The test tube is boiled for a minute and cooled. Brown colour of the solution will turn into black which shows the presence of cystine.

8. Sakaguchi Reaction

To 3ml of the solution in a test tube, add 1drop of 10% NaOH and 2 drops of 1% α-napthol in alcohol. After a few minutes add 1 drop of sodium hypobromite solution (Br_2 in NaOH). The appearance of intense red colour shows the presence of arginine.

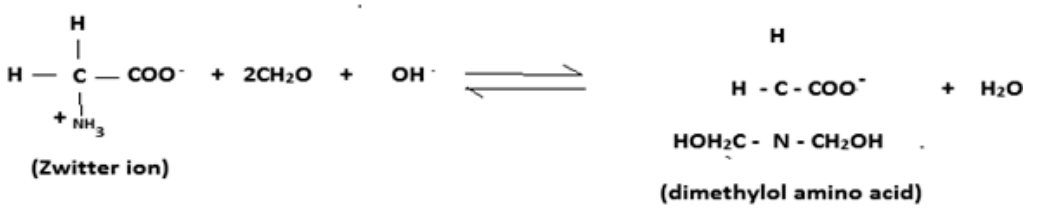
10.3 Estimation of Aminoacids (Glycine) by Formal Titration

10.3.1 Aim

To estimate the amount of glycine present in the whole of the given solution by formal titration. You are provided with exactly 0.1N oxalic acid solution and approximately decinormal solution of sodium hydroxide solution.

10.3.2 Principle

Amino acids contain amino group and carboxyl group. The carboxyl group of α-amino acids react with the basic amino groups to form zwitter ions. Zwitter ions are held together by electrostatic attraction. The zwitterions are not completely decomposed at the end point of alkaline indicators such as phenolphthalein. When amino acid solutions are treated with large excess of neutralysed formaldehyde the amino group combines with formaldehyde to form dimethylol amino acid. This reacts with alkali in the presence of phenolphthalein indicator to give a sharp end point.



$$\text{Equivalent weight of glycine} = \frac{\text{Molecular weight}}{1} = \frac{75}{1} = 75$$

10.3.3 Procedure

Titration I: Standardization of NaOH:

Pipette out 20mL solution hydroxide solution into a clean conical flask. Add a drop of phenolphthalein indicator. Titrate this solution against the standard oxalic acid taken in the burette. The end point is the disappearance of pink colour. Repeat the titration for concordant values. From the titre value the strength of sodium hydroxide solution can be determined.

Titration III

Estimation of Glycine

Titration II: Formaldehyde Versus Sodium hydroxide:

Pipette out 10mL of formalin and 20mL of water into a conical flask. Keep the mixture as such with occasional shaking. After 10 minutes, add a drop of phenolphthalein indicator. Titrate this mixture against the sodium hydroxide taken in the burette. The end point is the appearanceof pale permanent pink colour. Repeat the titration for concordant values.

Titration III: Estimation of Glycine:

Make up the given solution of glycine in a 100mL standard flask. Pipette out 20mL of this made up glycine into a conical flask. Add 10mL of formalin shake the contents well and allow the reaction to take place for 10 minutes. Now add a drop of phenolphthalein indicator. Titrate the contents against the sodium hydroxide taken in the burette. The end point is the appearance of pale, permanent pink colour. Repeat the titration for concordant values. From the titre value the strength and hence the weight of glycine in the whole of the given solution can be calculated.

10.3.4 Result

Weight of glycine present in the whole of the given solution = _____ g.

Titration I: Standardisation of NaOH Std Oxalic acid Vs NaOH

S.No	Volume of NaOH (mL)	Burette Reading (mL)		Volume of Oxalic acid (mL)	Indicator
		Initial	Final		
2.	20				

Volume of Oxalic acid (V_1) = _____ mL

Strength of Oxalic acid (N_1) = _____ 0.1N

Volume of Sodium hydroxide (V_2) = _____ mL

Strength of Sodium hydroxide (N_2) = _____ N

Strength of glycine (N_2) = $\frac{V_1 N_1}{V_2}$ = _____ N

Titration II: Formaldehyde Vs NaOH

S.No	Volume of formaldehyde (mL)	Burette Reading (mL)		Volume of NaOH (mL)	Indicator
		Initial	Final		
1.	20				
2.	20				

Titration III: NaOH Vs Given glycine

S.No	Volume of glycine (mL) + 10mL of formalin (mL)	Burette Reading (mL)		Volume of NaOH (V_y) (mL)	Indicator
		Initial	Final		
1.	20				
2.	20				

Volume of Sodium hydroxide (V_1) = (V_y – V_x) mL

Strength of Sodium hydroxide (N_1) = _____ N

Volume of glycine (V_2) = 20 mL

Strength of glycine (N_2) = $\frac{V_1 N_1}{V_2}$ =_____ N

Weight glycine present in the whole of the given solution = $\frac{N \times 75}{10} g$

= _____ g

From this, the amount of glycine present in the whole of the given solution is calculated.

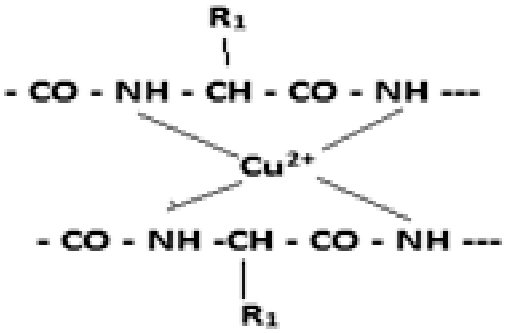
10.4 Estimation of Protein by Biuret Method

10.4.1 Aim

To estimate the amount of protein present in the whole of the given sample of serum by biuret method.

10.4.2 Principle

Cu^{2+} in alkaline solution complexes with nitrogen atoms of the peptide bonds in proteins. This complex gives a purple colour. The colour is measured at 520 n.m (green filter).



10.4.3 Procedure

Prepare a standard solution of protein (stock solution) by dissolving 15 g of Bovine serum albumin in 250 mL of distilled water. Prepare a working standard by diluting 10 mL of stock solution into 100 mL in a standard flask using distilled water. This working standard contains 6 mg of protein / mL. Pipette out into a series of tubes (S1 to S10) 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mL of the protein solution and make up the total volume to 5.0 mL with addition of distilled water. A blank tube (B) will contain only 5.0 mL of water. Add 6.0 mL of biuret reagent to each tube and mix well. Keep the test tubes at room temperature for 10 minutes. Measure the optical density of each tube at 520 n.m (green filter) using the reagent blank. Draw a standard graph using concentration along x-axis and optical density along y-axis. Make up the given serum to 100 mL in a standard flask. Pipette out 2 mL and 4 mL from this made up solution into different test tubes (T_1 and T_2) and make up the volume to 5 mL with water and repeat the above same procedure with these test solutions also. Cut the standard graph using the Optical density obtained for the test solutions. This gives the concentration of protein in the test solutions. From this calculate amount of protein present in the sample of serum given.

Estimation of Protein by Biuret method

Volume of Protein (mL)	Volume of Water (mL)	Volume of Biuret reagent (mL)	Optical Density (%T)	Concentration (mg)
1	4	6		
1.5	305	6		
2	3	6		
2.5	2.5	6		

3	2	6		
3.5	1.5	6		
4	1	6		
4.5	0.5	6		
5	0	6		
Unknown I		6		
Unknown II		6		

From the graph, the concentration of unknown protein is determined.

10.5 Estimation of Carbohydrate by Anthrone Method

10.5.1 Aim

To estimate the amount of carbohydrate present in the given sample.

10.5.2 Principle

Carbohydrates react with concentrated sulphuric acid to form furfural or 5- hydroxyl methyl furfurol. Then it condenses with anthrone to form a green coloured complex. Then the absorbance of the sample was measured colorimetrically at 620-640nm.

10.5.3 Procedure

100mg of glucose is dissolved in 100mL of water (stock solution). Pipette out 10mL from stock solution into an 100mL SMF (100µg/mL). Dissolve 0.2g anthrone in 5mL ethanol. Make up the solution to 100mL using 75% sulphuric acid.

0.1 to 1mL of the working standard solution is taken in a series of test tube. Make up the volume of all test tube to 1mL with distilled water. Keep the test tubes in an ice bath and slowly add 5mL of cold anthrone reagent. Mix well. Close the test tubes with aluminium foil and place it in a boiling water bath for 10 minutes.

Cool the test tubes . Measure OD at 620nm. Plot the graph between concentration and absorbance. Then the concentration of carbohydrate in the sample can be determined from the graph.

Estimation of Carbohydrate by anthrone method

Volume of working standard (mL)	Volume of Water (mL)	Concentration of working sample (µg/mL)	Volume of anthrone (mL)	OD at 620nm
0.1	0.9	10	5	

0.2	0.8	20	5	
0.3	0.7	30	5	
0.4	0.6	40	5	
0.5	0.5	50	5	
0.6	0.4	60	5	
0.7	0.3	70	5	
0.8	0.2	80	5	
0.9	0.1	90	5	
1	0	100	5	

From the graph, the concentration of unknown carbohydrate is calculated.

10.6 Techniques for Sample Preparation

Sample preparation is the series of steps required to prepare a sample in a suitable form for analysis. The faster these steps can be done, the more quickly the analysis will be completed. Sample preparation has been considered not as a part of the analytical process, rather the "procedure" that had do be done to develop and perform analytical methods.

Sample preparation may involve dissolution, extraction, reaction with some chemical species, pulverizing, treatment with a chelating agent (e.g. EDTA), masking, filtering, dilution, sub-sampling or many other techniques. Treatment is done to prepare the sample into a form ready for analysis by specified analytical equipment. Sample preparation could involve: crushing and dissolution, chemical digestion with acid or alkali, sample extraction, sample clean up and sample pre-concentration.

However, the significance of the sample preparation for the total analytical performance is nowadays widely recognized.

10.7 Ultrafiltration

Ultrafiltration (UF) is a membrane filtration process similar to Reverse Osmosis, using hydrostatic pressure to force water through a semi-permeable membrane. The pore size of the ultrafiltration membrane is usually 103 - 106 Daltons. Ultrafiltration (UF) is a pressure-driven barrier to suspended solids, bacteria, viruses, endotoxins and other pathogens to produce water with very high purity and low silt density.

Ultrafiltration (UF) is a variety of membrane filtration in which hydrostatic pressure forces a liquid against a semi permeable membrane. Suspended solids and

solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane. Ultrafiltration is not fundamentally different from reverse osmosis, microfiltration or nanofiltration, except in terms of the size of the molecules it retains.

A membrane or, more properly, a semi permeable membrane, is a thin layer of material capable of separating substances when a driving force is applied across the membrane. Once considered a viable technology only for desalination, membrane processes are increasingly employed for removal of bacteria and other microorganisms, particulate material, and natural organic material, which can impart color, tastes, and odors to the water and react with disinfectants to form disinfection by products (DBP).

10.8 Lyophilization

10.8.1 Introduction

Lyophilization is a water removal process typically used to preserve perishable materials, to extend shelf life or make the material more convenient for transport. Lyophilization works by freezing the material, then reducing the pressure and adding heat to allow the frozen water in the material to sublimate.

10.8.2 Phases of Lyophilization

Lyophilization occurs in three phases, with the first and most critical being the freezing phase. Proper lyophilization can reduce drying times by 30%.

i) Freezing Phase

There are various methods to freeze the product. Freezing can be done in a freezer, a chilled bath (shell freezer) or on a shelf in the freeze dryer. Cooling the material below its triple point ensures that sublimation, rather than melting, will occur. This preserves its physical form.

Lyophilization is easiest to accomplish using large ice crystals, which can be produced by slow freezing or annealing. However, with biological materials, when crystals are too large they may break the cell walls, and that leads to less-than-ideal freeze drying results. To prevent this, the freezing is done rapidly. For materials that tend to precipitate, annealing can be used. This process involves fast freezing, then raising the product temperature to allow the crystals to grow.

ii) Primary Drying (Sublimation) Phase

Lyophilization's second phase is primary drying (sublimation), in which the pressure is lowered and heat is added to the material in order for the water to sublimate. The

vacuum speeds sublimation. The cold condenser provides a surface for the water vapor to adhere and solidify. The condenser also protects the vacuum pump from the water vapor. About 95% of the water in the material is removed in this phase. Primary drying can be a slow process. Too much heat can alter the structure of the material.

iii) Secondary Drying (Adsorption) Phase

Lyophilization's final phase is secondary drying (adsorption), during which the ionically-bound water molecules are removed. By raising the temperature higher than in the primary drying phase, the bonds are broken between the material and the water molecules. Freeze dried materials retain a porous structure. After the lyophilization process is complete, the vacuum can be broken with an inert gas before the material is sealed. Most materials can be dried to 1-5% residual moisture.

10.8.3 Problems in Lyophilization

- Heating the product too high in temperature can cause melt-back or product collapse
- Condenser overload caused by too much vapor hitting the condenser.
  - Too much vapor creation
  - Too much surface area
  - Too small a condenser area
  - Insufficient refrigeration
- Vapor choking – the vapor is produced at a rate faster than it can get through the vapor port, the port between the product chamber and the condenser, creating an increase in chamber pressure.

10.8.4 Critical Temperature

During lyophilization, the maximum temperature of the product before its quality degrades by melt-back or collapse.

Lyophilization is a commonly used technique for formulation development of small molecules which are unstable in aqueous medium and are thermolabile in nature. Lyophilization of drug alone, however, presents certain formulation development challenges, which may be overcome by incorporation of excipients (e.g. bulking agents, buffering agents, tonicifying agent, wetting agent and cosolvents, preservatives and collapse temperature modifiers) in the formulation.

10.9 Quantitative Estimation of Lipid

10.9.1 Determination of Iodine Number

- The iodine number of a fat is the amount in gm. of iodine taken up by 100 gm. of fat. Not only iodine but also equivalent amounts of other halogens will add at double bonds; so bromine is often used instead of iodine because it is more reactive.
- The halogenating reagent used in this method is pyridine sulphate di-bromide. This reagent can be prepared by adding carefully 8.1 ml pyridine in 20 ml glacial acetic acid and making the volume up to 1 litre with glacial acetic acid.
- Weigh the bottle containing sample of oil plus a medicine dropper and then transfer about 0.1 to 0.3 gm. of oil to a flask. Reweigh the bottle containing oil and dropper to find out the exact quantity of the sample transferred. Add 10 ml of chloroform and then 25 ml of the pyridine sulphate di-bromide reagent.
- Shake thoroughly; allow standing for 5 minutes and then determine the residual bromine. To do this, add 10 ml of 10% KI and titrate the equivalent amount of iodine liberated by the residual bromine with the help of 0.1 (N) $Na_2S_2O_3$ (sodium thiosulphate). The titration can be done by adding sodium thiosulphate solution through a burette to the flask.
- When the colour of the solution in flask becomes light yellow add 1 ml of starch solution. It will become blue. Slowly add the thiosulphate solution again till it becomes colourless. Note the total volume of thiosulphate used.
- The total amount of bromine originally added is found by titrating 25 ml of the pyridine sulphate di-bromide reagent with thiosulphate after adding KI as in the previous case. The amount of bromine taken up by the fat sample can be determined by the difference between the two titers and then the iodine number can be calculated.

10.9.2 Quantitative Estimation of Cholesterol

- Shake the tubes well and keep them at room temperature for 30 minutes. Blue colour will develop in all the tubes except blank tube. Measure the absorbancies at 625 m|a. against the blank tube and plot these against the amount of cholesterol.
- Acetic anhydride-sulphuric acid reagent has to be freshly prepared before use. Acetic anhydride (20 ml) is taken in a glass stoppered flask which is then chilled in ice water. When cold, add 1 ml of conc. H_2SO_4 to it drop by drop.

- The contents are mixed and cooled during the addition. After completion of the addition the flask is stoppered and shaken vigorously for a few minutes. The solution has to be kept cold in ice and should be used within an hour.

10.10 Quantitative Estimation of Fatty Acid

Free fatty acids (FFA) in plant oils and fats (e.g. edible oils and fats) are a quality feature for these fats. Fats with high levels of FFA are more susceptible to oxidative aging, they become rancid more quickly. The FFA should be removed during a refining process.

Determination of the FFA in Oils and fats is done by potentiometric titration in Ethanol / Diethyl ether as solvent with KOH in Isopropyl alcohol.