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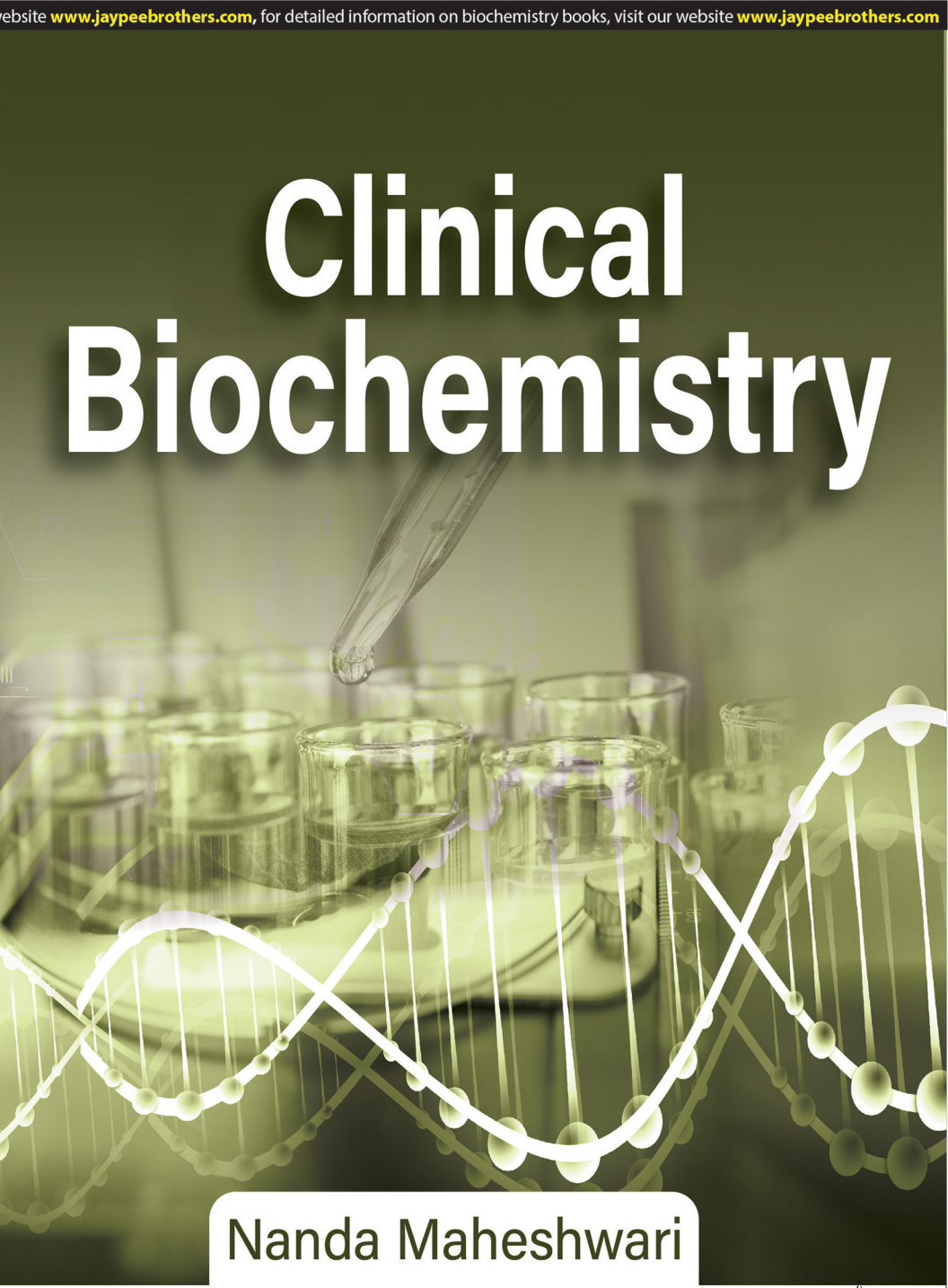
Clinical Biochemistry



Nanda Maheshwari

3rd Edition

Foreword
SM Arora

JAYPEE

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CHAPTER

8

Study of Carbohydrates

Keywords

Monosaccharides, oligosaccharide, polysaccharide, iodine test, Benedict's test, Fehling's test, mucic acid test, basal metabolic rate, glycogenesis, glycolysis, citric acid cycle, hexose monophosphate shunt (HMP pathway), galactosemia, fructose intolerance, disorders of pyruvate metabolism, biochemical importance of carbohydrate

INTRODUCTION

Carbohydrates constitute the greatest proportion of organic material on earth. They are the main components of the food. Carbohydrates are the important components of our clothing, housing furniture, etc.

Carbohydrates are defined as polyhydroxy aldehydes or ketones and their derivatives.

CLASSIFICATION

Carbohydrates are classified as **(Flowchart 8.1)**:

1. **Monosaccharides:** These are the simplest members of carbohydrate, which are represented by the general formula $C_nH_{2n}O_n$. Monosaccharides are grouped according to the number of carbon atoms present in a sugar molecule, such as trioses (3), tetroses (4), pentoses (5), and hexoses (6). Each of these can be further named as aldoses or ketoses depending on the presence of aldehyde or ketone group, respectively.

   For example, glucose—it is a hexose carbohydrate. It is an aldose sugar.
2. **Oligosaccharide:** These are condensation products of two to ten simple sugars or monosaccharide. They are represented by general formula $C_n(H_2O)_{n-1}$. Oligosaccharides are grouped according to number of monosaccharide units present in a molecule.

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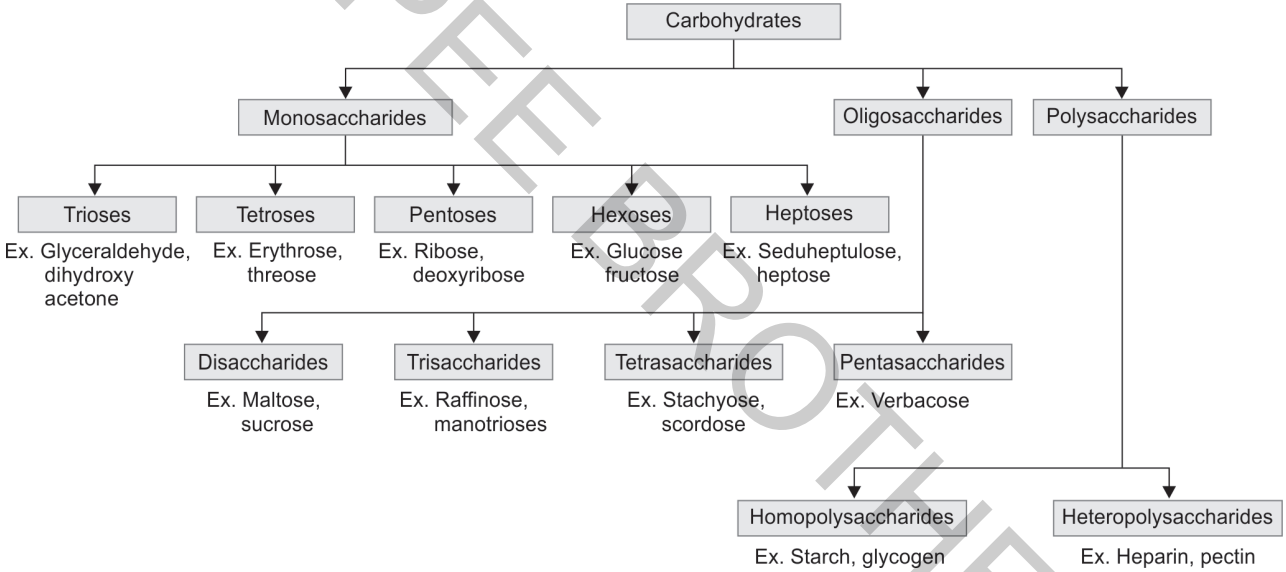
Flowchart 8.1: Classifications of carbohydrate.



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For example, maltose, lactose and sucrose are disaccharides and raffinose is trisaccharide.

3. **Polysaccharide:** These are the majority of carbohydrates found in natural sources. These are high molecular weight polymers of monosaccharides represented by general formula $(C_6H_{10}O_5)n$. They are classified as homopolysaccharide and heteropolysaccharide depending on the presence of either the same monosaccharide or more than one simple sugar.
   For example, cellulose and glycogen are homopolysaccharides while glucuronic acid is a heteropolysaccharide.

DETECTIVE TESTS FOR CARBOHYDRATE

1. **Dehydration:** Carbohydrates on dehydration give furfural or its derivatives. Concentrated sulfuric acid is used as dehydrating agent e.g., Moilsch test. It is general test for carbohydrate identification. Furfural or its derivatives formed during dehydration react with α-naphthol to give violet color.

$$\text{Ribose} \xrightarrow{\text{conc. } H_2SO_4} \text{Furfural}$$

2. **Reducing properties:** Carbohydrates having free carbonyl group acts as reducing agent. It reduces certain metal ions like copper, mercury, etc. based on this property following tests detect reducing sugars.
   - *Benedict's test:* Carbohydrates are heated with alkaline copper sulfate, copper ions get reduced and give red precipitate of Cu_2O.
   - *Fehling's test:* Reducing sugar reduces copper ions present in Fehling's solution so as to give red precipitates of Cu_2O.
   - *Formation of osazone:* When reducing sugar is heated with phenylhydrazine, yellow crystalline compounds called osazones are formed. Definite crystalline forms of osazone are obtained from different reducing sugars. For example, glucose phenylhydrazone react with two molecules of phenylhydrazine to give glucosazone, aniline and ammonia.
3. **Reduction:** The carbonyl group of sugar can be reduced by variety of reagents such as H_2 and Pt to an alcohol. Such carbohydrate derivatives are called alditols. For example,

$$\text{Glucose} \xrightarrow{H_2 \text{ - Pt}} \text{Sorbitol}$$

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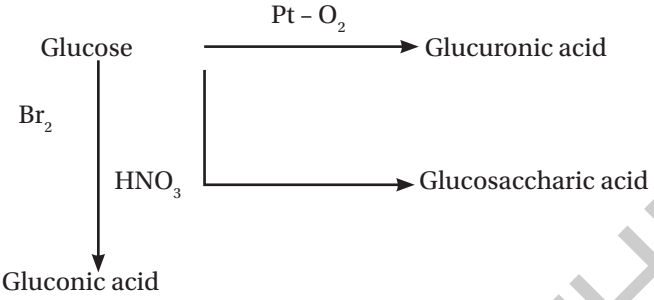
4. **Oxidation:** Sugar on oxidation gives acid. The oxidation product depends upon oxidizing agent used in the reaction. For example,



5. **Mucic acid test:** This test is used for identification of galactose and lactose. Galactose/lactose on oxidation in presence of concentrated nitric acid gives galactosaccharic acid (mucic acid). The mucic acid is insoluble and gets crystallized. The crystals are colorless and broken glass type.
6. **Iodine test:** Iodine reacts with different sugars to form different colors. For example,

Types of polysaccharide	*Color with iodine*
Starch	Blue
Dextrin	Brown
Glycogen	Pink
Amylase	Deep blue
Amylopectin	Purple

METABOLISM

Metabolism of a substance is defined as series of biochemical reactions occurring within the living organism from the time of incorporation until its excretion. Metabolism consists of anabolism and catabolism.

Anabolism: Anabolism or synthesis is defined as series of biochemical reactions involved in the process of synthesis of larger molecules from smaller ones.

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Catabolism: Catabolism or analysis is defined as series of reactions involved in the process of breakdown of larger molecules into smaller ones for supply of energy.

Energy metabolism: There will be continuous exchange of energy between living body and its surrounding environment. This energy exchange is based on first law of thermodynamics which states that energy is neither gained nor lost when it is converted from one form to another form. These forms are chemical, electrical, mechanical, thermal, etc.

Basal metabolism: Energy produced as by-product of cellular metabolism is essential for sustenance of life. Although, there is variation in requirement of amount of energy, rate of production of energy in body remains more or less constant under some standard conditions known as basal metabolism.

Basal metabolic rate: The rate of production of energy at basal conditions per hour per meter square of body surface area is called basal metabolic rate. Its normal value in adult male is 40 calories per hour per meter square and 37 calories per hour per meter square in adult female.

Unit of energy: It is mainly calorie. It is defined as amount of heat energy which can raise the temperature of 1 g of water from 15°C to 16°C. Amount of heat can also be expressed as joule.

1 kilocalorie = 4.19 kilojoules

Uses of energy in body: Energy obtained from food sources is used in body for several purposes such as:

- Synthesis of protoplasmic constituents like DNA, RNA, phospholipids, etc.
- Functioning of different organs like heart, kidneys, etc.
- Maintenance of body temperature.
- Generation of electrical potentials in central nervous system (CNS), autonomic nervous system (ANS), heart, etc.
- Transport of substances against concentration gradient.
- Growth and maintenance.

Food sources that give us energy are mainly carbohydrate, protein and fat.

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CARBOHYDRATE METABOLISM

Carbohydrates are converted to monosaccharides in the process of digestion in the digestive system. Mainly they are glucose, fructose and galactose. They are absorbed from the small intestines into portal circulation. Major function of carbohydrates is release of energy by undergoing oxidation. Carbohydrates are mainly used by cells in the form of glucose. Especially brain cells use glucose only. Fructose and galactose are also converted to glucose in liver. Different metabolic processes undergone by carbohydrates in the body are given below:

- **Glycogenesis:** It is the synthesis of glycogen from glucose.

  Glucose ⟶ glycogen
- **Gluconeogenesis:** It is formation of glucose from non-carbohydrates as glycerol.

  Glycerol ⟶ glucose
- **Glycogenolysis:** It is the conversion of glycogen to glucose.

  Glycogen ⟶ glucose
- **Glycolysis:** It is the oxidative pathway of glycogen or glucose.
- **Citric acid cycle:** It is also oxidative pathway of glucose.
- Hexose monophosphate shunt is alternative to glycolysis and citric acid cycle for oxidation of glucose.

Glycolysis

Glycolysis is also known as Embden Meyerhoff pathway. Anaerobic glycolysis is anaerobic degradation of glucose to yield lactic acid.

Stages of glycolysis: There are two stages in glycolysis **(Flowchart 8.2)**. In the first stage, glucose is converted to glyceraldehyde 3-phosphate, a three carbon compound. In the second stage, glyceraldehyde 3-phosphate is converted into pyruvate. It is regarded as the preparatory (or investment) phase, since they consume energy to convert the glucose into two three-carbon sugar phosphates. This pyruvate enters citric acid cycle in aerobic conditions. The second half of glycolysis is known as oxidative (or the pay-off phase) it is characterized by oxidation of glucose to pyruvate, with a net gain of the energy-rich molecules ATP and NADH. In anaerobic conditions, pyruvate is converted to lactate.

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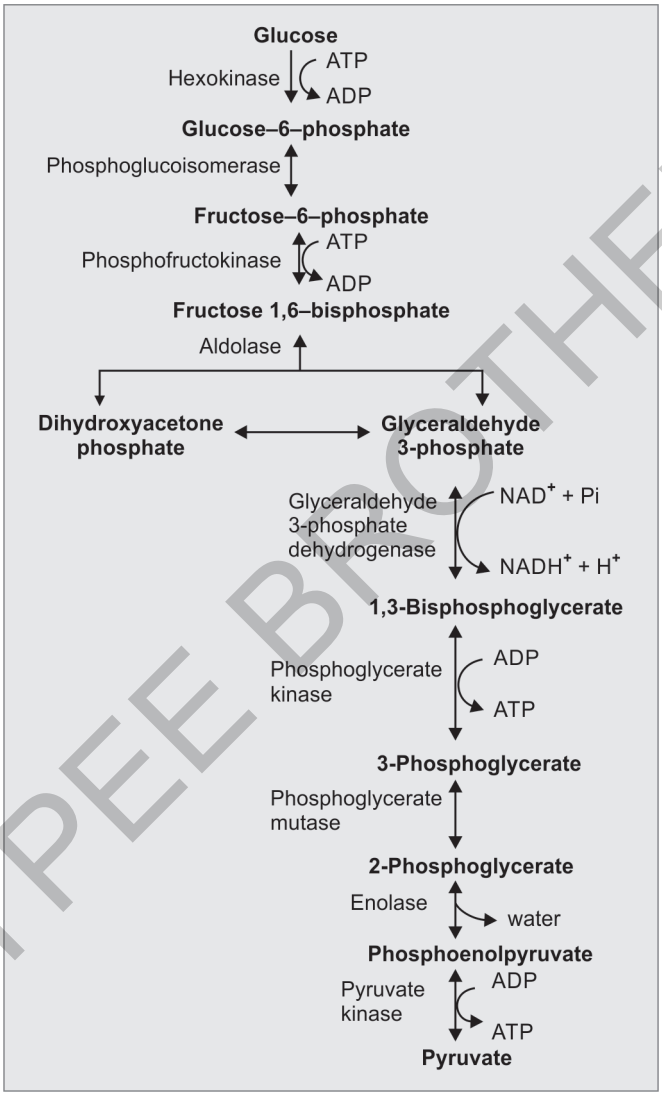
Flowchart 8.2: Glycolysis.



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Steps in Preparatory Phase of Glycolysis

1. Glucose $\xrightarrow[\text{ATP}]{\text{Hexokinase}}$ Glucose 6-phosphate $\underset{\text{ADP}}{}$

In this step, phosphorylation of D-Glucose to glucose 6-phosphate by ATP takes place. It is catalyzed by enzyme hexokinase.

2. Glucose 6-phosphate $\xrightarrow{\text{Isomerase}}$ Fructose 6-phosphate

   In this step, isomerization of glucose 6-phosphate to fructose 6-phosphate takes place. It is catalyzed by enzyme isomerase.

3. Fructose 6-phosphate $\xrightarrow[\text{ATP}]{\text{Hexokinase}}$ Fructose 1,6-bisphosphate $_{\text{ADP}}$

4. Fructose 1,6-bisphosphate $\xrightarrow{\text{Aldolase}}$ Dihydroxyacetone phosphate + Glyceraldehyde 3-phosphate

   In this step, fructose 1,6-bisphosphate is broken down to dihydroxy acetone phosphate and glyceraldehyde 3-phosphate (3 carbon compounds). This reaction is catalyzed by enzyme aldolase.

Steps in Oxidative Phase of Glycolysis

1. Glyceraldehyde-3-phosphate (2) + NAD^+ + Pi $\xrightarrow{\text{Dehydrogenase}}$ 1,3-Bisphosphoglycerate (2) + NADH + H^+

   In this step, oxidation of glyceraldehyde 3-phosphate (2) to 1,3-Bisphosphoglycerate (2) takes place. It is catalyzed by enzyme glyceraldehyde 3-phosphate dehydrogenase.

2. 1,3-Bisphosphoglycerate (2) $\xrightarrow[\text{Kinase}]{\text{2ADP}}$ 3-Phosphoglycerate (2) + 2 ATP

   In this step, 2ATP molecules are produced by transfer of phosphates from 2 molecules of 1,3-Bisphosphoglycerate. It is catalyzed by enzyme kinase.

3. 3-Phosphoglycerate (2) $\xrightarrow{\text{Mutase}}$ 2-Phosphoglycerate (2)

   In this step, conversion of 3-Phosphoglycerate(2) to 2-Phosphoglycerate (2) takes place. It is catalyzed by enzyme mutase.

4. 2-Phosphoglycerate (2) $\xrightarrow{\text{Enolase}}$ Phosphoenolpyruvate(2) + H_2O

   In this step, conversion of 2-Phosphoglycerate(2) to phosphoenolpyruvate (2) takes place. It is catalyzed by enzyme enolase.

5. Phosphoenolpyruvate (2) + 2ADP $\xrightarrow{\text{Kinase}}$ Pyruvate (2) + 2 ATP

   In this step, conversion of phosphoenolpyruvate (2) to pyruvate (2) takes place. It is catalyzed by enzyme kinase.

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2 ATP molecules are produced by transfer of phosphates from phosphoenolpyruvate.

6. $\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$

In anaerobic conditions, pyruvate is converted to lactate by enzyme lactate dehydrogenase (LDH). In aerobic conditions, pyruvate enters citric acid cycle.

Glycolysis yields two molecules of ATP (4 ATPs are produced, 2 ATPs are consumed. Net yield of ATP is 2), two molecules of pyruvic acid and two "high energy" electron carrying molecules of NADH.

Tricarboxylic Acid Cycle

Tricarboxylic acid cycle (TCA) cycle is a cyclic sequence of reactions in which pyruvate formed in glycolysis is completely oxidized to CO_2 and H_2O.

It was postulated by HA Krebs in 1937. Its original name is citric acid cycle.

TCA cycle is the common pathway for degradation of two carbon acetyl residues derived not only from carbohydrates but also from fatty acids and, which is amino acids. TCA cycle actually takes place in intact cells. It accounts quantitatively for oxidation of carbohydrates, fatty acids and amino acids.

TCA cycle is both catabolic and anabolic pathway. It also generates precursors for anabolic pathways. α-ketoglutarate and oxaloacetate serve as precursors of amino acids. α-ketoglutarate and oxaloacetate are intermediates of this cycle. Citrate can be removed from this cycle and can be used as precursor of extramitochondrial acetyl-CoA for fatty acid biosynthesis. Succinyl-CoA can also be removed from the cycle for biosynthesis of heme.

The Net Equation

$\text{Acetyl-CoA} + 3\ \text{NAD}^+ + \text{FAD} + \text{GDP} + \text{HPO}_4^{-2} \longrightarrow 2\text{CO}_2 + 3\ \text{NADH} + \text{FADH}_2 + \text{GTP} + \text{CoA}$

Reaction 1: Formation of Citrate

The first reaction of the cycle is the condensation of acetyl-CoA with oxaloacetate to form citrate, catalyzed by citrate synthase.

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Once oxaloacetate is joined with acetyl-CoA, a water molecule attacks the acetyl leading to the release of coenzyme A from the complex. It is catalyzed by citrate synthatase forming citrate.

$$\text{Acetyl-CoA + Oxaloacetate + } H_2O \xrightarrow{\text{Citrate synthase}} \text{Citrate + CoA}$$

Reaction 2: Formation of Isocitrate

The citrate is rearranged to form an isomeric form, isocitrate by an enzyme aconitase.

The effect of this conversion is that the –OH group is moved from the 3′ to the 4′ position on the molecule.

$$\text{Citrate} \xrightarrow{\text{Aconitase}} \textit{Cis}\text{-Aconitate} \xrightarrow{\text{Aconitase}} \text{Isocitrate}$$

Reaction 3: Oxidation of Isocitrate to α-Ketoglutarate

In this step, isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to form α-ketoglutarate.

In the reaction, generation of NADH from NAD is seen. The enzyme isocitrate dehydrogenase catalyzes the oxidation of isocitrate to yield an intermediate which then has a carbon dioxide molecule removed from it to yield α-ketoglutarate.

$$\text{Isocitrate + } NAD^+ (NADP^+) \longrightarrow \alpha\text{-Ketoglutarate + } CO_2 + \text{NADH (NADPH)} + H^+$$

Reaction 4: Oxidation of α-Ketoglutarate to Succinyl-CoA

Alpha-ketoglutarate is oxidized, carbon dioxide is removed, and coenzyme A is added to form the 4-carbon compound succinyl-CoA. During this oxidation, NAD^+ is reduced to NADH and H^+. The enzyme that catalyzes this reaction is α-ketoglutarate dehydrogenase.

$$\alpha\text{-ketoglutarate + } NAD^+ + \text{CoA} \longrightarrow \text{Succinyl-CoA+}CO_2\text{+ NADH + } H^+$$

Reaction 5: Conversion of Succinyl-CoA to Succinate

Coenzymes A is removed from succinyl-CoA to produce succinate. The energy released is used to make guanosine triphosphate (GTP) from guanosine diphosphate (GDP) and Pi by substrate-level

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phosphorylation. GTP can then be used to make ATP. The enzyme succinyl-coA synthase catalyzes this reaction of the citric acid cycle.

$$\text{Succinyl-CoA} + \text{Pi} + \text{GDP} \xrightarrow{\text{Succinyl-CoA synthetase}} \text{Succinate} + \text{GTP} + \text{CoA-SH}$$

Reaction 6: Oxidation of Succinate to Fumarate

Succinate is oxidized to fumarate. During this oxidation, FAD is reduced to $FADH_2$. The enzyme succinate dehydrogenase catalyzes the removal of two hydrogens from succinate.

$$\text{Succinate} + \text{E-FAD} \xrightarrow{\text{Succinate dehydrogenase}} \text{Fumarate} + \text{E-FADH}_2$$

Reaction 7: Hydration of Fumarate to Malate

The reversible hydration of fumarate to L-malate is catalyzed by fumarase (fumarate hydratase).

Fumarase continues the rearrangement process by adding hydrogen and oxygen back into the substrate that had been previously removed.

$$\text{Fumarate} + H_2O \xrightarrow{\text{Fumarase}} \text{L-malate}$$

Reaction 8: Oxidation of Malate to Oxaloacetate

Malate is oxidized to produce oxaloacetate, the starting compound of the citric acid cycle by malate dehydrogenase. During this oxidation, NAD^+ is reduced to NADH and H^+.

Significance of Krebs Cycle

1. Amino acids are formed from α-ketoglutaric acid, pyruvic acids and oxaloacetic acid.
2. Krebs cycle (citric acid cycle) releases plenty of energy (ATP) required for various metabolic activities of cell **(Fig. 8.1)**.
3. By this cycle, carbon skeleton are got, which are used in process of growth and for maintaining the cells.
4. The potential of NADH and $FADH_2$ is converted to more ATP through an electron transport chain with oxygen as the "terminal electron acceptor".*

*Number of adenosine triphosphate (ATP) formed from a molecule of glucose.

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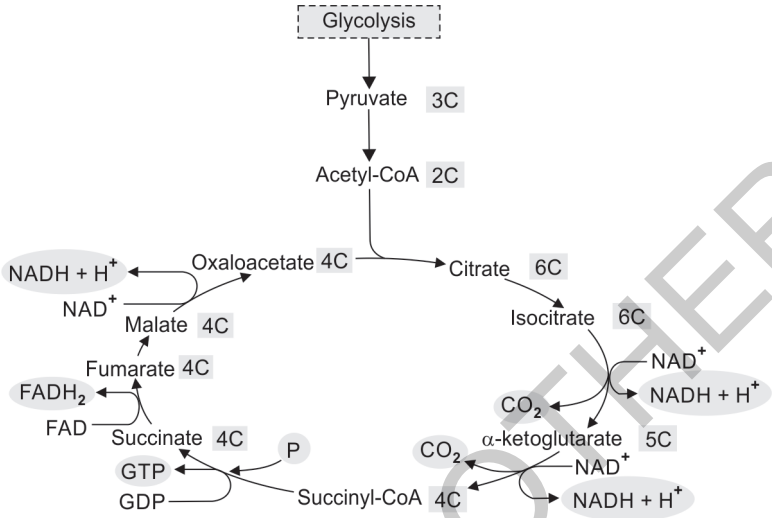

Fig. 8.1: Cyclic representation of TCA cycle.

The stoichiometry of coenzyme reduction and ATP formation in the aerobic oxidation of a molecule of glucose via glycolysis, the pyruvate dehydrogenase reaction, and the citric acid cycle

Reaction	*Number of ATP or reduced coenzymes directly formed*	*Number of ATP ultimately formed**
Glucose to glucose-6-phosphate	-1 ATP	-1
Fructose-6-phosphate to fructose-1,6-bisphosphate	-1 ATP	-1
2 glyceraldehyde-3-phosphate to 2 1,3-bisphosphoglycerate	2 NADH *	6
2 1,3-bisphosphoglycerate to 2 3-phosphoglycerate	2 ATP	2
Phosphoenolpyruvate to 2 pyruvate	2 ATP	2
2 pyruvate to 2 acetyl-CoA	2 NADH *	6
2 isocitrate to α-ketoglutarate	2 NADH*	6
2 α-Ketoglutarate to 2 succinyl-CoA	2 NADH *	6
2 succinyl-CoA to 2 succinate	2 ATP (or 2 GTP)	2
2 succinate to 2 fumarate	2 $FADH_2$ *	4
2 malate to 2 oxaloacetate	2 NADH *	6
Total		38

*One molecule of glucose metabolizes to give 38 ATPs.

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COMPARISONS BETWEEN GLYCOLYSIS AND KREBS CYCLE

Glycolysis	*Krebs Cycle*
It is the first step of respiration yielding two molecules of pyruvic acids after the partial breakdown of a glucose molecule in a set of enzymatic processes	Krebs cycle is the second step of aerobic respiration in which pyruvate is oxidized completely into inorganic substances forming carbon dioxide
Occurs in all the living organisms	Occurs in aerobes
Occurs inside the cytoplasm	Occurs inside the mitochondria
No carbon dioxide evolved	Carbon dioxide evolved
Oxygen not required for glycolysis	Oxygen is required for Krebs cycle
Four adenosine triphosphate (ATP) molecules are produced in the glycolysis for each glucose molecule	One ATP or guanosine triphosphate (GTP) molecule is produced by substrate-level phosphorylation in each turn of the Krebs cycle
Consumes two molecules of ATP for initial phosphorylation of substance molecules	Does not consume ATP
Net gain of two molecules of ATP and two molecules of nicotinamide adenine dinucleotide (NADH) gained for every molecule of glucose broken down	Each turn of the Krebs cycle yields three molecules of NADH and two molecules of flavin adenine dinucleotide ($FADH_2$)
Occurs as a linear sequence	Occurs as a cyclic sequence

Both glycolysis and the Krebs cycle are enzyme-mediated and are under constant regulation based on the energy requirement of the cell/organism. The rates of these processes vary under various conditions, such as the well-fed state, fasting state, exercised state, and starvation state.

DISORDERS OF CARBOHYDRATE METABOLISM

As discussed above, carbohydrates must be broken down by the body into simpler constituents. This process needs many enzymes. If an enzyme needed to process a certain sugar is missing, the sugar can accumulate in the body, causing problems.

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- **Glycogen storage diseases:** Glycogen is made of many glucose molecules linked together. The sugar glucose is the body's main source of energy for the muscles (including the heart) and brain. Any glucose that is not used immediately for energy is held in reserve in the liver, muscles, and kidneys in the form of glycogen and is released when needed by the body.
  There are many different glycogen storage diseases (also called glycogenoses), each identified by a roman numeral. These diseases are caused by a hereditary lack of one of the enzymes that is essential to the process of forming glucose into glycogen and breaking down glycogen into glucose. About 1 in 20,000 infants has some form of glycogen storage disease **(Table 8.1)**.
  - *Symptoms:* Some of these diseases cause few symptoms. Others are fatal. The specific symptoms, age at which symptoms start, and their severity vary considerably among these diseases. For types II, V, and VII, the main symptom is usually weakness. For types I, III, and VI, symptoms are low levels of sugar in the blood and protrusion of the abdomen (because excess or abnormal glycogen may enlarge the liver). Low levels of sugar in the blood cause weakness, sweating, confusion, and sometimes seizures and coma. Other consequences for children may include stunted growth, frequent infections, and sores in the mouth and intestines.
    Glycogen storage diseases tend to cause uric acid (a waste product) to accumulate in the joints, which can cause gout, and in the kidneys, which can cause kidney stones). In type I glycogen storage disease, kidney failure is common.
  - *Diagnosis and treatment:* The specific type of glycogen storage disease is diagnosed by examining a piece of muscle or liver tissue under a microscope (biopsy).
    Treatment depends on the type of glycogen storage disease. For most types, eating many small carbohydrate-rich meals everyday helps prevent blood sugar levels from dropping.
- **Galactosemia:** Galactose is a sugar that is present in milk and in some fruits and vegetables. A deficient enzyme or liver dysfunction can alter the metabolism, which can lead to high levels of galactose in the blood (galactosemia). There are different forms of galactosemia, but the most common and the most severe form is referred to as classic galactosemia.

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